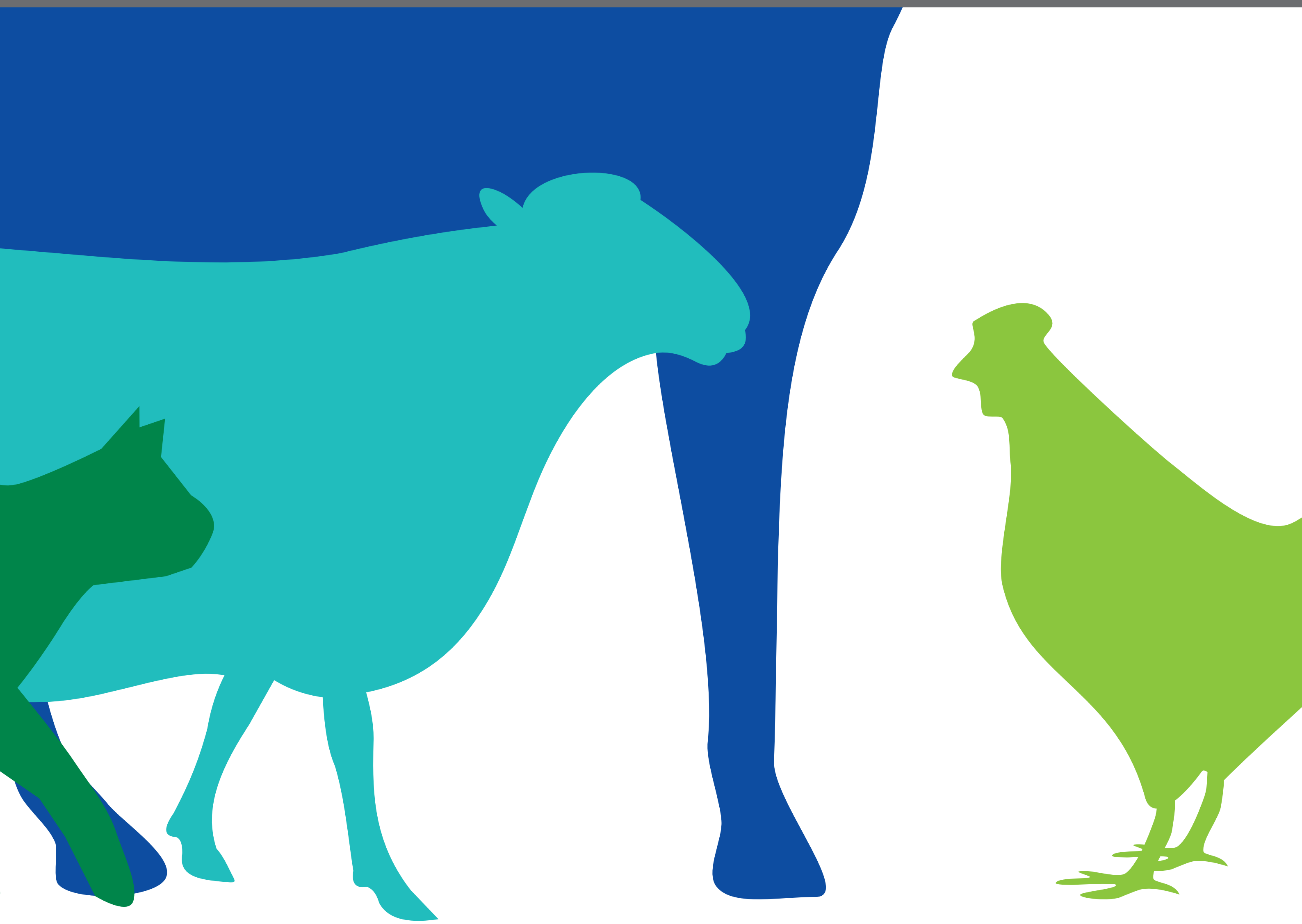
WORKING DOGS: FORM AND FUNCTION, 2nd Edition

EDITED BY: Cynthia M. Otto, Erik Wilsson and Mia Cobb
PUBLISHED IN: Frontiers in Veterinary Science



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-636-5
DOI 10.3389/978-2-88963-636-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

WORKING DOGS: FORM AND FUNCTION, 2nd Edition

Topic Editors:
Cynthia M. Otto, University of Pennsylvania, United States
Erik Wilsson, Swedish Armed Forces, Sweden
Mia Cobb, Working Dog Alliance, Australia

Publisher's note: In this 2nd edition, the following article has been updated: Otto CM, Cobb ML and Wilsson E (2019) Editorial: Working Dogs: Form and Function. Front. Vet. Sci. 6:351. doi: 10.3389/fvets.2019.00351

Citation: Otto, C. M., Wilsson, E., Cobb, M., eds. (2020). Working Dogs: Form and Function, 2nd Edition. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-636-5

Table of Contents

frontiers in Veterinary Science

EDITORIAL
published: 18 October 2019
doi: 10.3389/fvets.2019.00351

Editorial: Working Dogs: Form and Function

Cynthia M. Otto[1,2]*, *Mia L. Cobb*[3,4] *and Erik Wilsson*[5†]

[1] Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania, Philadelphia, PA, United States, [2] Penn Vet Working Dog Center, Philadelphia, PA, United States, [3] Working Dog Alliance, Melbourne, VIC, Australia, [4] School of Psychological Sciences, Monash University, Melbourne, VIC, Australia, [5] Swedish Armed Forces, Stockholm, Stockholm, Sweden

Keywords: heatstress, selection, assistance dog, canine athlete, hydration, gastritis, lameness

Editorial on the Research Topic

Working Dogs: Form and Function

Dogs have a long history of serving mankind as hunters and protectors. In the last century the unique roles of dogs working in concert with humans has expanded. Dogs now fulfill vital roles to support the concept of One Health, where the health of animals, humans and the environment intersect. These working dogs include assistance dogs that serve to guide and support individuals in their daily lives, detection dogs that use their powerful noses to identify hazards or odors of interest (e.g., explosives, drugs, diseases, and invasive or endangered species) and law enforcement canines that work with police and the military for our safety. In addition to these lines of work, canine athletes compete in professional (e.g., racing) and amateur events. This Research Topic addresses topics relevant to Working Dogs: **Form** and **Function**. As we ask dogs to provide a wide variety of functions, new information to ensure that they are physically and behaviorally equipped for the tasks is vital.

With the need for dogs to work in a wide variety of conditions, emphasis on factors that support optimal performance will enhance the ability of these dogs to have long and healthy careers. In this special issue; Working Dogs: Form and Function, of the 14 papers, eight address key aspects on preservation of physical health (form) and the remaining six highlight topics ranging from olfaction to service dog selection (function).

In military working dogs, heat stroke is one of the most common preventable cause of death (1) or early retirement (2). For working dogs and other canine athletes, heat stress is a well-recognized threat. Dogs generate heat through muscle activity during exercise, often work in environmentally challenging conditions and rely on panting to eliminate heat. Heat stress is a hazard for working dogs and can result in serious injury or death. In this special issue, temperature regulation was the most common topic and also the one that has generated the most interest from the community. Clinically, canine heat stroke is defined by core body temperatures exceeding 41°C (105.8°F) accompanied by central nervous system (CNS) dysfunction (3). Traditionally, rectal temperatures in excess of 105.8°F have been considered consistent with heat stroke, however this data is derived from hospital records rather than field assessments. It is now recognized that dogs actively exercising can reach body temperatures in excess of 105.8°F without any medical consequences or neurologic dysfunction (Robbins et al.; McNicholl et al.). Monitoring temperature can be challenging in the field setting in which dogs are exercising. Rectal temperature is considered the standard for body temperature measurement, however, medical conditions, physiologic states (4) and aversion to thermometer placement can impair the ability to collect this data. Several studies employed the research tool of telemetric core intestinal temperature monitoring (Robbins et al.; Otto et al.). Alternative ways to monitor for increased body temperature include thermography and physiologic signs.

OPEN ACCESS

Edited and reviewed by:
Mary M. Christopher, University of California, Davis, United States

***Correspondence:**
Cynthia M. Otto
cmotto@vet.upenn.edu

†Present address:
Erik Wilsson, Retired, Stockholm, Sweden

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: 07 September 2019
Accepted: 25 September 2019
Published: 18 October 2019

Citation:
Otto CM, Cobb ML and Wilsson E (2019) Editorial: Working Dogs: Form and Function. Front. Vet. Sci. 6:351. doi: 10.3389/fvets.2019.00351

In the study by Zanghi, rectal temperature, non-contact infrared thermography of the eye and contact thermography of the ear were evaluated in 16 Beagles and 16 Labrador retrievers after 30 min of play indoors with controlled temperature of (78–80°F) 25.6–26.7°C. Breed specific variations in temperature were identified at rest and after exercise, with Labradors maintaining higher temperatures and having slower post-exercise temperature recovery than Beagles. Ear and eye temperatures typically underestimated rectal temperature at rest and the relationship between eye temperature and rectal temperature diverged after activity. Play resulted in an average temperature of only 103.8°F (39.9°C), suggesting that the level of exertion was less than typically seen in exercising working dogs (Robbins et al.) but is consistent with dogs doing controlled on leash searches in a shaded environment (Otto et al.). Even with this moderate level of hyperthermia, after 15 min rectal temperatures in the Beagles had returned to baseline, while it required 30 min for Labrador rectal temperatures to return to baseline. Labrador ear and eye temperatures had not reached baseline by 30 min post-exercise.

Although the least invasive measure (eye temperature) does not track rectal temperature after exercise, other clinical signs can be used to identify dogs with hyperthermia and at risk of heat stress. In a field study of exercising working dogs, Robbins et al., evaluated core body temperature (using ingested temperature sensing capsules) and stamina (defined as the ability to withstand high energy demanding activity over extended periods of time). Dogs were exercised for 30 min outdoors in a median temperature of 28.9°C (84°F) and median humidity of 47%. Core body temperature which reached as high as 42.4°C (108.3°F) did not predict stamina, rather respiratory efficiency or ability to eliminate heat without creating profound acid/base disturbances was a major factor influencing stamina. Clinical signs of heat stress included an extended flat ("spade" shaped) tongue, retracted ears, squinty eyes, panting, and shade seeking.

Strategies to reduce heat stress may provide important ways to improve the performance and safety of working dogs that are required to exert themselves under adverse conditions. Two approaches to prevention are to decrease the heat of work and to improve the efficiency of heat exchange. One strategy is to provide more efficient burning fuels through nutritional modification. Current recommendations suggest that protein should represent 24% of metabolizable energy for working dogs. Protein, when fed in excess will be utilized as an energy source. Fats are considered a primary energy source for dogs. Feeding higher fat diets may improve stamina and olfactory ability, but the source of the fat is also important. Saturated fats (i.e., coconut oil) are reported to decrease olfactory acuity, while polyunsaturated fats (i.e., corn oil) improved olfactory efficiency (5, 6). Dietary fat may also impact thermodynamics. Compared to protein, which requires energy, thus generation of heat, to be utilized, fats are metabolized with close to 100% efficiency. The effect of a high fat (57%; corn oil supplemented), low protein diet (18%) on treadmill exercised detection dogs was compared to a high protein, high fat diet (27%:57% ME) and a high protein: low fat (27%:32% ME) (Ober et al.). The dogs fed the low protein, high fat diet maintained a lower core temperature after exercise compared to dogs fed the high protein, low fat diet. Altering dietary components that may help reduce heat generation is one strategy to optimize thermal balance.

A second approach is to maximize heat loss. Dogs eliminate heat through panting; increased salivation and lingual blood flow allow for convection and evaporative cooling (7). As dogs become more dehydrated, salivation is reduced dramatically (8), thus eliminating most of the cooling effect of salivation. Strategies to enhance hydration and prevent dehydration in working dogs have included providing water or flavored electrolyte solution orally or preloading with subcutaneous electrolyte solutions. None of these approaches had been evaluated under field conditions until a cross-over study was conducted with Border Patrol dogs screening vehicles at the Southern Border of the United States (Otto et al.). The Border Patrol dogs that were provided a flavored oral electrolyte solution consumed over twice the volume of liquid on a per weight basis. The dogs that received electrolyte solutions, whether oral or subcutaneous, had indirect markers of improved hydration (higher total CO_2, and lower packed cell volume and total plasma protein) at the end of the day. No effect of hydration strategy, however, was seen on core temperature.

Environmental conditions play an important role in the development of heat stress in dogs. The conditions during the Border Patrol study were not extreme (median temperature 84.8°F (29.3°C); median humidity 70%). Similarly, the Robbins et al., study evaluated exercise in dogs under median temperature [28.9°C (84°F)] but lower median humidity (47%) and found stamina was influenced by temperature but not humidity. In the Robbins study, core body temperature was not predicted by either temperature or humidity. In a study of racing greyhounds performing anaerobic exercise (running for 15–45 s over 300–731 m), there was an association between environmental temperature and post-race increase in rectal temperature (McNicholl et al.). When the ambient temperature reached 38°C (100.4°F), over one-third (39%) of greyhounds had a rectal temperature >41.5°C (106.7°F). Darker coat color, heavier body weight and male greyhounds were associated with higher post-race rectal temperatures. Humidity was not a predictor of core temperature in dogs in these studies.

Exercise induced hyperthermia represents a spectrum of injury, if severe enough to cause heat stroke, it can be a deadly. Less severe temperature elevations can lead to subtle physiologic changes that accumulate with repeated episodes of exercise induced hyperthermia and can also have serious consequences. In racing sled dogs, gastric dysfunction and development of ulcers (exercise induced gastric disease; EIGD), has been identified as a major cause of death (Davis and Williamson). The mechanism of injury is not proven; however, repeated exercise-induced hyperthermia, stress and excessive gastrin secretion are current hypotheses thought to contribute to altered mucosal permeability and ulcer formation. EIGD is not limited to endurance athletes. In one study, 84% of explosive detection Labradors had evidence of gastric ulcers after 5 consecutive days of exercise (9).

Exercise depends on the coordinated physiology to deliver oxygen to functioning muscles; without an intact musculoskeletal system, the dogs are unable to achieve the physical demands of mobility. Lameness is the asymmetric use of one or more limbs and can result from injury to muscles, bones, joints and nerves. The etiologic diagnosis of lameness has traditionally relied on physical palpation and radiographs, with options for advanced imaging such as magnetic resonance imaging (MRI), computed tomography (CT) and musculoskeletal ultrasound. In working and athletic dogs, subtle lameness can lead to an observable decrement of performance. In a study of guide dogs (Lloyd et al.), musculoskeletal disorders were the most common reason for dogs being withdrawn from service prior to retirement. Frequently physical examination and traditional imaging are not sensitive enough to detect the site of subtle injuries, and whole-body imaging is not economical or practical in most cases. New advances in imaging, include positron emission tomography (PET) imaging and novel applications of traditional computed tomography (CT), may help improve diagnosis and monitoring of musculoskeletal disease or injury. In PET scans, the radiopharmaceutical, fluorine- 18-fluorodeoxyglucose (18F-FDG), is taken up by hypermetabolic cells, allowing identification of regions of disease, inflammation or cellular activity. Traditionally, PET has been used for tracking cancerous cells, studying glucose metabolism or muscle activity. In a report by Mann et al., PET-CT imaging was utilized to pinpoint the source of a subtle lameness in a dog. The injury was then further characterized by musculoskeletal ultrasound. The addition of PET-CT to the diagnostic armamentarium provides novel information on both anatomic location and cellular activity, which is likely to assist with the characterization of elusive and dynamic lameness in canine athletes.

One common cause of mobility disorders in working dogs is lumbosacral (LS) or low back pain. This debilitating condition, like in humans, can be the result of structural abnormalities of the spine or inadequate muscular support in a highly dynamic region subject to repetitive motion during search behavior. Cain et al. compared CT images from working dogs with LS pain ($n = 11$) to those without LS pain ($n = 5$). Using quantitative transverse area ratios of the paraspinal muscles in the LS region, the investigators were able to compare the association of relative muscle mass and presence of muscle asymmetry to the presence of LS pain. Muscle asymmetry was not different between groups, but dogs with LS pain had significantly lower muscle area ratios. The reduction of muscle area could result from pain and disuse but could also present a target for physical rehabilitation. CT screening may be able to identify dogs at risk of injury and allow development of preventative physical conditioning plans.

The **function** of working dogs depends on their career path. Detection dogs rely on efficient and sensitive olfaction to perform their jobs. As reviewed by Angle et al., applications of canine olfaction to the detection of biological volatile organic compounds (VOCs) has been applied to medical diagnosis, therapeutic monitoring, disease outbreak containment, and disease prevention. Endogenous VOCs are generated through metabolic processes of the host and commensal organisms, transported through the blood and released into the environment through various routes including breath, sweat, feces, and urine. The VOC profile can be altered by numerous factors including diet, genetics, environment, and disease states. Dogs are able to detect odors in concentrations as low as one part per trillion, compared to traditional analytic chemistry instruments which are only sensitive in the order of parts per billion. In addition to the recent focus on cancer detection, biomedical detection dogs have been utilized to identify other diseases and pathogens. The approach has been versatile, with dogs working in laboratory environments, hospital environments and in the field. In the laboratory, *in vivo* and *in vitro* samples have been utilized. In this Research Topic, Angle et al. described how dogs could detect the virus that causes bovine viral diarrhea and discriminate it from bovine herpesvirus 1 and bovine parainfluenza virus 3, in cell cultures (*in vitro*). One of the important field applications of canine olfaction is the detection of diseased, invasive or endangered species. In a review of scent detection dogs in conservation settings Beebe et al., described the scope of conservation including detection of live wildlife, carcass detection for birds, and bats around wind turbines, detection of scats, pathogens, and other biological materials, and detecting the presence/absence, and relative abundance, of plants and wildlife.

A hurdle faced by all disciplines of working dogs is the ability to identify the best dog for the job. It is not unusual to screen hundreds of shelter dogs to identify those with the physical and behavioral traits necessary for success (10). Breeding programs offer some advantages through the ability to select for heritable health and behavioral traits and control the early developmental period of potential working dogs. There is a movement to develop a National Breeding Cooperative in the United States to provide a larger pool of available working dogs to meet the growing demands (11). Selection criteria are important for both breeding programs and for dogs selected from shelters. In the review by Beebe et al., selection parameters identified for conservation detection dogs share several similarities with other types of detection and some assistance dogs. The categories for selection include biological traits, psychological processes and social contexts. Most detection dogs and traditional assistance dogs are medium to large in stature, however, in the US, as the number of psychiatric dogs has increased, the number of small breed assistance dogs has increased dramatically (Walther et al.). Detection dogs (e.g. conservation, search and rescue, explosives, narcotics etc.) are often selected from working lines with strong olfactory capability and an unflagging desire to work for toys or in some cases, food. Normal hearing is necessary for optimal communication between dogs and handlers in most working careers. Assistance dogs rely more on visual cues than detection dogs, but good vision is essential for all working dogs. Conservation and detection dogs are often selected based on the psychological dimensions of persistence (the ability to sustain working motivation) and boldness (the response to unfamiliar stimuli and environments); although validated quantitative measures of these traits are generally lacking. A standardized, validated, and widely used survey instrument that measures the frequency and/or severity of most common behavior problems in dogs has been used to characterize assistance (Serpell and Duffy) and search dogs (12). In a study by Serpell and Duffy, predictors of behavioral traits in Guide Dogs being raised in volunteer's homes were retrospectively evaluated; German shepherd dogs had an increase in aggression toward strangers, but not familiar individuals between 6 and 12 months. Traumatic events during the 6–12 month stage were associated with more fear in the dogs. Being raised in a multidog household was associated with more positive behavioral traits.

The third aspect (social characteristics) of selection of conservation dogs, which is likely applicable to all working dog teams, focuses on correctly pairing dog-handler teams based on the experience and psychological traits of both the dog and the handler (Beebe et al.). In addition to their role as a mobility aid, guide dogs provide also provide other benefits that influence the success of the match. A successful match of guide dog and handler relies on the mobility and orientation teamwork, social interactions with the handler, other people and animals, and adaptation to the home environment. Over one quarter of guide dogs were identified as mismatched with handlers. Generally, the mismatch was apparent within 10 months of the match. The most common behavioral reason for return of a dog was poor home/social behavior. As with the importance of the role of the handler, the puppy raiser's experience also has an important impact on positive behavioral traits of the dog; the more experienced the puppy raiser, the more likely the dog will exhibit positive behavioral traits (Serpell and Duffy).

Preparing dogs and handlers for any type of working career is challenging. There is an increasing demand for both assistance and detection dogs. In response to this demand, many new organizations have cropped up, particularly in the field of assistance dogs (Walther et al.). In the United States, there are no published national standards for assistance dogs. Although the international organizations, Assistance Dogs International (ADI; https://assistancedogsinternational.org/) and International Guide Dog Federation (IGDF; https://www.igdf.org.uk/), have established basic guidelines and an accreditation process, these are voluntary. Many of the smaller organizations are not accredited and appear to have a high turnover (Walther et al.). There is a global shortage of detection dogs that is attributed to increased international demand (11). The National Institute of Standards and Technology (NIST; https://www.nist.gov/topics/forensic-science/dogs-sensors-subcommittee) has been working to convert Scientific Working Group on Dogs and Orthogonal detector Guidelines (SWGDOG; http://swgdog.fiu.edu/) into standards for detection dogs to ensure that the dogs that are serving in these important roles meet defined performance and care standards. In order to create standards, continued research into the form and function of these dogs is vital. These 14 studies published in this Research Topic have added to our knowledge to support the advancement of working dog science, but as all studies do, they raise more questions that we need to address. We are proud to be able to promote the science and dissemination of knowledge in the Frontiers open source venue and invite you to contribute your science to the next Working Dog Research Topic.

AUTHOR CONTRIBUTIONS

This editorial was authored by CO with review and input by MC and EW.

REFERENCES

1. Moore GE, Burkman KD, Carter MN, Peterson MR. Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993-1996). *J Am Vet Med Assoc.* (2001) 219:209–14. doi: 10.2460/javma.2001.219.209
2. Evans RI, Herbold JR, Bradshaw BS, Moore GE. Causes for discharge of military working dogs from service: 268 cases (2000-2004). *J Am Vet Med Assoc.* (2007) 231:1215–20. doi: 10.2460/javma.231.8.1215
3. Drobatz KJ, Macintire DK. Heat-induced illness in dogs: 42 cases (1976-1993). *J Am Vet Med Assoc.* (1996) 209:1894–9.
4. Goic JB, Reineke EL, Drobatz KJ. Comparison of rectal and axillary temperatures in dogs and cats. *J Am Vet Med Assoc.* (2014) 244:1170–5. doi: 10.2460/javma.244.10.1170
5. Angle CT, Wakshlag JJ, Gillette RL, Steury T, Haney P, Barrett J, et al. The effects of exercise and diet on olfactory capability in detection dogs. *J Nutr Sci.* (2014) 3:e44. doi: 10.1017/jns.2014.35
6. Altom EK, Davenport GM, Myers LJ, Cummins KA. Effect of dietary fat source and exercise on odorant-detecting ability of canine athletes. *Res Vet Sci.* (2003) 75:149–55. doi: 10.1016/S0034-5288(03)00071-7
7. Crawford EC Jr. Mechanical aspects of panting in dogs. *J Appl Physiol.* (1962) 17:249–51. doi: 10.1152/jappl.1962.17.2.249
8. Baker MA, Doris PA, Hawkins MJ. Effect of dehydration and hyperosmolality on thermoregulatory water losses in exercising dogs. *Am J Physiol.* (1983) 244:R516–21. doi: 10.1152/ajpregu.1983.244.4.R516
9. Davis MS, Willard MD, Bowers D, Payton ME. Effect of simulated deployment patrols on gastric mucosa of explosive detection dogs. *Comp Exercise Physiol.* (2014) 10:99–103. doi: 10.3920/CEP14002
10. Hurt A, Smit DA. Conservation dogs. In: Helton WS, editor. *Canine Ergonomics: The Science of Working Dogs*. Boca Raton, FL: CRC Press (2009). p. 175–94. doi: 10.1201/9781420079920.ch9
11. Leighton EA, Hare E, Thomas S, Waggoner LP, Otto CM. A solution for the shortage of detection dogs: a detector dog center of excellence and a cooperative breeding program. *Front Vet Sci.* (2018) 5:284. doi: 10.3389/fvets.2018.00284
12. Hare E, Kelsey KM, Serpell JA, Otto CM. Behavior differences between search-and-rescue and pet dogs. *Front Vet Sci.* (2018) 5:118. doi: 10.3389/fvets.2018.00118

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 08 January 2016
doi: 10.3389/fvets.2015.00079

Real-Time Detection of a Virus Using Detection Dogs

T. Craig Angle[1], Thomas Passler[2], Paul L. Waggoner[1], Terrence D. Fischer[1], Bart Rogers[1], Patricia K. Galik[3] and Herris S. Maxwell[2]*

[1] *Canine Performance Sciences Program, Auburn University, Auburn, AL, USA,* [2] *Department of Clinical Sciences, Auburn University, Auburn, AL, USA,* [3] *Animal Health Research, Auburn University, Auburn, AL, USA*

Viral infections are ubiquitous in humans, animals, and plants. Real-time methods to identify viral infections are limited and do not exist for use in harsh or resource-constrained environments. Previous research identified that tissues produce unique volatile organic compounds (VOC) and demonstrated that VOC concentrations change during pathologic states, including infection, neoplasia, or metabolic disease. Patterns of VOC expression may be pathogen specific and may be associated with an odor that could be used for disease detection. We investigated the ability of two trained dogs to detect cell cultures infected with bovine viral diarrhea virus (BVDV) and to discriminate BVDV-infected cell cultures from uninfected cell cultures and from cell cultures infected with bovine herpes virus 1 (BHV 1) and bovine parainfluenza virus 3 (BPIV 3). Dogs were trained to recognize cell cultures infected with two different biotypes of BVDV propagated in Madin–Darby bovine kidney cells using one of three culture media. For detection trials, one target and seven distractors were presented on a scent wheel by a dog handler unaware of the location of targets and distractors. Detection of BVDV-infected cell cultures by Dog 1 had a diagnostic sensitivity of 0.850 (95% CI: 0.701–0.942), which was lower than Dog 2 (0.967, 95% CI: 0.837–0.994). Both dogs exhibited very high diagnostic specificity (0.981, 95% CI: 0.960–0.993) and (0.993, 95% CI: 0.975–0.999), respectively. These findings demonstrate that trained dogs can differentiate between cultured cells infected with BVDV, BHV1, and BPIV3 and are a realistic real-time mobile pathogen sensing technology for viral pathogens. The ability to discriminate between target and distractor samples plausibly results from expression of unique VOC patterns in virus-infected and -uninfected cells.

Keywords: bovine viral diarrhea virus, dog, odor, virus detection, volatile organic compounds

Edited by:
Cynthia M. Otto, University of Pennsylvania, USA

Reviewed by:
Erik Wilsson, Swedish Armed Forces, Sweden
Claire Marie Guest, Medical Detection Dogs, UK
Esther Schalke, Lupologic GmbH, Germany

****Correspondence:***
T. Craig Angle
angletc@auburn.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *20 October 2015*
Accepted: *14 December 2015*
Published: *08 January 2016*

Citation:
Angle TC, Passler T, Waggoner PL, Fischer TD, Rogers B, Galik PK and Maxwell HS (2016) Real-Time Detection of a Virus Using Detection Dogs. Front. Vet. Sci. 2:79. doi: 10.3389/fvets.2015.00079

INTRODUCTION

Globally, infectious diseases continue to be leading causes of morbidity and mortality. Efforts to control infectious disease in human, animal, and plant populations would benefit from real-time screening technologies, which could be effectively deployed in areas of strategic interest for pathogen transmission. Current surveillance methods rely on the collection of diagnostic samples from individuals or contaminated environments, transportation of samples to a laboratory, and subsequent laboratory testing to demonstrate the presence of the pathogen of interest, resulting in a significant delay in response times and containment efforts. Development of a sensitive, easily deployable real-time mobile pathogen sensing technology (RMST) would be useful in border security, public health, wildlife management, and agriculture to aid in the detection and containment of disease outbreaks and to prevent acts of bioterrorism and agro terrorism.

The abilities of trained dogs to identify odors associated with explosives and munitions in operational environments are superior to other currently available detection technologies. The highly sensitive canine olfactory sensory system can detect some target substances at concentrations as low as parts per trillion (1), three orders of magnitude more sensitive than available instruments which reliably identify substances at concentrations of parts per million or billion. Canine detection systems are inherently mobile and can trace an odor to its source. To be comparable to the detection ability of dogs, a detection system would need to be (1) extraordinarily sensitive, (2) mobile, and (3) able to move toward a target source. No currently available system meets those criteria. If dogs can be trained to detect odors associated with specific pathogens, canine-based RMST systems could provide a method for pathogen detection in frontline operational environments and complement the development of detection instrumentation for pathogen detection, analytical chemistry, and metabolomics.

Pathogen Biomarkers for Scent Detection

Strong scientific evidence supports the release of stable volatile organic compounds (VOC) from tissues that are present in exhaled breath, urine, feces, and sweat. The VOC that are released are known as the volatilome (2). VOC are volatile at ambient temperatures, may be associated with an odor, and may provide the continuous signal needed for real-time detection (3).

Tissues infected with pathogens release unique volatile metabolic biomarkers, which become part of detectable VOC disease signatures (4, 5). Disease-specific VOC show potential for use in medical diagnosis and therapeutic monitoring (2). Multiple studies demonstrated that VOC patterns may be unique to a specific pathogen or an infection with a specific pathogen. In a review of 31 publications, Bos et al. (6) concluded that many pathogenic bacteria have distinct metabolisms that produce species-specific VOC and suggested that the presence of these VOC in patients indicated infection. In a cell culture model, Schivo et al. (7) demonstrated different VOC expression patterns in primary human tracheobronchial cells infected or uninfected with human rhinovirus. Aksenov et al. (8) determined that VOC produced by B lymphoblastoid cells following infection with three live influenza virus subtypes were unique for each virus subtype. In addition, Abd El Qader et al. (9) examined the VOC released from cultures of five viruses (influenza A, influenza B, adenovirus, respiratory syncitial virus, and parainfluenza 1 virus) and four bacteria (*Moraxella catarrhalis*, *Haemophilus influenza*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*). The researchers detected 12 and 6 VOC that were associated with bacterial and viral growth and identified 2 VOC that were differentiated between bacterial and viral infection (9). Lastly, Mashir et al. (10) administered live attenuated H1N1 vaccine (FluMist®) to humans and demonstrated that exhaled breath VOC increased for 7 days after the vaccination. These studies suggest that unique VOC profiles associated with viral pathogens exist and that they may be detected in patients. If pathologic processes such as infections, neoplasia, and metabolic disorders influence the type, ratio, and strength of VOC emitted from a biological system, then unique VOC patterns may create a specific signature odor (11). Currently, VOC analysis requires expensive and sophisticated stationary analytical chemical instrumentation, such as gas chromatography–mass spectrometry.

Dogs as a Pathogen and Disease Sensor

Trained dogs have consistently demonstrated value as sensitive real-time chemical sensing detectors for narcotic, explosive, and select biological targets. Similarly, dogs have been used to detect disease biomarkers in humans, including cancer and bacterial infections. Potential VOC biomarker concentrations are reported to be in the part per billion to part per trillion range for breath and part per million to part per billion range in blood and urine (12). Canids are capable of detecting some substances in concentrations as low as parts per trillion (1). Therefore, VOC biomarkers are within the detection range of the canine olfaction system. In some cases, dogs have been able to detect disease states in exhaled breath that contains the lowest known VOC concentrations. Sonoda et al. (13) trained a dog to detect human patients with colon cancer using samples of exhaled breath and watery stool. The dog's sensitivity and specificity for cancer detection in breath samples were 0.91 and 0.99, respectively. The sensitivity and specificity for detection in stool samples were 0.97 and 0.99, respectively (13).

Another study demonstrated that the overall sensitivity of canine scent detection of lung cancer utilizing exhaled breath samples was 0.99, with a specificity of 0.99 (14). In the same report utilizing trained dogs to evaluate breath samples from breast cancer patients and controls, the sensitivity of detection was 0.88 and specificity was 0.98 (14). These reports suggest that VOC or similar compounds from diseased internal tissues are released externally and may be detected on the body or in the surrounding air with the aid of trained dogs with a high degree of diagnostic accuracy.

Pathogen detection methods utilizing the keen canine sense of smell may offer a viable option for developing a rapidly deployable disease screening tool and provide valuable information about a subject's pathophysiological condition (3, 15). The use of trained detection dogs offers certain inherent advantages. Unlike a deployable instrument, dogs can examine thousands of samples or scan large surface areas efficiently, which is important in detecting pathogens in large herds of animals, crowds of people, objects (e.g., ships, airplanes, buildings), or areas of land. Diagnostic testing using laboratory instrumentation in an operational environment is often impeded by the lack of cleanliness, interference by air particulates, presence of non-target VOC produced by various substances in the environment, and constantly changing variables, such as temperature, humidity, wind, and thermal plumes. By contrast, purpose-bred detection dogs have a demonstrated ability to search for unique odor patterns and identify specific targets in field conditions amidst substantial "odor noise" (i.e., varied and/or strong odors). Although at least 381 unique VOC are emitted from human feces (2), a trained dog was able to detect *Clostridium difficile* in human stool samples (16). The dog detected *C. difficile* with high diagnostic sensitivity and specificity in stool samples and hospitalized patients, correctly identifying 25 of the 30 *C. difficile* cases and 265 of 270 control cases (16). This emphasizes the dog's ability to detect a specific odor pattern among the myriad of odors from other bacteria, fungi, and viruses naturally present in feces.

There are no reported attempts to train dogs to detect viral pathogens. Detection of infection or disease by trained dogs could provide advantages over other VOC detection technologies such as mass spectrometry by providing a real-time binary response, avoiding the need for trained personnel in the processing and interpretation of mass spectrometry samples, and avoidance of testing-associated delays in response efforts. Dogs are mobile, adapted to difficult work environments, can track a plume of airborne target material to its source and can eliminate the need to collect and transport surface or air samples to a centralized laboratory. If dogs can be trained to locate target pathogens, they could be employed to detect pathogens or be deployed at strategic locations to prevent entry and transmission of disease.

The use of dogs to detect odors associated with viral infection and sensitivity and specificity of a canine detection model has not been described. The purpose of this study was to evaluate the ability of trained dogs to detect viral pathogen-associated odors in real-time, alert a handler to the presence of these pathogens, and discriminate those odors from those associated with other viral pathogens. Specifically, we examined the dog's ability to identify bovine viral diarrhea virus (BVDV) infected cell cultures and to discriminate those cell cultures from those infected with other bovine viral pathogens.

MATERIALS AND METHODS

Animals

Two healthy adult male Labrador Retrievers were trained to detect BVDV-infected cell cultures. The dogs were purpose-bred for detection work from a colony of detection dogs developed at the Auburn University Canine Performance Sciences Breeding Program. Each dog had over 3 years of operational experience as an explosives detection dog in our Canine Performance Sciences Research Program. The dogs were selected based on their previous experience as explosives detection dogs and because they had calculated and methodical microsearch techniques that are important for the detection of viral targets. The dogs received 2 months of proprietary viral target detection training prior to data collection. The dogs were trained by a Master Detection Dog Trainer who had over 35 years of experience training detection dogs. The dogs were trained in a Bio Safety Lab Level 2 (BSL2) during the training period and received up to 15–30 trials per day, 4–5 days/week. All activities for this project were approved by an Institutional Animal Care and Use Committee and a Biological Use Authorization was granted by an Institutional Biosafety Committee.

Testing Apparatus

A 12 × 12 foot, climate and humidity controlled, indoor, BSL2 isolation room was used for scent testing. In the center of the room was a scent wheel with eight arms that are designed to each hold a small metal basket. For each trial, one target odor and seven distracting odors (or eight distracting odors for blank trials) (**Table 1**) were each placed in separate glass Petri dishes, covered by a mesh screen, and then individually placed in a metal basket, one per arm of the scent wheel. All target odors were randomly assigned a position (1–8) on the scent wheel. Dogs were brought into the room and allowed to search, starting at position 1 and working to position 8. When the dog found the target odor, it alerted by sitting, and was rewarded with a toy.

Targets and Distractors

Bovine viral diarrhea virus (BVDV) was chosen as the target virus, because it provides a pathogen model that has been extensively studied and is easily propagated in different types of cells and media and is not pathogenic to humans or dogs. A non-cytopathic strain of BVDV (1b AU526) and a cytopathic strain of BVDV (1a NADL) were used as target viruses. The BVDV targets were propagated in Madin–Darby bovine kidney (MDBK) cells using one of three variations of culture media. The dogs were presented with 0.5 ml of target sample containing either cytopathic or non-cytopathic BVDV [1×10^5–1×10^6 $CCID_{50}$ (cell culture infective doses, 50% endpoint)/ml]. The distractor viruses used in this study were bovine herpesvirus 1 (BHV 1) Colorado strain and bovine parainfluenza virus 3 (BPIV

TABLE 1 | Target viruses (BVDV AU526 and BVDV NADL) and distractor viruses (BHV 1 and BPI 3) were propagated on Madin–Darby bovine kidney (MDBK) cells using one of three media.

Targets	Distractors
1A (*n* = 19): BVDV AU526 **(Non-cytopathic)** + MDBK + **EQS** (SPT = 3)	1B = MDBK + **EQS**
2A (*n* = 21): BVDV AU526 **(Non-cytopathic)** + MDBK + **FBS** (SPT = 1)	2B = MDBK + **FBS**
3A (*n* = 15): BVDV AU526 **(Non-cytopathic)** + MDBK + EQS + **Gentamicin** (SPT = 2)	3B = MDBK + **EQS** + **Gentamicin**
4A (*n* = 2): BVDV NADL **(Cytopathic)** + MDBK + **EQS** (SPT = 0)	7A = **BHV-1 (Cytopathic)** + MDBK + **EQS**
5A (*n* = 2): BVDV NADL **(Cytopathic)** + MDBK + FBS (SPT = 1)	8A = **BHV-1(Cytopathic)** + MDBK + **FBS**
6A (*n* = 5): BVDV NADL **(Cytopathic)** + MDBK + EQS + **Gentamicin** (SPT = 0)	9A = **BHV-1 (Cytopathic)** + MDBK + **Gentamicin**
	10A = **BPIV-3 (Cytopathic)** + MDBK + **EQS**
	11A = **BPIV-3 (Cytopathic)** + MDBK + **FBS**
	12A = **BPIV-3 (Cytopathic)** + MDBK + **EQS** + **Gentamicin**

While cell culture media 1, 3, 4, and 6 contained equine serum (EQS), fetal bovine serum (FBS) was used in the preparation of medium 2 and 5. Cell culture medium 3 and 6 were prepared using the antibiotic gentamicin as an additional distractor. The n values represent the total number of samples used in the target column and the SPT values equal the total number of search past targets for each sample. The underlined text emphasizes the different characteristics of the two strains of BVDV that were used; the text in bold emphasizes differences among the three types of media.

3) SF/4. Preparation of all samples was performed by the same laboratory technician wearing identical nitrile gloves to prevent odor differences among samples caused by differences in sample handling. Target and distractor viruses were propagated in 75 cm^2 cell culture flask that had been seeded with MDBK cells 24 h earlier. Three hundred microliters of stock virus were added to each flask in 3 ml of media. Cell culture media contained purified water, Minimal Essential Media with Earle's salts (GIBCO® MEM, 10×, 11430, Thermo Fisher Scientific, Life Technologies, Grand Island, NY, USA), L-glutamine [GIBCO® L-glutamine (200 mM), Thermo Fisher Scientific, Life Technologies, Grand Island, NY, USA], PSF [GIBCO® Antibiotic-Antimycotic (100×), Thermo Fisher Scientific, Life Technologies, Grand Island, NY, USA], sodium bicarbonate (GIBCO® Sodium Bicarbonate 7.5% solution, Thermo Fisher Scientific, Life Technologies, Grand Island, NY, USA), and serum. Three different cell culture media were prepared that differed either in the type of serum added (equine serum (1 and 4) or fetal bovine serum (FBS) (2 and 5)), or contained gentamicin as an additional antibiotic (3 and 6). Following 1 h of adsorption, 20 ml of additional medium was added. Flasks were incubated until cytopathic effect in approximately 60% of cells was observed (BVDV 1 NADL, BHV 1, and BPI 3) or for 3 days when propagating the non-cytopathic BVDV AU526. Virus was harvested by a single freeze–thaw cycle by placing the flask in a −80°C freezer. Following thawing, contents of each flask were aliquoted into plastic snap-top tubes and stored (−80°C) until needed. Virus-free distractor samples underwent identical preparation including 24 h incubation prior to addition of 20 ml of media, incubation for 2–3 days following addition of media, and submission to a single freeze–thaw cycle prior to aliquoting and storage. For training and testing of dogs, 0.5 ml of each sample was placed into glass Petri dishes.

Distractors, or "non-target odors," are used to provide scents that are similar to or slightly different than the positive target to ensure that the dog is truly indicating on the positive target. Distractors in this study are listed in **Table 1**.

The duration of exposure to target and non-target odors was very short, typically <0.25 s (i.e., the amount of time needed to sniff a basket), and determined by individual dog search behavior; dogs were never manually forced to sample the vapor from any target source (i.e., the dog is always free to repel from the source of the odor). The dogs typically searched all eight scent wheel positions in 3.5–4.0 s. Targets and distractors were presented in a manner to prevent dogs from physically contacting or ingesting the sample.

Extensive efforts were made to reduce confounding factors that could lead to false positive results or inflated measures of detection performance unrelated to detection/non-detection of the virus odor outside that of the dog smelling the target virus odor. Only one target sample was used per trial and its position among the eight arms of the scent wheel on each trail was randomly assigned. One hundred percent of targets and distractors and their holding containers were changed after each trial. Baskets, basket holders, scent wheel apparatus, and Petri dishes were only handled using nitrile gloves and metal forceps to eliminate human scent. Baskets were sanitized on high heat at least daily in a commercial dishwasher, without soap. All targets and distractors were handled by the same person to eliminate the dog's ability to identify a single person associated with the target. Each arm of the scent wheel is identical to negate any visual cues that may enable detection capabilities. Dogs were monitored for characteristic changes in behavior related to detection of a target odor, including pausing and turning head abruptly at a position or emitting its trained final response of sitting at a target. All dogs were operated off lead by the handler. The dogs where handled by a Lead Canine Instructor who had 6 years of canine training and handling experience. This canine instructor did assist in training the dogs for this project. The handler was blinded to the target location and upon releasing the dog into the room, the handler stood at the door and stared straight ahead to avoid influencing the dog. If the dog indicated on a basket, the test moderator who was in the test room told the handler to reward or withhold the reward. This process insured that the dogs were not rewarded for indicating on a distractor virus and then being subsequently imprinted on the distractor virus because it received its reward. Blank trials contained eight distractors and no BVDV. Blank trials were utilized to insure that the dogs did not find a live target every time they searched the scent wheel, which reduces the propensity of the dog to alert to the final position regardless of whether it contains a target.

The dogs were taught to search position 1 to position 8 and then exit the room. The test moderator at the end of the trial notated the number of distractors that the dog searched in each trial. This provided the ability to calculate the total number of distractors searched by the dog.

Data Analysis

Search results for each trial were recorded and entered into a spreadsheet (Microsoft Excel, Microsoft, Redmond, WA, USA). The sensitivity, specificity, and associated 95% confidence intervals were calculated using an online calculator (http://vassarstats.net).

RESULTS

The results for this study are shown in **Table 2**. Dog 1 alerted to 28 of 34 total target presentations and emitted 6 alerts across a total of 317 distractor presentations. Dog 2 alerted to 30 of 31 total target presentations and emitted 2 alerts across a total of 287 distractor presentations. The diagnostic sensitivity of Dog 1 [0.850, 95% confidence interval (CI): 0.701–0.942] was lower than that of Dog 2 (0.967, 95% CI: 0.837–0.994), and both dogs exhibited very high diagnostic specificity (0.981, 95% CI: 0.960–0.993) and (0.993, 95% CI: 0.975–0.999), respectively.

TABLE 2 | Individual dog results.

	Dog 1	Dog 2
Sensitivity	0.85	0.967
Specificity	0.981	0.993
Total number of search past targets (i.e., misses)	6	1
Total number of positive indications	28	30
Total number of positive trials	34	31
Total number of blank trials (i.e., no BVDV present)	24	20
Total number of negative samples searched	317	287
Total number of false indications	6	2

A total of 65 positive and 44 blank (i.e., no BVDV present) trials were conducted. Each trial had a possibility of up to seven or eight distractors. There were 109 total trials in the study and after back calculating for the total number of distractors experienced, there were 604 distractors encountered by the dogs. There were eight total false responses that occurred while discriminating the 604 distractors used during this study. This demonstrates that the dogs did discriminate a large number of distractors and maintained a high rate of specificity. The dogs did not alert on seven targets and false responded on eight distractors. No search past target was attributed to a specific target (i.e., type of BVDV, see **Table 1**). Data for false responses were not collected for a specific distractor; therefore, it is unknown if the dogs false responded on any one distractor. Observations made by the test moderator were that the dogs false indicated on multiple distractors.

DISCUSSION

We hypothesized that dogs were able to detect and differentiate BVDV-infected cell cultures utilizing odors associated with different VOC expression patterns. Several recent reports indicate that cellular infections with viruses are associated with unique profiles of VOC (7–10, 17), but no published studies equate these VOC with an odor that is detectable by trained dogs. In this study, we were able to train dogs to discriminate cell cultures infected with BVDV from uninfected cell cultures and from cell cultures infected with one of two other viruses demonstrating the potential to detect the presence of odors associated with a target virus. The detection and discrimination of infected cell cultures by trained dogs supports our hypothesis of a unique odor profile associated with BVDV infection. As detection by the dog is immediate, this method has potential for use in real-time detection of viral pathogens in field situations.

Biochemical mechanisms underlying the release of disease-related VOC are largely unknown (4, 5, 12). No published studies define which, if any, VOC are detectable as an odor by dogs and the identity of the substances which alerted our dogs to infection is speculative. It is possible that VOC profiles associated with viral infections are analogous to the odor profile detected by our dogs. Previous studies demonstrated that compounds induced by pathogen infection may be detected as an odor (11), but current chemical analytical techniques have not defined the full odor profile which would control the alerting behavior of a dog.

Our study utilized purpose-bred detection dogs and training methods proven to be successful for the detection of other target substances, such as explosives. To the best of our knowledge, no previous attempts to detect and discriminate viral targets utilizing trained dogs have been made. The sensitivity and specificity of detection in the current study are similar to studies using dogs to detect the presence of neoplastic disease. Two studies evaluating the ability of dogs to detect prostate cancer-associated compounds in urine have yielded conflicting results. Elliker et al. (18) reported that trained dogs did not generalize on a prostate cancer odor and did not alert to new cancer samples following training on 50 unique prostate cancer samples and 67 controls. However, this study did not use purpose-bred detection dogs and the dogs lacked any previous detection experience. By contrast, Cornu et al. (19) trained a single dog to alert to positive prostate cancer samples with a sensitivity of 0.91. The differing results suggest that training methods, individual canine capabilities, and trial evaluations can affect outcomes and must be rigorous and scientifically sound to allow valid conclusions.

Results of our study suggest that trained dogs have the potential to function as a highly sensitive, highly specific, and mobile sensory technology to detect pathogen targets. Trained dogs could bridge the gap in pathogen and disease detection until other technologies are developed. The use of trained detector dogs has proven to be successful as a real-time mobile sensory technology in many inclement environments in the world during military, police, rescue, and other operations. Conversely, current analytical technologies capable of detecting diseases or pathogens in real-time in operational scenarios are unavailable, and there is no reported technology that can follow odor to its source like a dog.

Limitations of the current study include the use of cell culture-derived BVDV, use of a small number of trained dogs, a single-blind experiment, and the completion of the experiment in a climate-controlled environment. We did not evaluate the lowest level of detection of BVDV; however, our 0.5 ml samples of cell cultures were well within the range of detection by the dogs. We do not know at what level the sample becomes undetectable. Future research should focus on multiple target viruses, utilize a greater number of trained canids, and include a greater number of distractors. The test moderator was in the BSL2 with the dog making this a single-blind experiment. It is not known if the dog or handler was influenced by the moderator's knowledge of the target position and how that may/may not have influenced the results. The dogs were off lead and the only command given by the handler was to "come" (i.e., out of the BSL2 room). Future projects should be conducted in a double-blind fashion. Virus detection should be conducted in different environmental conditions to assess the effects of temperature and humidity on sensitivity and specificity. The ability of dogs trained on cell culture-derived BVDV to correctly identify samples collected from infected hosts should be explored to determine if dogs can successfully detect viral infection utilizing a variety of sample types (e.g., breath, nasal discharge, sweat, and saliva).

The potential for detector dogs or their handlers becoming infected or harmed by a potential pathogen or transmitting the pathogen should be considered. Specific target viruses selected for study should be evaluated for risk to the detector dogs or transmission to humans, animals, and plants.

CONCLUSION

Our study indicates that dogs can detect and discriminate virus-infected cell cultures. Apparently, unique odors associated with viral infections allow the dogs to obtain high rates of sensitivity and specificity. This finding demonstrates the potential for utilizing dogs to detect pathogens in real-time, which would be useful to identify or contain pathogen outbreaks, deter acts of bioterrorism, facilitate immediate treatment and containment of pathogen outbreaks, and reduce the need to transport samples for laboratory testing. Given that this demonstrated capability of dogs to detect odors associated with viruses and act as a RMST, additional research and development are warranted. In addition, dogs offer a platform for discovery to advance disease and chemical sensing machine technologies. Dogs provide a three order of magnitude more sensory capacity than most current diagnostic instruments. Therefore, they could be used for identifying specific VOC biomarkers related to disease which could aid metabolomics discoveries in other fields, such as analytical chemistry and wildlife biology.

Manufacturer Information

a Corning® cell culture flask, Corning Inc., Corning, NY, USA.
b GIBCO® MEM, 10×, 11430, GIBCO by Life Technologies Corp., Grand Island, NY, USA.
c Seradigm, Providence, UT, USA.
d HyClone Laboratories Inc., Logan, UT, USA.
e GIBCO® L-glutamine (200 mM), GIBCO by Life Technologies Corp., Grand Island, NY, USA.
f GIBCO® Antibiotic-Antimycotic (100×), GIBCO by Life Technologies Corp., Grand Island, NY, USA.
g GIBCO® Sodium Bicarbonate 7.5% solution, GIBCO by Life Technologies, Grand Island, NY, USA.
h Gentamicin sulfate powder, AMRESCO, Solon, OH, USA.

AUTHOR CONTRIBUTIONS

CA, TP, and PW developed the research idea and experimental design. TP and PG propagated the viruses and prepared training and testing aids. TF and BR performed canine training and testing. CA, TP, PW, and HM co-wrote the manuscript and all authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors express sincere appreciation for advice and technical support by Dr. Kay Riddell and Dr. Paul Walz of Auburn University Animal Health Research.

FUNDING

Funding for this project was provided by the Auburn University Canine Performance Sciences Program through the Richard G. and Dorothy A. Metcalf Endowment.

REFERENCES

1. Walker DB, Walker JC, Cavnar PJ, Taylor JL, Pickel DH, Hall SB, et al. Naturalistic quantification of canine olfactory sensitivity. *Appl Anim Behav Sci* (2006) **97**:241–54. doi:10.1016/j.applanim.2005.07.009
2. Amann A, Costello B, Miekisch W, Schubert J, Buszewski B, Pleil J, et al. The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res* (2014) **8**:034001. doi:10.1088/1752-7155/8/3/034001
3. Sethi S, Nanda R, Chakraborty T. Clinical application of volatile organic compound analysis for detecting infectious diseases. *Clin Microbiol Rev* (2013) **26**:462–75. doi:10.1128/CMR.00020-13
4. Buljubasic F, Buchbauer G. The scent of human diseases: a review on specific volatile organic compounds as diagnostic biomarkers. *Flavour Frag J* (2015) **30**:5–25. doi:10.1002/ffj.3219
5. Wilson AD. Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath. *Metabolites* (2015) **5**:140–63. doi:10.3390/metabo5010140
6. Bos LDJ, Sterk PJ, Schultz MJ. Volatile metabolites of pathogens: a systematic review. *PLoS Pathog* (2013) **9**:e1003311. doi:10.1371/journal.ppat.1003311
7. Schivo M, Aksenov AA, Linderholm AL, McCartney MM, Simmons J, Harper RW, et al. Volatile emanations from in vitro airway cells infected with human rhinovirus. *J. Breath Res* (2014) **8**:037110. doi:10.1088/1752-7155/8/3/037110
8. Aksenov AA, Sandrock CE, Zhao WX, Sankaran S, Schivo M, Harper R, et al. Cellular scent of influenza virus infection. *Chembiochem* (2014) **15**:1040–8. doi:10.1002/cbic.201300695
9. Abd El Qader A, Lieberman D, Shemer Avni Y, Svobodin N, Lazarovitch T, Sagi O, et al. Volatile organic compounds generated by cultures of bacteria and viruses associated with respiratory infections. *Biomed Chromatogr* (2015) **29**:1783–90. doi:10.1002/bmc.3494
10. Mashir A, Paschke KM, van Duin D, Shrestha NK, Laskowski D, Storer MK, et al. Effect of the influenza A (H1N1) live attenuated intranasal vaccine on nitric oxide (FENO) and other volatiles in exhaled breath. *J Breath Res* (2011) **5**:037107. doi:10.1088/1752-7155/5/3/037107
11. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. *J Biochem* (2011) **150**:257–66. doi:10.1093/jb/mvr090
12. Schmidt K, Podmore I. Current challenges in volatile organic compounds analysis as potential biomarkers of cancer. *J Biomark* (2015) **2015**:981458. doi:10.1155/2015/981458
13. Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, et al. Colorectal cancer screening with odour material by canine scent detection. *Gut* (2011) **60**:814–9. doi:10.1136/gut.2010.218305
14. McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K, Janecki T. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. *Integr Cancer Ther* (2006) **5**:30–9. doi:10.1177/1534735405285096
15. Van den Velde S, Nevens F, Van Hee P, van Steenberghe D, Quirynen M. GC-MS analysis of breath odor compounds in liver patients. *J Chromatogr B Analyt Technol Biomed Life Sci* (2008) **875**:344–8. doi:10.1016/j.jchromb.2008.08.031
16. Bomers MK, van Agtmael MA, Luik H, van Veen MC, Vandenbroucke-Grauls CMJE, Smulders YM. Using a dog's superior olfactory sensitivity to identify *Clostridium difficile* in stools and patients: proof of principle study. *Br Med J* (2012) **345**:e7396. doi:10.1136/bmj.e7396
17. Cheung WHK, Pasamontes A, Peirano DJ, Zhao W, Grafton-Cardwell EE, Kapaun T, et al. Volatile organic compound (VOC) profiling of citrus tristeza virus infection in sweet orange citrus varietals using thermal desorption gas chromatography time of flight mass spectrometry (TD-GC/TOF-MS). *Metabolomics* (2015) **11**:1514–25. doi:10.1007/s11306-015-0807-6
18. Elliker KR, Sommerville BA, Broom DM, Neal DE, Armstrong S, Williams HC. Key considerations for the experimental training and evaluation of cancer odour detection dogs: lessons learnt from a double-blind, controlled trial of prostate cancer detection. *BMC Urol* (2014) **14**:22. doi:10.1186/1471-2490-14-22
19. Cornu JN, Cancel-Tassin G, Ondet V, Girardet C, Cussenot O. Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. *Eur Urol* (2011) **59**:197–201. doi:10.1016/j.eururo.2010.10.006

Conflict of Interest Statement: The authors declare that they have no conflicts of interest. There are no MTAs, patents, or patent applications that apply to reagents, methods, or data in the paper.

frontiers in Veterinary Science

REVIEW
published: 04 April 2016
doi: 10.3389/fvets.2016.00030

Gastritis and Gastric Ulcers in Working Dogs

Michael S. Davis[1]* *and Katherine K. Williamson*[2]

[1] *Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA,* [2] *Purina Animal Nutrition, Land O'Lakes Inc, Gray Summit, MO, USA*

Gastritis and gastric ulcers are an important cause of morbidity and mortality in canine athletes. Although the majority of scientific work on this condition has been performed in ultraendurance racing sled dogs, this condition has been identified in other canine athletes, including sled dogs competing in shorter events and dogs performing off-leash explosive detection duties. The cause of the syndrome is unknown, but current hypotheses propose a link between exercise-induced hyperthermia and loss of gastric mucosal barrier function as an early event in the pathogenesis. Treatment is focused on prevention of clinical disease using acid secretion inhibitors, such as omeprazole, which has excellent efficacy in controlled clinical studies.

Keywords: exercise, stomach ulcers, gastritis, sled dogs, hyperthermia, omeprazole, working dogs, retrievers

OPEN ACCESS

Edited by:
Cynthia M. Otto,
University of Pennsylvania, USA

Reviewed by:
Bess Janine Pierce,
Virginia Maryland College of Veterinary Medicine, USA
Brian Michael Zanghi,
Nestle Purina PetCare, USA
Joseph Wakshlag,
Cornell University, USA

Correspondence:
Michael S. Davis
michael.davis@okstate.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *08 February 2016*
Accepted: *24 March 2016*
Published: *04 April 2016*

Citation:
Davis MS and Williamson KK (2016) Gastritis and Gastric Ulcers in Working Dogs. Front. Vet. Sci. 3:30. doi: 10.3389/fvets.2016.00030

INTRODUCTION

Gastritis and gastric ulcers can be an important cause of morbidity and mortality in canine athletes, most notably racing sled dogs but also other athletic dog populations. This review will outline the important points regarding this condition, including prevalence and risk factors, clinical syndrome, pathophysiology, and treatment and prevention strategies.

PREVALENCE AND IMPORTANCE

The importance of exercise-induced gastric disease (EIGD) in dogs is highlighted by both the frequency with which this syndrome is directly or indirectly related to mortality, but also the more insidious effect on performance and overall thriftiness. For the past two decades, organized ultraendurance sled dog racing has required the meticulous documentation of sled dog deaths in an attempt to provide transparency to the fans and critics of the sport as well as identify areas of canine health and well-being that require additional investigation. These reports showed that from 1994 to 2006, 23 dogs died during the 13 Iditarod races held during that time span (1). Eleven of those deaths were either directly or indirectly related to gastric disease (blood loss or vomiting and aspiration of gastric contents, respectively). Comprehensively collated statistics such as these are not available for other major races, but anecdotal evidence supports a similar proportional pattern (albeit with smaller numbers overall due to the fewer numbers of dogs involved in these other events). These statistics do not reflect the unknown number of dogs that may have been affected in a less severe manner, and were dropped off at checkpoints along the racecourse. Strenuous exercise requires the consumption of considerable amounts of food and water – ultraendurance racing sled dogs will burn from 8000 to 12000 kcal/day (2, 3) and turnover 5 l of bodywater/day (4). Any illness that reduces a dog's appetite or makes them reluctant to eat or drink or promotes vomiting/regurgitation – as gastric disease is known to do – will rapidly cause poor performance and dehydration under these conditions.

Some of the earliest studies of the prevalence of gastric disease in athletic dogs were done in association with the Iditarod Sled Dog race. In 2000, a small pilot study was performed to follow-up on anecdotal work done by Drs. Jack Morris and Phil Meyer, in which they reported frequently finding gastric lesions in dogs following the race. The 2000 study was conducted on dogs returning from the race, and found a gastric lesion prevalence of approximately 35% in dogs that were examined from 3 to 7 days post-exercise (5). Some of these subjects had completed the race, but most had been "dropped" for various medical reasons (not always due to suspected gastrointestinal disease). The first systematic evaluation of gastric health in racing sled dogs was performed the following year. Gastric endoscopy was performed on 73 dogs within 24 h of finishing the race (5). Using the visible presence of at least one area of erosion or ulceration in the gastric mucosa as the criterion, nearly half of the dogs had endoscopically visible lesions that were considered clinically significant. This percentage has held up through seven different studies: unmedicated racing sled dogs can be expected to have between 50 and 70% prevalence of clinically significant lesions after at least a single day of exercise (6), whether it is a long training day (7), a mid-distance race (8), or one of the ultraendurance races (5, 9–11). It seems intuitive that exercise intensity has some influence on disease severity, but further discussion of this type requires more careful definition of "exercise intensity," which can be quantified many different ways. Within the scope of a 1000+-mile race, finishing place does not seem to have a major influence on prevalence – teams finishing in 12 days (averaging 83 miles/day) had similar prevalence values to teams finishing in 9 days (averaging 111 miles/day) (5). Although data are not available from this study to assess whether the difference in daily distance was due to higher speeds or shorter rests in the teams finishing in 9 days, in general the lower-placing teams do so by resting longer as well as traveling slower. Teams competing in mid-distance races averaging 150 miles/day, during which substantially less rest/day is taken compared to the longer distance races, had noticeably higher prevalence and average gastric endoscopy severity scores (ESSs), suggesting that intensity of exercise (or something that is strongly correlated to intensity of exercise) has an influence on EIGD (8).

There have been studies of other athletic dog populations that have helped clarify the scope of this issue in the world of canine athletes. A study of field trial retrievers participating in a simulated single-day competition found a statistically significant worsening of the gastric endoscopy scores, but the scores stayed within the range that is considered not clinically significant (12). Thus, this population is probably not at risk for exercise-induced gastritis/gastric ulcers as a primary disease process, but consideration should be given to taking preventive measures if an individual competitive dog has additional risk factors for gastric disease. On the other hand, retrievers used for off-leash explosive detection work (during which the dogs may be exercising intermittently for up to 9 h/day) had an 84% prevalence of clinically severe gastric ulcers after five consecutive days of exercise, with the mean endoscopy score of the exercising dogs being higher than any other group of exercising dogs examined in other studies (13). Thus, it is clear that dogs other than racing sled dogs are at risk for EIGD if they perform sufficiently strenuous exercise.

DEFINITION OF CLINICAL SYNDROME

Perhaps the most frustrating aspect to this disease is that the overwhelming majority of affected dogs are subclinical, as evidenced by the fact that all endoscopy studies to date have been done in dogs that were clinically normal – having completed a grueling competition and/or having passed the physical examination that preceded the general anesthesia for endoscopy (5–11, 13). At the opposite end of this clinical spectrum are the dogs that are withdrawn from competition due to unequivocal evidence of gastric disease (i.e., repeated vomition or vomiting of fresh blood, aspiration pneumonia), but these dogs are relatively rare. The proportion of dogs that are withdrawn from competition that may have EIGD as an underlying cause (for example, due to lack of appetite or dehydration) is unknown, but it is likely that these "dropped" dogs have a prevalence at least as high as those dogs that continue to perform well.

There are no clinical pathology measurements that have been shown to characterize EIGD reliably. With the exception of the relatively rare instance in which the disease progresses to severe, acute, or chronic blood loss, decreased erythrocyte and serum protein concentrations are common in dogs performing endurance exercise (14), but not consistently associated with EIGD. Hematocrit may decrease in response to plasma volume expansion, which is a known and predictable conditioning response (15). Thus, differentiating mild hemodilution secondary to a desirable conditioning effect from occult moderate blood loss can be challenging in the athletic dog population. Serum cortisol predictably increases in exercise challenged dogs (16–19), likely due to the need for gluconeogenesis to offset the increased demand for glucose as a metabolic fuel (20), thus decreasing the usefulness of assessing serum cortisol as an indicator of physiological stress that could predispose to EIGD. In the studies in which this marker has been reported in the same dogs for which gastric examinations were performed, there was no association between serum cortisol and gastric disease on an individual dog basis (even though there was concurrent increase in serum cortisol and gastric disease in athletic dogs as a group) (11), suggesting that the increases in serum cortisol were not related either causally or as an effect of EIGD.

Endoscopy remains the gold standard for the diagnosis of EIGD in dogs due to the lack of consistent clinical signs or laboratory indices in the majority of the affected dogs. The range of severity of endoscopic findings can be substantial, from a few submucosal petechia to multiple actively bleeding lesions. A subjective severity scoring system has been established in which a stomach completely free of visible lesions is scored as 0; a stomach with a few submucosal petechia but no visible defects in the mucosa is scored as 1, a dog with extensive areas of erosions OR a single bleeding ulcer is scored as 2, and a dog with multiple bleeding ulcers is scored as 3 (**Figure 1**). Lesions can be found in all regions of the stomach, and through hundreds of examinations a particular bias in lesion location within the stomach has not been identified. Endoscopic assessment of motility, while subjective, is noteworthy in that as opposed to impaired motility (which can be associated with gastric lesions), the typical athletic dog appears to have enhanced gastrointestinal motility. The stomach of a trained

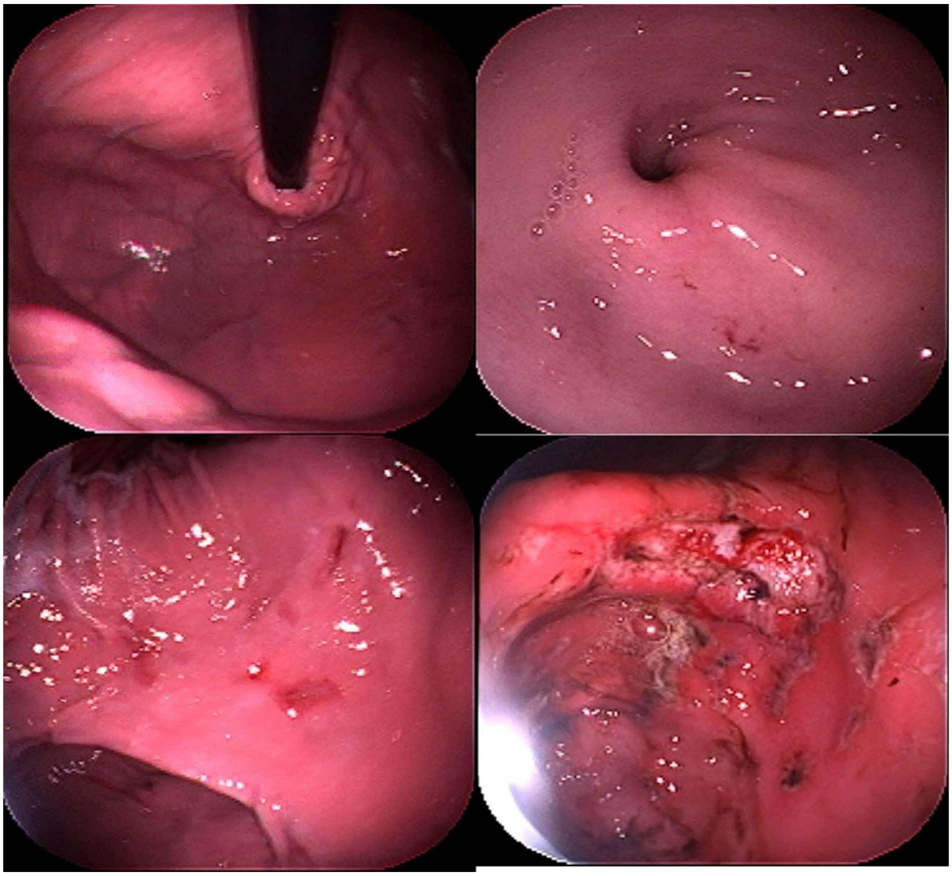


FIGURE 1 | **Endoscopy severity score (ESS) guide**. **(A)** ESS = 0; **(B)** ESS = 1; **(C)** ESS = 2; **(D)** ESS = 3.

working dog routinely can be examined, free of ingesta, after only a 12-h fast. Though of minimal scientific value, this observation may have considerable logistical value in deciding whether to perform gastric endoscopy on a dog with suspected EIGD.

Histopathology of dogs affected with EIGD has provided important clues regarding the pathophysiology of the disease. As a general practice, biopsies are obtained from normal-appearing areas of the gastric mucosa to avoid the secondary inflammation and necrosis that is expected in areas of actively bleeding lesions. Nevertheless, these biopsies have consistently demonstrated chronic mononuclear inflammation (5, 21), suggesting that although the gross lesions may have appeared in <24 h, the underlying pathological process has been occurring much longer – often predating the exercise session that appears to have caused the gastric mucosal disruption. Of particular interest is histopathological evidence of extensive apoptosis of grossly normal mucosa, suggesting that the terminal event in the pathophysiology is the programed cell death and sloughing of the mucosal epithelium, rather than chemical erosion and damage of the cells by stomach acid. Evidence of chronic mononuclear inflammation is present even in dogs that have been rested for up to 4 months (21). This type of chronic inflammation is not present in field trial retrievers (12), supporting both the contention that this population of dogs is not affected routinely by EIGD and the possible role of chronic mononuclear inflammation in the development of EIGD.

PATHOPHYSIOLOGY

There are few studies that specifically address the mechanisms underlying the development of EIGD in dogs. Increased permeability of the gastrointestinal mucosa has been measured in response to exercise in racing sled dogs (6, 10, 11). Although it is impossible to determine whether the loss of barrier function measured in these studies reflects an early pathophysiological event or is the result of the disease, it is worthwhile to note that the magnitude of increase in serum sucrose (a marker of permeability of the gastric mucosa) did not correlate with the endoscopically assessed severity of gastric mucosal disruption. With increased paracellular permeability, virtually anything that is in the lumen can find its way into the walls of the respective organs. In the stomach, the primary noxious material for diffusion is acid. With chronic, repeated periods of hyperthermia, the intermittent paracellular acid leak not only causes chronic subclinical inflammation (demonstrated in clinically normal, fully trained sled dogs prior to competitive exercise) but also sets the stage for acute exacerbation of the disease on the first day of competition.

The possibility of increased mucosal permeability as an early event is supported by studies in rodents and humans, demonstrating that physiological hyperthermia comparable to the magnitude routinely documented in exercising dogs will result in increased transmucosal permeability of other segments of the gastrointestinal tract (22–24). A primary role of hyperthermia-induced loss of gastrointestinal mucosal barrier function is further supported by the consistency between disease severity and the time-weighted duration of hyperthermia when all studies are considered: explosive detection dogs will achieve and sustain remarkably high rectal temperatures during simulated deployment activities (13), higher than is routinely found in racing sled dogs. In turn, racing sled dogs that are participating in relatively shorter distance events (300 miles) will tend to maintain higher speeds and rest less often than dogs competing in the ultraendurance events of 1000 miles. Finally, training runs tend to be slower and shorter in duration than racing, presumably resulting in less hyperthermia. Although this correlation is admittedly crude, its general fidelity with the population-wide variations in gastric disease severity, combined with the mechanistic data available in other species, makes hyperthermia-induced mucosal permeability a leading candidate for the initiating step in this syndrome.

The effects of "stress" are often cited as the cause of gastric ulcers in a variety of animals and circumstances. Commonly, physiological stress is quantified through the measurement of serum or salivary cortisol – an approach that may be too simplistic to provide a clear explanation or mechanistic basis. Glucocorticoids have a very wide range of biological activities, including the upregulation of hepatic gluconeogenesis (20), so it is not surprising that increases in serum cortisol are measured routinely during and following strenuous exercise (16–19) when the rate of glucose oxidation by working muscle greatly increases (25). Although the association between large doses of exogenous corticosteroids and gastric ulcers has been documented (26), a similar association between endogenous corticosteroids and gastric disease is more problematic for the simple reason that when investigators document concurrent hypercortisolemia and gastric disease, it is impossible to determine whether the increased release of cortisol is causing the disease or a response to the disease (or both). Similarly, in exercising animals – particularly those with modest increases in serum cortisol that are consistent with the requirement for increased glucose availability, it is difficult to make a firm association between hypercortisolemia and gastric disease. Indeed, in the single study reporting concurrent measurements, there was not an individual association between the magnitude of gastric disease and serum cortisol concentration (11). Thus, the role of glucocorticoids in the pathophysiology of exercise-induced gastrointestinal disease remains unconfirmed.

Increased secretion of gastrin (a potent gastric acid secretagogue) has been documented in both human (27–29) and equine athletes (30), and is believed to contribute to EIGD in these species through increased gastric acidity. Although a potential role for gastric acid in the pathogenesis of EIGD in dogs can be suggested due to the documented protective effects of acid suppressing medications, neither increased gastrin nor gastric hyperacidity has been documented in athletic dogs at this time. Therefore, the potential role for increased gastrin secretion in the pathogenesis of EIGD remains possible, but speculative.

There are many proposed causes of EIGD in dogs, but none that have been definitively proven at this time. Many, however, can be regarded as unlikely as major contributing factors to the disease in general. For example, the distribution of lesions observed during gastroscopy (widely distributed with no particular region predominantly affected) would tend to discount the possibilities of direct tissue trauma such as might occur when sled dogs are fed frozen snacks (5). The possibility of trauma secondary to ingestion of frozen food can be further disregarded in light of the prevalence of gastric lesions in studies in which the dietary intake of the dogs was closely controlled (8, 13). The fact that severe lesions have been found in individual dogs in one year and virtually nothing the following year (or vice versa) tends to reduce the likelihood of individual susceptibility as a major contributing factor. While the possibility that a high-fat diet may contribute to susceptibility cannot be discounted (since virtually all athletic dogs are consuming a high-fat diet in order to match energy intake with expenditure, even the military explosive detection dogs), minor variations in diet composition within that broad category of "high energy" diets do not seem to have a major influence on disease prevalence. Whether the high-fat diets routinely used in these types of canine athletes predispose to EIGD has not been examined. Gastric pathogens, such as *Helicobacter pylori*, are a major cause of gastric disease in humans and, therefore, the possibility of these organisms as a contributing cause to canine EIGD has been investigated. Special stains on biopsies obtained in three different studies have failed to demonstrate consistently the presence of helical bacteria, and in the instances in which these genera were detected, there was no association with either visible or histopathological evidence of disease (5, 21). Finally, exercise-induced ischemia due to the redistribution of visceral blood flow is possible in dogs for which the intensity of exercise exceeds the capacity of the cardiovascular system. However, direct measurements of visceral blood flow in trained sled dogs exercising at typical ultraendurance racing speeds found no reduction in splanchnic blood flow (31), suggesting that at least in these populations (as well as populations exercising at a similar intensity or lower such as explosive detection dogs), splanchnic ischemia is not a major factor in the development of EIGD.

Non-steroidal anti-inflammatory drugs (NSAIDs) deserve special mention due to their well-known ulcerogenic potential (32–34). Although it is possible that concurrent administration of NSAIDs could have an additive or synergistic role in promoting gastric disease in exercising dogs, it should be noted that all of the studies to date that have demonstrated EIGD in dogs have been done in circumstances in which NSAID use was prohibited [either in organized competition in which random drug testing is a prominent feature (5, 8–10) or in controlled studies in which the investigators could exclude the use of NSAIDs (6–8, 13)]. Thus, it can be safely concluded that NSAIDs are not a necessary component of the pathophysiology of EIGD in dogs.

TREATMENT AND PREVENTION

The treatment of EIGD in dogs is not dissimilar to the treatment of gastric ulcers of any cause, with the sole exception of cessation of the inciting cause (exercise) being a prominent aspect of EIGD treatment. Acid suppression is the most important component in the treatment and prevention of gastric ulcers. There are two main drug classes currently in use in veterinary medicine for acid suppression. Histamine-2 (H2) receptor antagonists, such as cimetidine, ranitidine and famotidine, act to suppress gastric acid secretion by binding the H2 receptors of gastric parietal cells, thus, preventing the secretion of both hydrochloric acid and pepsin. Proton pump inhibitors, such as omeprazole, act to block the H^+–K^+ATPase proton pump that is the final stage of gastric acid secretion. While proton pump inhibitors are considerably more potent antacids than H2 blockers, the realities of dosing should be considered in drug selection. Unlike proton pump inhibitors, H2 antagonists have good oral bioavailability when administered with food, and, therefore, need not be administered on an empty stomach as is the case with omeprazole. This fact is significant when one considers the difficulties of manual oral administration to dogs in extreme conditions or situations, and that frequent meals are required to meet the caloric demands of heavily exercising dogs making administration on an empty stomach problematic. To further illustrate this point, the efficacy of omeprazole in preventing gastric ulcers in sled dogs under racing conditions was the first preventive therapy investigated by Davis et al. (9), and the results were disappointing, showing that omeprazole was only moderately effective in preventing clinically significant gastric ulcers in dogs finishing the Iditarod Sled Dog race. Further unpublished research later determined that the absorption of proton pump inhibitors administered with a meal to dogs was extremely poor. With this in mind, the authors undertook a series of studies to determine the effectiveness of famotidine (which is well absorbed in the presence of food) in preventing gastric ulcers in racing sled dogs. The initial study indicated that under relatively modest conditions (training runs up to 100 miles in a day), famotidine was effective in preventing gastric ulcers (7). However, a subsequent study showed that, under actual racing conditions, famotidine was not sufficiently effective in preventing severe EIGD (8). A further study was then conducted, which compared the efficacy high-dose famotidine (40 mg PO BID/~25 kg dog) with omeprazole (20 mg PO SID/~25 kg dog) in preventing EIGD under racing conditions. This study showed that, with carefully timed administration, near the conclusion of a long exercise bout during which minimal snacking has occurred, omeprazole is more effective in reducing the number and severity of gastric lesions in racing sled dogs than famotidine (8). If an additional 30 min is allowed to pass prior to feeding the dog, efficacy can approach 100% in preventing clinically significant lesions during even the most strenuous exercise events.

Sucralfate, a GI protectant that binds to proteinaceous exudates found at ulcer sites, should be administered to dogs with confirmed or suspected gastric ulcers. The primary benefit of sucralfate is that it protects the ulcerated areas from further damage from gastric acid, bile, and pepsin. It has also been shown to have cytoprotective and antacid effects. Supportive care may include ensuring adequate hydration and nutrition, and transfusions in the case of excessive blood loss. Specific treatment for sequelae such as aspiration pneumonia may be necessary. Canine athletes, like any athlete, occasionally suffer from musculoskeletal injury for which NSAIDs are indicated. The potential contribution of NSAIDs to EIGD is unknown, but given the ulcerogenic potential of NSAIDs, these drugs should not be used before or during the types of exercise known or suspected to predispose a dog to the development of EIGD. NSAIDs should be used judiciously in dogs who are at risk of or suspected to have EIGD and concurrent acid suppression therapy should be considered.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The authors would like to thank the dedicated dog owners and collaborators in the many different studies that have contributed to our knowledge of this syndrome, including most importantly the successful prevention of clinical disease in working dogs.

REFERENCES

1. Dennis MM, Nelson SN, Cantor GH, Mosier DA, Blake JE, Basaraba RJ. Assessment of necropsy findings in sled dogs that died during Iditarod Trail sled dog races: 23 cases (1994-2006). *J Am Vet Med Assoc* (2008) **232**:564–73. doi:10.2460/javma.232.4.564
2. Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA. Metabolizable energy intake and sustained energy expenditure of Alaskan sled dogs during heavy exertion in the cold. *Am J Vet Res* (1997) **58**:1457–62.
3. McKenzie EC, Hinchcliff KW, Valberg SJ, Williamson KK, Payton ME, Davis MS. Intramuscular triglyceride and glycogen utilization during prolonged repetitive exercise by Alaskan sled dogs. *Am J Vet Res* (2008) **69**:1097–103. doi:10.2460/ajvr.69.8.1097
4. Hinchcliff KW, Reinhart GA, Burr JR, Swenson RA. Exercise-associated hyponatremia in Alaskan sled dogs: urinary and hormonal responses. *J Appl Physiol (1985)* (1997) **83**:824–9.
5. Davis MS, Willard M, Nelson S, Mandsager RE, Mckiernan BC, Mansell JK, et al. Prevalence of gastric lesions in racing Alaskan sled dogs. *J Vet Intern Med* (2003) **17**:311–4. doi:10.1111/j.1939-1676.2003.tb02453.x
6. Davis MS, Willard M, Williamson KK, Royer CM, Payton ME, Steiner JM, et al. The temporal relationship between gastrointestinal protein loss, gastric ulceration or erosion, and strenuous exercise in racing Alaskan sled dogs. *J Vet Intern Med* (2006) **20**:835–9. doi:10.1111/j.1939-1676.2006.tb01794.x
7. Williamson KK, Willard MD, Mckenzie EC, Royer CM, Payton ME, Davis MS. Efficacy of famotidine for the prevention of exercise-induced gastritis in racing Alaskan sled dogs. *J Vet Intern Med* (2007) **21**:924–7. doi:10.1111/j.1939-1676.2007.tb03044.x
8. Williamson KK, Willard MD, Payton ME, Davis MS. Efficacy of omeprazole versus high-dose famotidine for prevention of exercise-induced gastritis in racing Alaskan sled dogs. *J Vet Intern Med* (2010) **24**:285–8. doi:10.1111/j.1939-1676.2009.0454.x

9. Davis MS, Willard M, Nelson S, Mccullough S, Mandsager RE, Roberts JE, et al. Efficacy of omeprazole for the prevention of exercise-induced gastritis in racing Alaskan sled dogs. *J Vet Intern Med* (2003) **17**:163–6. doi:10.1111/j.1939-1676.2003.tb02453.x
10. Davis MS, Willard MD, Williamson KK, Steiner JM, Williams DA. Sustained strenuous exercise increases intestinal permeability in racing Alaskan sled dogs. *J Vet Intern Med* (2005) **19**:34–9. doi:10.1111/j.1939-1676.2005.tb02655.x
11. Royer CM, Willard MD, Williamson KK, Steiner JM, Williams DA, Davis MS. Exercise stress, intestinal permeability, and gastric ulceration in racing Alaskan sled dogs. *Equine Comp Exercise Physiol* (2005) **2**:53–9. doi:10.1079/ECP200446
12. Davis MS, Willard MD, Day MJ, Mccann J, Payton ME, Cummings SL. Effect of exercise on gastric health in field retrievers. *Comp Exercise Physiol* (2016) **12**:4. doi:10.3920/CEP150036
13. Davis MS, Willard MD, Bowers D, Payton ME. Effect of simulated deployment patrols on gastric mucosa of explosive detection dogs. *Comp Exercise Physiol* (2014) **10**:4. doi:10.3920/CEP14002
14. Davis MS, Davis WC, Ensign WY, Hinchcliff KW, Holbrook TC, Williamson KK. Effects of training and strenuous exercise on hematologic values and peripheral blood leukocyte subsets in racing sled dogs. *J Am Vet Med Assoc* (2008) **232**:873–8. doi:10.2460/javma.232.6.873
15. McKeever KH, Schurg WA, Convertino VA. Exercise training-induced hypervolemia in greyhounds: role of water intake and renal mechanisms. *Am J Physiol* (1985) **248**:R422–5.
16. Wakshlag JJ, Snedden K, Reynolds AJ. Biochemical and metabolic changes due to exercise in sprint-racing sled dogs: implications for postexercise carbohydrate supplements and hydration management. *Vet Ther* (2004) **5**:52–9.
17. Durocher LL, Hinchcliff KW, Williamson KK, Mckenzie EC, Holbrook TC, Willard M, et al. Effect of strenuous exercise on urine concentrations of homovanillic acid, cortisol, and vanillylmandelic acid in sled dogs. *Am J Vet Res* (2007) **68**:107–11. doi:10.2460/ajvr.68.1.107
18. Angle CT, Wakshlag JJ, Gillette RL, Stokol T, Geske S, Adkins TO, et al. Hematologic, serum biochemical, and cortisol changes associated with anticipation of exercise and short duration high-intensity exercise in sled dogs. *Vet Clin Pathol* (2009) **38**:370–4. doi:10.1111/j.1939-165X.2009.00122.x
19. Tharwat M, Al-Sobayil F, Buczinski S. Influence of racing on the serum concentrations of acute-phase proteins and bone metabolism biomarkers in racing greyhounds. *Vet J* (2014) **202**:372–7. doi:10.1016/j.tvjl.2014.08.027
20. Fujiwara T, Cherrington AD, Neal DN, Mcguinness OP. Role of cortisol in the metabolic response to stress hormone infusion in the conscious dog. *Metabolism* (1996) **45**:571–8. doi:10.1016/S0026-0495(96)90026-8
21. Ritchey JW, Davis MS, Breshears MA, Willard MD, Williamson KK, Royer CM, et al. Gastritis in Alaskan racing sled dogs. *J Comp Pathol* (2011) **145**:68–76. doi:10.1016/j.jcpa.2010.11.008
22. Shapiro Y, Alkan M, Epstein Y, Newman F, Magazanik A. Increase in rat intestinal permeability to endotoxin during hyperthermia. *Eur J Appl Physiol Occup Physiol* (1986) **55**:410–2. doi:10.1007/BF00422742
23. Coltart RS, Howard GC, Wraight EP, Bleehen NM. The effect of hyperthermia and radiation on small bowel permeability using 51Cr EDTA and 14C mannitol in man. *Int J Hyperthermia* (1988) **4**:467–77. doi:10.3109/02656738809027692
24. Prosser C, Stelwagen K, Cummins R, Guerin P, Gill N, Milne C. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. *J Appl Physiol* (2004) **96**:650–4. doi:10.1152/japplphysiol.00295.2003
25. Miller BF, Drake JC, Peelor FF III, Biela LM, Geor R, Hinchcliff K, et al. Participation in a 1,000-mile race increases the oxidation of carbohydrate in Alaskan sled dogs. *J Appl Physiol (1985)* (2015) **118**:1502–9. doi:10.1152/japplphysiol.00588.2014
26. Neiger R, Gaschen F, Jaggy A. Gastric mucosal lesions in dogs with acute intervertebral disc disease: characterization and effects of omeprazole or misoprostol. *J Vet Intern Med* (2000) **14**:33–6. doi:10.1111/j.1939-1676.2000.tb01496.x
27. Banfi G, Marinelli M, Bonini P, Gritti I, Roi GS. Pepsinogens and gastrointestinal symptoms in mountain marathon runners. *Int J Sports Med* (1996) **17**:554–8. doi:10.1055/s-2007-972894
28. Gritti I, Banfi G, Roi GS. Pepsinogens: physiology, pharmacology pathophysiology and exercise. *Pharmacol Res* (2000) **41**:265–81. doi:10.1006/phrs.1999.0586
29. Sliwowski Z, Lorens K, Konturek SJ, Bielanski W, Zoładź JA. Leptin, gastrointestinal and stress hormones in response to exercise in fasted or fed subjects and before or after blood donation. *J Physiol Pharmacol* (2001) **52**:53–70.
30. Furr M, Taylor L, Kronfeld D. The effects of exercise training on serum gastrin responses in the horse. *Cornell Vet* (1994) **84**:41–5.
31. Van Citters RL, Franklin DL. Cardiovascular performance of Alaska sled dogs during exercise. *Circ Res* (1969) **24**:33–42. doi:10.1161/01.RES.24.1.33
32. Papich MG. Antiulcer therapy. *Vet Clin North Am Small Anim Pract* (1993) **23**:497–512. doi:10.1016/S0195-5616(93)50301-7
33. Johnston SA, Budsberg SC. Nonsteroidal anti-inflammatory drugs and corticosteroids for the management of canine osteoarthritis. *Vet Clin North Am Small Anim Pract* (1997) **27**:841–62. doi:10.1016/S0195-5616(97)50083-0
34. Johnston SA, Fox SM. Mechanisms of action of anti-inflammatory medications used for the treatment of osteoarthritis. *J Am Vet Med Assoc* (1997) **210**:1486–92.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

METHODS
published: 12 May 2016
doi: 10.3389/fvets.2016.00034

Feasibility for Measuring Transverse Area Ratios and Asymmetry of Lumbosacral Region Paraspinal Muscles in Working Dogs Using Computed Tomography

Bethany Cain[1†], Jeryl C. Jones[1], Ida Holášková[2], Larry Freeman[3] and Bess Pierce[4]*

[1] *Division of Animal and Nutritional Sciences, Davis College of Agriculture, Natural Resources, and Design, West Virginia University, Morgantown, WV, USA,* [2] *Office of Statistics, Davis College of Agriculture, Natural Resources, and Design, West Virginia University, Morgantown, WV, USA,* [3] *Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA,* [4] *Department of Population and Health Science, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA*

OPEN ACCESS

Edited by:
Cynthia M. Otto, University of Pennsylvania, USA

Reviewed by:
Howard H. Erickson, Kansas State University College of Veterinary Medicine, USA
Janice Lauren Baker, Veterinary Tactical Group, USA

***Correspondence:**
Jeryl C. Jones
jerylj@clemson.edu

†Present address:
Bethany Cain, Class of 2018, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *24 January 2016*
Accepted: *18 April 2016*
Published: *12 May 2016*

Citation:
Cain B, Jones JC, Holášková I, Freeman L and Pierce B (2016) Feasibility for Measuring Transverse Area Ratios and Asymmetry of Lumbosacral Region Paraspinal Muscles in Working Dogs Using Computed Tomography. Front. Vet. Sci. 3:34. doi: 10.3389/fvets.2016.00034

Objectives: Describe computed tomographic (CT) anatomy of canine lumbosacral (LS) paraspinal muscles, a method for measuring paraspinal muscle transverse area ratios and asymmetry using CT, and application of this method in a small sample of working dogs with versus without LS pain.

Methods: Published anatomy references and atlases were reviewed and discrepancies were resolved by examination of anatomic specimens and multiplanar reformatted images to describe transverse CT anatomy of LS region paraspinal muscles. Sixteen Belgian malinois military working dogs were retrospectively recruited and assigned to LS pain positive versus negative groups based on medical record entries. A single observer unaware of dog group measured CT transverse areas of paraspinal muscles and adjacent vertebral bodies, in triplicate, for L5–S1 vertebral locations. A statistician compared muscle transverse area ratios and asymmetry at each vertebral location between groups.

Results: The relative coefficient of variation for triplicate CT area measurements averaged 2.15% ($N = 16$). Multifidus lumborum (L6–7), psoas/iliopsoas (L5–6, L6–7), and sacrocaudalis dorsalis lateralis (L6–7, L7–S1) transverse area ratios were significantly smaller in dogs with LS pain ($n = 11$) versus without LS pain ($n = 5$) ($p \leq 0.05$). Muscle asymmetry values were not significantly greater in dogs with versus without LS pain.

Clinical relevance: Computed tomographic morphometry of LS region paraspinal muscles is a feasible objective method for use in future evidence-based research studies in working dogs. Potential future research applications include determining whether decreased paraspinal muscle area ratios and/or increased paraspinal muscle asymmetry could be used as markers for preclinical LS pain in stoic dogs or risk factors for other injuries in high performance canine athletes, or determining whether core muscle strengthening exercise prescriptions for dogs with LS pain have an effect on paraspinal muscle area ratios and asymmetry.

Keywords: CT, core muscle, canine, lower back, cauda equina

INTRODUCTION

Lower back [lumbosacral (LS)] pain (LBP) is an important cause of debilitation and early retirement in working dogs (1–3). The standard diagnostic test is clinical detection of a painful reaction to palpation of the LS junction and/or dorsal extension of the tail (tail jack). For stoic, high drive, or aggressive working dogs, clinical detection of LBP may be difficult to demonstrate. For these dogs, diagnosis of LBP may be based on observed performance deficits, such as altered LS region posture during working tasks, reluctance to perform tasks requiring hyperextension of the LS spine, and/or altered movement of the tail (4). Commonly reported causes of LBP in dogs have included degenerative LS stenosis/disk disease, sacroiliac degenerative joint disease, and/or soft tissue injury in the LS region (1–6). Human and canine studies have indicated that chronic LBP often leads to maladaptive patterns of movement and abnormal resultant ground reaction forces, which may put patients at increased risk for injury and chronic, referred pain syndromes (7–9). In order to minimize risks of these complications, core muscle-strengthening and conditioning exercise prescriptions are increasingly being recommended and implemented for preventing or treating LBP in canine athletes (10–14) However, there are few evidence-based research studies supporting these prescriptions. A non-invasive, repeatable technique for objectively quantifying characteristics of LS region paraspinal muscles would be helpful for supporting development of these evidence-based research studies.

The anatomy and functions of canine LS region paraspinal muscles have been described in standard anatomic reference textbooks (15, 16). The lumbar epaxial spinal muscles include the following (from medial-to-lateral and dorsal to the level of transverse processes): multifidus lumborum (ML), longissimus lumborum (LL), and iliocostalis (IC) lumborum. All three of the epaxial spinal muscle systems serve, bilaterally, to extend the vertebral column. Unilaterally, they bend (flex) the column to that side such that the concavity of the bend faces to that side. The lumbar hypaxial muscles (medial-to-lateral and ventral to the level of transverse processes) include the following: psoas minor, psoas major [combines with the iliacus at the ventral ilium and becomes the iliopsoas (IP)], and the quadratus lumborum (QL). All these hypaxial muscles flex the lumbar portion of vertebral column and unilaterally serve to bend the column, so that the convexity of the bend faces to that side. The medial and lateral dorsal sacrocaudal muscles function bilaterally to extend/raise/lift the tail. If they contract unilaterally, they raise and deviate the tail toward the same side. The sacrocaudalis dorsalis medialis (SDM) is the continuation of the medial epaxial system, hence of the ML, into the sacral and tail region. It functions as the medial and short elevator of the tail in contrast to the lateral and long tail elevator, which is the sacrocaudalis dorsalis lateralis (SDL). Its cranial extent is its origin on the dorsolateral aspect of L7 vertebra. The SDL, the long elevator of the tail, is composed of muscle bundles that come together to form essentially the continuation of the LL into the sacral and tail region of the vertebral column. It originates *via* tendons from the first or second to seventh lumbar vertebrae as well as from the sacrum and tail vertebrae. Transverse sectional anatomy of canine lumbar and LS muscles has been described in veterinary anatomy atlases (17–20); however, identifications for some paraspinal muscles have been contradictory in these publications.

Computed tomography (CT) has been established as a method for quantifying cross-sectional area of lumbar region paraspinal muscles in humans with LBP (21–28). Similar intra- and interrater reliability has been reported for magnetic resonance imaging (MRI) and CT measures of paraspinal muscle cross-sectional areas in humans (24). A previous CT morphometry study of the canine vertebral canal in dogs with versus without cauda equina syndrome described the use of transverse vertebral canal area ratios calculated with area of the adjacent vertebral body as a correction factor for reducing variations due to differences in dog body sizes (29). A recently published study of canine paraspinal muscles in dogs with versus without degenerative LS stenosis described a MRI method for measuring transverse muscle area ratios and symmetry of the SDL, ML, and longissimus lumborum muscles at L7–S1 (30). Measurements of other muscles at other LS vertebral levels have not been reported in dogs.

We hypothesized that CT would be a feasible method for quantifying paraspinal muscle transverse area ratios and asymmetry in the canine LS region. Objectives of this pilot study were to describe (1) transverse CT anatomy of LS region (L5–S1) paraspinal muscles, (2) CT methods for measuring canine LS region paraspinal muscle transverse area ratios and asymmetry, and (3) application of these CT measurement methods in a small sample population of Belgian malinois military working dogs with versus without LBP.

MATERIALS AND METHODS

Patient Selection Criteria

With hospital director approval, dogs were retrospectively recruited from medical record and computed tomographic (CT) image archives at the Daniel Holland Military Working Dog Veterinary Hospital at Lackland Air Force Base, TX, USA. All hospital requirements for ensuring confidentiality of patient data were maintained throughout the study. The search period for data retrieval was from April 2005 to July 2011. Inclusion criteria were as follows: Belgian malinois breed, CT scan that included the LS region, and available medical records describing clinical examination findings at the time of CT scanning. All CT images and medical records for dogs meeting these inclusion criteria were retrieved. A board-certified veterinary radiologist (Jeryl C. Jones) reviewed CT scans and excluded dogs if LS paraspinal muscles were not included in the scan field of view or if there was evidence of LS fractures, infection, neoplasia, or previous surgery.

Transverse CT Anatomy Study

All digital CT images for included dogs were uploaded directly to a password-protected image analysis workstation (MacPro 12-core with 30″ Apple Cinema HD display, 1 Infinite Loop, Cupertino, CA, USA). Hard copy CT images for included dogs were first converted to DICOM format using a digital scanner system (Vidar Sierra Advantage, Sound Eklin, Carlsbad, CA, USA) and then transferred to the same image analysis workstation. All images reviews were performed using the same image analysis freeware (OsiriX version 4.1.2, http://www.osirix-viewer.com).

A veterinary radiologist and veterinary anatomist reviewed anatomic reference textbooks and transverse sectional atlases and compared these to retrieved CT images (15–20). Discrepancies in transverse sectional atlas muscle identifications were resolved based on dissection of anatomic specimens (**Figure 1**), evaluation of multiplanar reformatted CT images, and a consensus agreement between both expert readers. For this study, the ML muscle was defined as the muscle lateral to the L5, L6, and cranial L7 spinous processes in transverse CT images (**Figures 2–5**). At the level of L7–S1, the muscle lateral to the caudal L7 spinous process was defined as a combination of ML and SDM. The SDL was defined as the muscle lateral to the ML and SDM. The combined LL/IC lumborum was defined as the muscle group lateral and ventral to the SDL (**Figures 6–9**). The QL was defined as the muscle lateral and ventral to the transverse processes of L5, L6, and L7, and that terminated on the medial margin of the ilium (**Figures 10–13**). The psoas was defined as the muscle medial to the QL and ventral to the L5, L6, and cranial L7 vertebral bodies. At the level of L7–S1, the muscle medial to the QL and ventral to the caudal L7 and cranial S1 vertebral bodies was identified as the IP (combined iliacus and psoas).

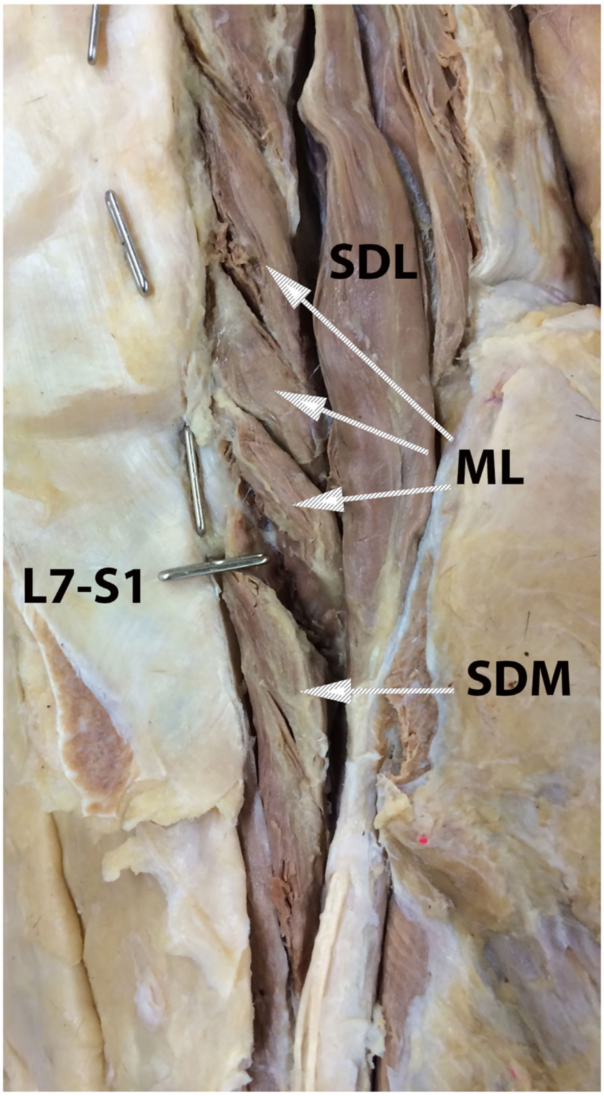


FIGURE 1 | Anatomic dissection photograph illustrating identification of the multifidus lumborum (ML), sacrocaudalis dorsalis medialis (SDM), and sacrocaudalis dorsalis lateralis (SDL) muscles. Cranial is at the top of the image and the dog's right is on the viewer's right. Metal pins oriented in the sagittal plane mark the L7 and L6 spinous processes. The metal pin oriented in the transverse plane marks the L7–S1 junction. Attaching near the summit of the L7 spinous process is the SDM muscle bundle that continues caudally into the sacral region and on caudally, along with other muscle bundles into the tail region. This is the so-called short elevator of the tail. A longer muscle than is the SDM, the SDL originates as far craniad as from the cranial lumbar vertebrae and continues caudally into the sacral and tail regions. This muscle, which serves as the so-called long elevator of the tail, is readily dissectable as separate from the longissumus lumborum.

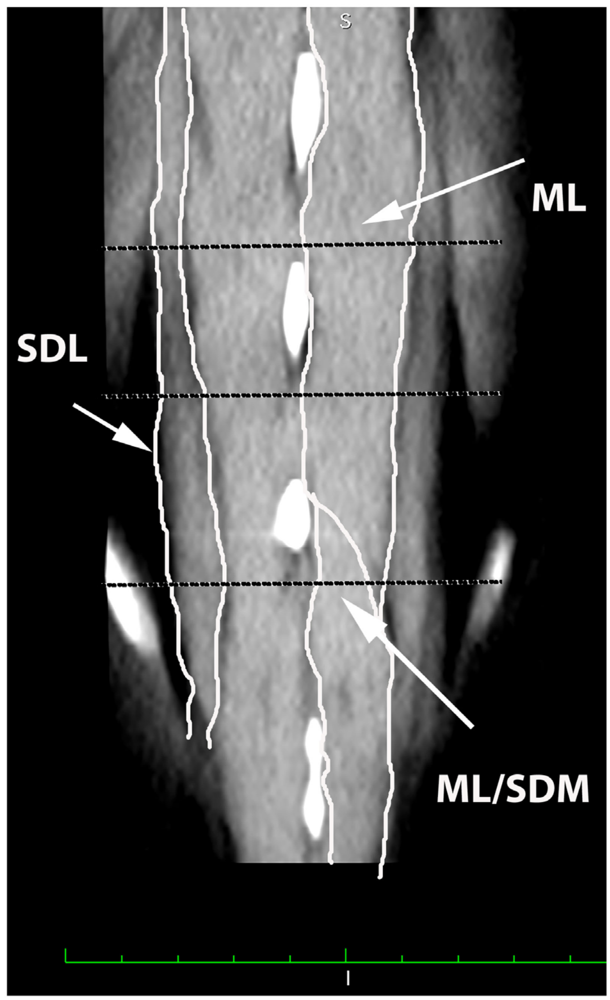


FIGURE 2 | Dorsal oblique multiplanar CT image at the level of the L5–S1 spinous processes, illustrating margins of the multifidus lumborum (ML), sacrocaudalis dorsalis lateralis (SDL), and sacrocaudalis dorsalis medialis (SDM). Cranial is at the top of the image and the patient's right is on the viewer's left. Transverse dotted lines illustrate the locations of L5–6, L6–7, and L7–S1.

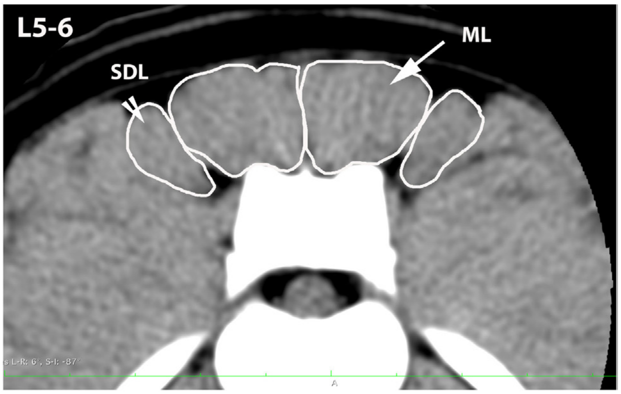


FIGURE 3 | **Transverse CT image at the level of L5–6, illustrating margins of the multifidus lumborum (ML) and sacrocaudalis dorsalis lateralis (SDL)**.

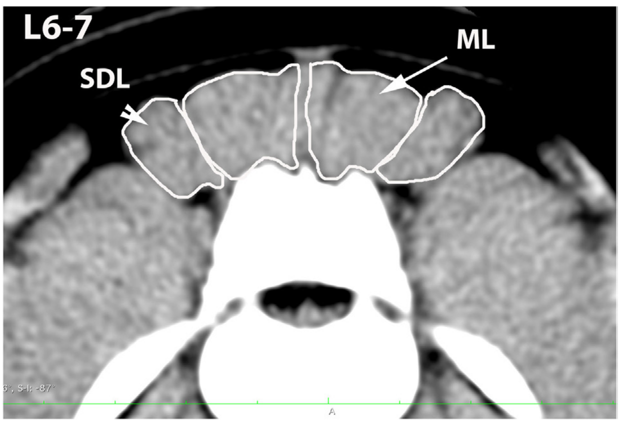


FIGURE 4 | **Transverse CT image at the level of L6–7, illustrating margins of the multifidus lumborum (ML) and sacrocaudalis dorsalis lateralis (SDL)**.

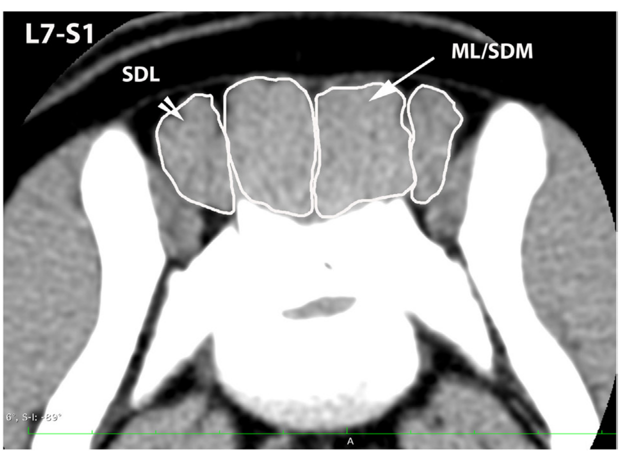


FIGURE 5 | **Transverse CT image at the level of L7–S1, illustrating margins of the sacrocaudalis dorsalis lateralis (SDL) and the combined multifidus lumborum/sacrocaudalis dorsalis medialis (ML/SDM)**.

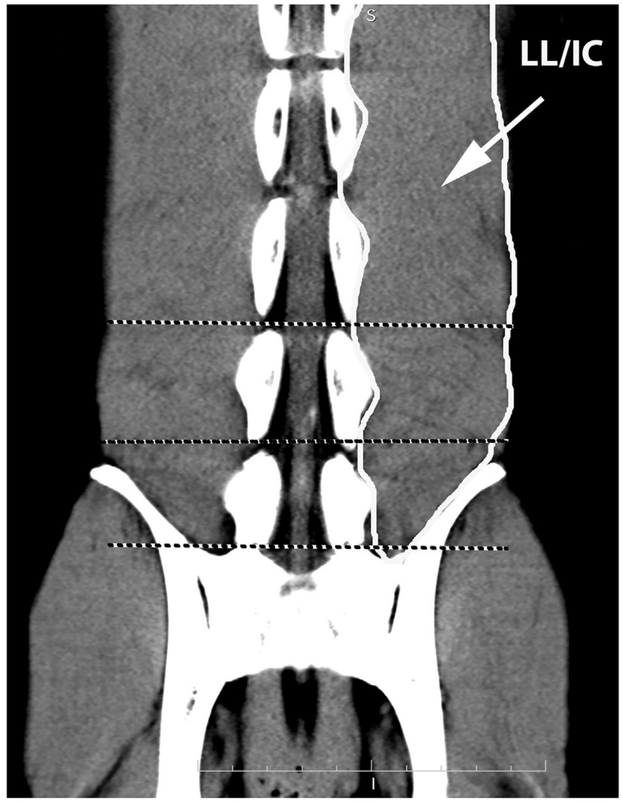


FIGURE 6 | **Dorsal oblique multiplanar CT image at the level of the L5–S1 vertebral canal, illustrating margins of the combined longissimus lumborum/iliocostalis (LL/IC) muscle group**. Notice that this muscle group tapers at the level of L7–S1.

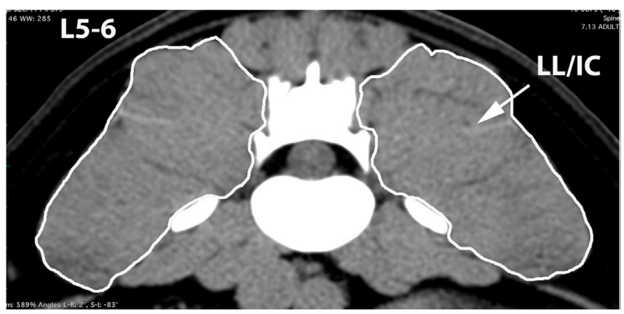


FIGURE 7 | **Transverse CT image at the level of L5–6, illustrating margins of the combined longissimus lumborum/iliocostalis (LL/IC) muscle group**.

Computed Tomographic Morphometry Technique

A single observer (Bethany Cain) performed all quantitative analyses of paraspinal muscles without knowledge of dog LS pain status. Centimeter scale tools in the image analysis freeware were used for calibration of area measurements in hard copy

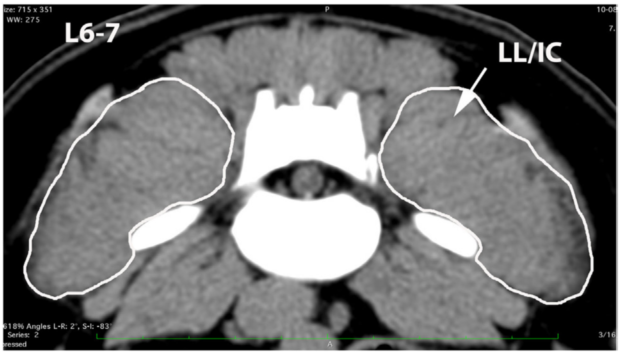


FIGURE 8 | **Transverse CT image at the level of L6–7, illustrating margins of the combined longissimus lumborum/iliocostalis (LL/IC) muscle group**.

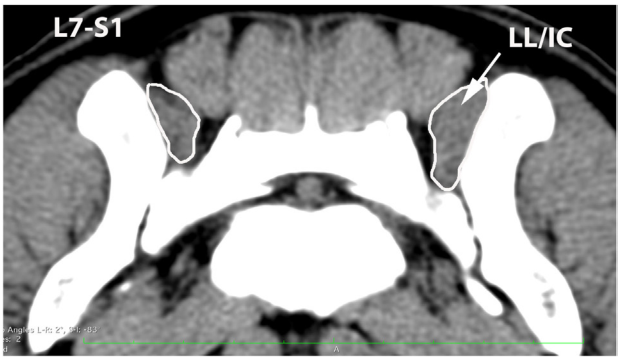


FIGURE 9 | **Transverse CT image at the level of L7–S1, illustrating margins of the combined longissimus lumborum/iliocostalis (LL/IC) muscle group**.

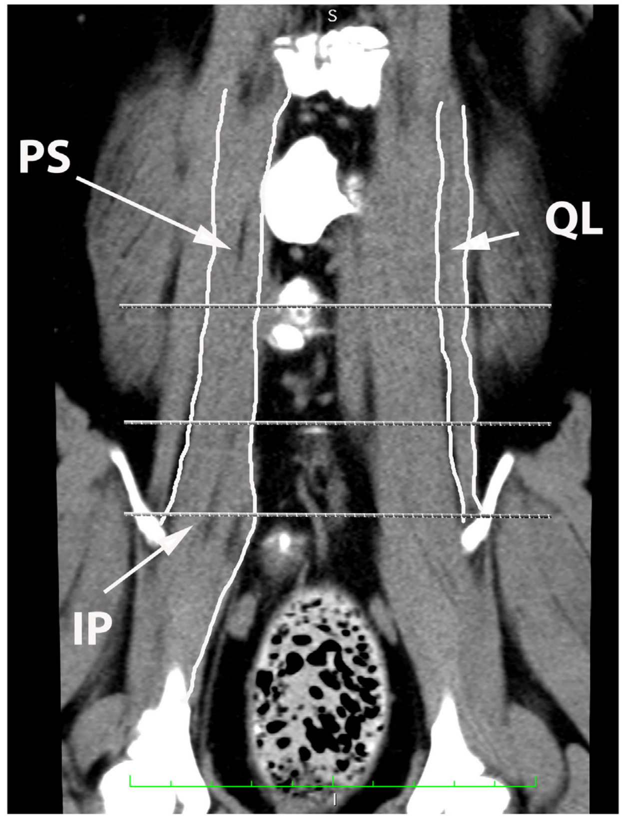


FIGURE 10 | **Dorsal oblique multi-planar CT image at the level of the ventral vertebral bodies, illustrating margins of the quadratus lumborum (QL), psoas (PS), and iliopsoas (IP) muscles**.

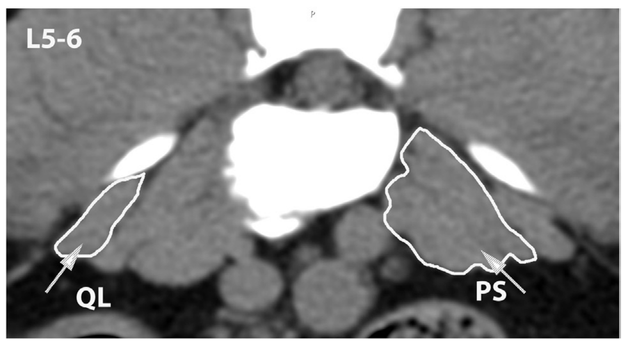


FIGURE 11 | **Transverse CT image at the level of L5–6, illustrating margins of the quadratus lumborum (QL) and psoas (PS) muscles**.

images. To perform the calibration for each scanned set of CT hard copy images, the observer first used the software's line tool to mark locations of adjacent centimeter marks displayed in one of the image frames and the software automatically recorded this value as the number of pixels. The software's centimeter scale tool was then used to assign that number of pixels the value of 1 cm. Once this calibration was performed, area measurements were converted from pixels to centimeters by the software program. The software's "thick slab, mean" tool was used to standardize all transverse images to a 5-mm slice thickness before measurements were made. The observer used the image analysis freeware's pencil tool to hand trace regions of interest (ROIs) around the outer margins of each of the paraspinal muscles defined by the anatomy study at the L5–6, L6–7, and L7–S1 vertebral levels. The slice location for measurements was chosen based on the transverse image that displayed the maximum height of the intervertebral foramen and complete caudal vertebral endplate margins. A standardized soft tissue window display setting (350 width, 40 level) was used for all muscle measurements. If the margins between adjacent muscles were not distinguishable, the observer extrapolated intermuscular margins by drawing a straight perpendicular line from the peripheral muscle margin to the adjacent vertebral margin. If the outer margin of a muscle was not completely included in the scan field of view, the muscle was excluded from the analyses. ROIs were also traced around vertebral bodies at the same locations as muscle ROIs and these were used as correction factors for variations in dog size (**Figure 14**). A standardized bone window display setting (1500 width, 300 level) was used for all vertebral body measurements. Areas for each muscle and adjacent vertebral body were measured in triplicate. After all ROI

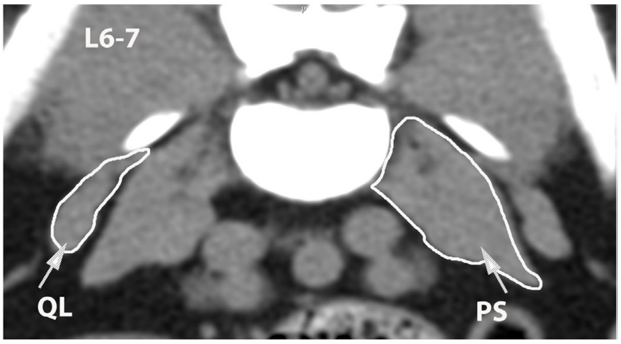


FIGURE 12 | **Transverse CT image at the level of L6–7, illustrating margins of the quadratus lumborum (QL) and psoas (PS) muscles**.

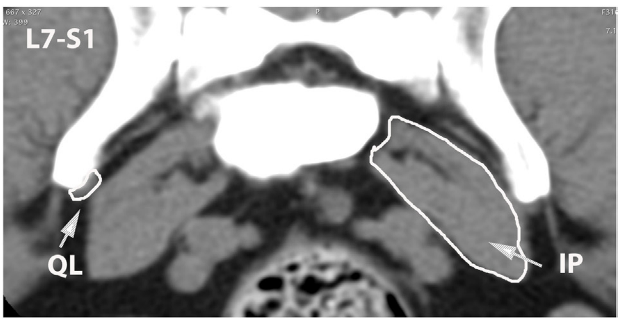


FIGURE 13 | **Transverse CT image at the level of L7–S1, illustrating margins of the quadratus lumborum (QL) and iliopsoas (IP) muscles**.

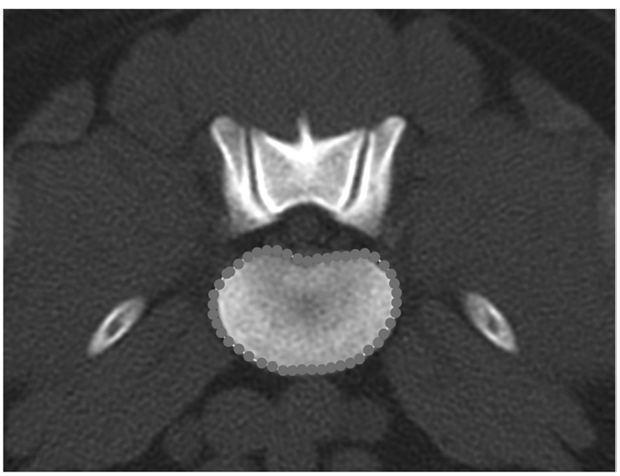


FIGURE 14 | **Transverse bone window CT image at the level of L6–7, illustrating the hand-traced region of interest and calculated area value for the vertebral body**.

measurements were completed, mean area ratios for each muscle, each vertebral location, and each dog were calculated by the same observer using commercial software (Excel Office for Mac 2011, version 14.4.3) and the following formula (30):

$$\text{Transverse area ratio} = [(\text{average of 3 right muscle area measurements} + \text{average of 3 left muscle area measurements})/\text{average of 3 vertebral endplate area measurements}]$$

Application of CT Morphometry Technique for Comparing Dogs with versus without Lumbosacral Pain

The same observer (Bethany Cain) reviewed medical record data after all CT mean area and area ratio calculations were completed. Dogs were assigned to the LS pain positive group if at least one of the following phrases was found in the medical record at the time the dog was presented for CT scanning: "pain/reaction on palpation of the LS junction," "pain/reaction on elevation of the tail/tail jack," or "LS hyperesthesia." A statistician (Ida Holásková) selected and performed all statistical tests using commercial software (JMP®, Version Pro 11, SAS Institute, Inc., Cary, NC, USA, Copyright ©2013; SAS®, Version 9.3, SAS Institute, Inc., Cary, NC, USA, Copyright ©2002–2010). Intra-observer repeatability (relative coefficient of variation %, CV) for triplicate area measurements was calculated for each dog, each side, and each variable using the following formula:

$$CV = [(SD/\text{mean}) \times 100]$$

Each response variable was first tested for normality using the Shapiro–Wilk *W* test. For variables that were not normally distributed, a log 10 or a square root transformation was applied. Variables with fewer than two available values were excluded from the analyses. Muscle asymmetry values for each muscle and each vertebral location were calculated for each dog using the following formula (28):

$$\text{Asymmetry value} = [(\text{Average of 3 right area measurements}) - (\text{Average of 3 left area measurements})]$$

Analysis of covariance (ANCOVA) was performed on the area ratio and asymmetry, to adjust the effect of LS pain for the possible dog-specific covariates such as age, weight, and sex. To test the hypothesis that means muscle transverse area ratios would be smaller in dogs with LBP, a lower tail *t*-test was performed for normally distributed data. In order to control for the type I error rate, when analyzing 13 muscle areas simultaneously, the Benjamini–Hochberg adjustment with 15% false discovery rate was applied to *p* values obtained from the *t*-tests (31). To test the hypothesis that muscle asymmetry would be greater in dogs with LBP, the upper tail *t*-test was performed. Power analysis was done after the aforementioned statistical tests. For each test, and before adjustment, statistical significance was defined as $p \leq 0.05$.

RESULTS

Description of Sample Population

A total of 16 dogs met all inclusion criteria for the study. Eleven dogs were assigned to the LS pain positive group and five dogs were assigned to the LS pain negative group (**Table 1**). Dogs in the LS pain negative group had been presented for CT scanning for the following reasons: hindlimb lameness ($n = 2$) and another research project ($n = 3$). Eight digital and eight hard copy CT studies were used in the analyses. All dogs had been sedated or anesthetized and positioned in dorsal recumbency for scanning. All dogs were positioned with the LS spine in an extended position. All scans were acquired on site at the MWD hospital using multidetector CT scanners with a 512 × 512 matrix (HiSpeed Advanced System No. HSA2 or LightSpeed VCT, GE Medical Systems, Milwaukee, WI, USA). Other CT technical parameters had varied at the discretion of the veterinary radiologist overseeing the case.

The relative coefficient of variation (CV, intra-observer repeatability) for all triplicate CT area measurements averaged 2.15% (range 0.7–4.3%). When calculated by dog group, the average CV for triplicate measures was 1.45% (0.57–2.82%) for the LS pain positive group and 2.85% (1.85–3.45) for the control group. Dogs' age was a significant covariate in one of the 13 muscle areas (QL at L5–6) for both area ratio and asymmetry; however, the low sample size at this location ($n = 7$) lead to very low statistical power for the ANCOVA (<20%). Weight was also detected as significant covariate in one of the 13 muscle locations (SDL at L7–S1, **Figure 15A**), with negative slope ($p = 0.03$) and power of 67% for transverse area ratio. There was no significant interaction detected between LS pain and weight of dogs in this vertebral region. For asymmetry, dog's weight was found as significant covariate in ML at L5–6 with significant interaction of body weight and LS pain ($p = 0.018$). However, the power of this test was only 25% (data not shown).

Results from the one-tailed *t*-test after Benjamini–Hochberg adjustment indicated that dogs with LS pain had significantly smaller transverse area ratios for the following muscles and locations: psoas at L5–6 ($p = 0.007$) and L6–7 ($p = 0.049$; **Figures 15B,C**), ML at L6–7 ($p = 0.025$; **Figure 15C**), and SDL at L6–7 ($p = 0.012$) and L7–S1 ($p = 0.035$; **Figures 15C,D**). There were no significant differences detected in mean transverse area ratios for longissimus and quadratus muscles. Results from the one-tailed *t*-test and Benjamini–Hochberg adjustment indicated that paraspinal muscle asymmetry was not significantly greater in any of the muscle areas in dogs with versus without LS pain. However, with a large variability in asymmetry data, the power of the *t*-test was <31% for all muscle areas.

TABLE 1 | Description of sample population of 16 Belgian malinois military working dogs included in the study.

Characteristics		Lumbosacral pain positive ($n = 11$)	Lumbosacral pain negative ($n = 5$)
Sex	Female	2	2
	Male	9	3
Age (years)	Mean (SD)	6.5 (3.1)	5 (2.3)
	Median (range)	7 (2–11)	4 (3–8)
Weight (kg)	Mean (SD)	29.8 (4.7)	27.3 (3.8)
	Median (range)	29 (25–40)	27 (23–32)

Age and weight data were normally distributed.

DISCUSSION

The intention of the current pilot study was to develop and describe an objective method for quantifying LS paraspinal muscles in working dogs, with the long-term goal of supporting evidence-based research studies. Application of the method was illustrated in a small sample of working dogs with versus without clinically detected LS pain. Utility of these measures as a diagnostic tool for individual patients was not tested. Findings indicated that CT is a feasible method for measuring LS paraspinal muscle transverse area ratios and asymmetry in groups of dogs for research purposes. The use of multiplanar reformatting and anatomic dissections was helpful for clarifying muscle anatomy in transverse CT images. Inclusion of L5–6 and L6–7 in the measurements allowed detection of muscle area differences that would have been missed if only the L7–S1 level was measured. Paraspinal muscles measured at the L5–6 and L6–7 vertebral levels were those primarily responsible for flexion, extension, and lateral movements of the caudal lumbar spine. Paraspinal muscles measured at L7–S1 also included those responsible for movement of the tail and rear limbs.

Vertebral body transverse area measurements were used as correction factors for muscle transverse area measurements in order to minimize effects of dog body size variations for group comparisons. Intra-observer repeatability for muscle and vertebral body measurements was high for dogs in both LS pain positive and negative groups. We identified the evidence that dog weight or age were possibly covariates for transverse area ratios or asymmetry in some muscle areas. However, for age covariate analyses, the power of the tests was low due to the small sample size. The negative slope between the transverse area ratio and weight indicated that the area ratio may decrease with increasing weight regardless of the pain category, but more dogs over 35 kg without pain should be included in the analysis in order to more definitively test this theory. Significant differences in transverse area ratios were identified for groups of dogs with versus without LS pain. Results of comparisons were consistent with those reported in previous CT morphometry studies of humans with versus without LBP (22, 26, 27), and a previous MRI morphometry study of dogs with versus without degenerative LS stenosis (30). Therefore, either MRI or CT could be used for measuring muscles in future research studies and the selection of modality could be based on availability and cost.

Limitations of the current study included a small sample size, mixture of digital and hard copy CT images, and variable CT technical parameters. We attempted to minimize outside sources of measurement variation by standardizing the CT image analysis workstation/software, CT slice thickness, and window/level display settings; and using an average of triplicate area measures for group comparisons. Authors acknowledge that there was a sample population bias for this study in that only Belgian malinois military working dogs presenting to a tertiary referral MWD hospital for CT scans that included the LS region were sampled. Whether the findings from this study would be generalizable for other dog breeds and for non-working dogs, therefore, remains unknown. Belgian malinois were chosen for the study because they are one of the most commonly used breeds for military service.

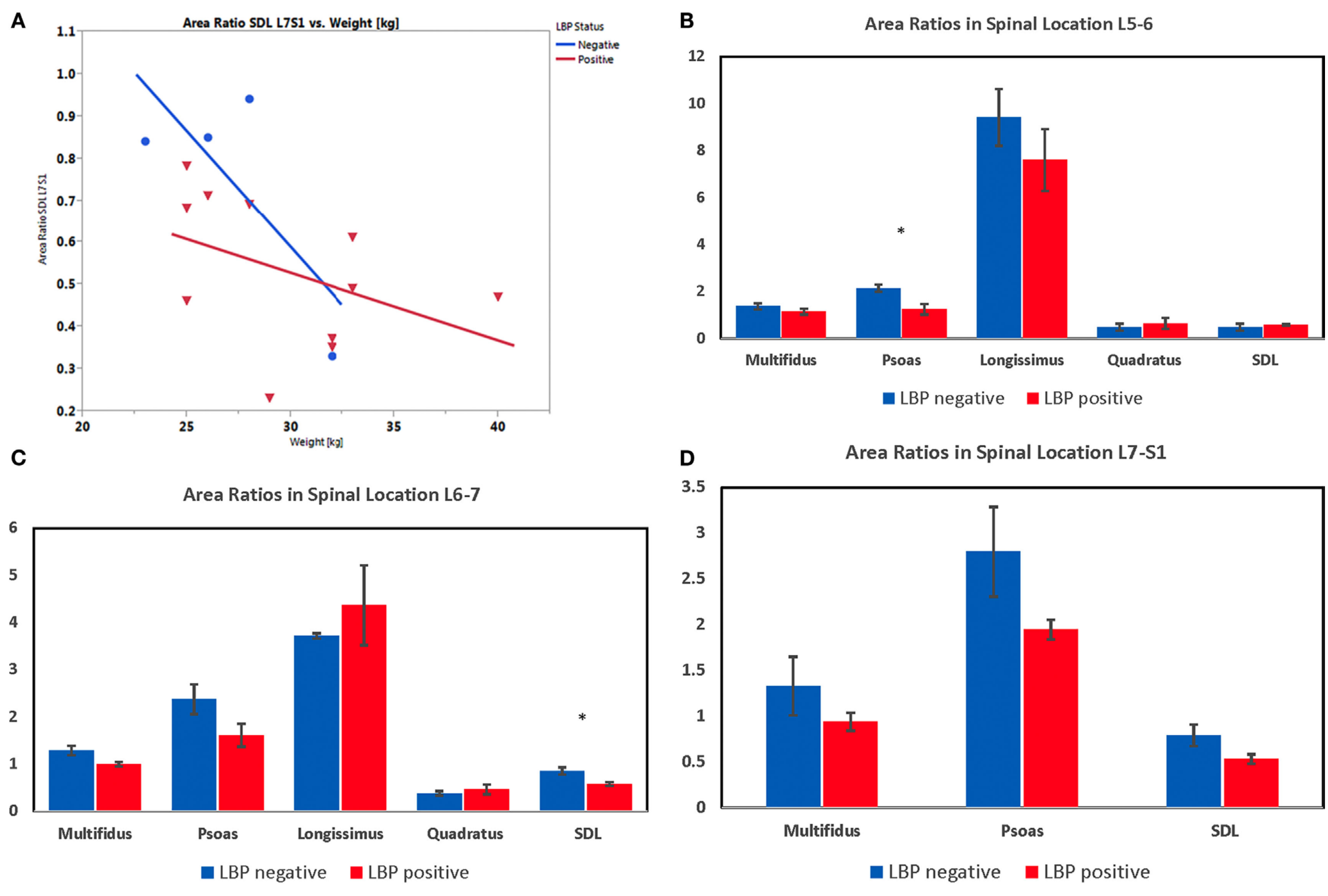


FIGURE 15 | (A) Plot representing the ANCOVA depicting the effect of lower back pain (LBP) and weight (covariate) on transverse area ratio of the sacrocaudalis dorsalis lateralis muscle at L7–S1 in Belgian malinois working dogs (n = 15). One dog was excluded from these analyses, because the SDL was partially cut off in the scan field of view at L7–S1. Weight is a significant covariate (p = 0.029), and there was no significant interaction of LBP and weight on transverse area ratio. **(B)** Transverse area ratios of muscles measured at the level of the disc space between the fifth and sixth lumbar vertebrae. **(C)** Transverse area ratios of muscles measured at the level of the disc space between the sixth and seventh lumbar vertebrae. **(D)** Transverse area ratios of muscles measured at the level of the disc space between the seventh lumbar and first sacral vertebrae. (*indicates significant at $p < 0.05$ for the particular spinal location detected by lower-tail t-test after Benjamini–Hochberg adjustment.)

Potential future research applications for methods described in the current pilot study could include determining whether decreased paraspinal muscle area ratios and/or increased paraspinal muscle asymmetry could be used as markers for preclinical LS pain in stoic dogs or risk factors for other injuries in high performance canine athletes. Another potential research application could include determining whether core muscle strengthening exercise prescriptions for dogs with LS pain have an effect on paraspinal muscle area ratios and asymmetry. Effects of other possible factors for decreased muscle transverse area ratios and increased asymmetry, such as positioning variation, observer expertise for determining pain status, presence of concurrent diseases, prior or ongoing use of medications, duration of signs, sex, and type of work, may also warrant further investigation.

In conclusion, findings from the current pilot study indicated that CT measurements of transverse area ratios and asymmetry are feasible methods for objective, quantitative characterization of LS region paraspinal muscles for use in future canine research studies. Additional studies are needed to test the effects of other clinical factors on muscle quantitative characteristics.

ETHICS STATEMENT

All dogs were owned by the Department of Defense, and use of the archived medical record data was approved by the Director of the Military Working Dog hospital.

AUTHOR CONTRIBUTIONS

BC contributed all of the recorded data for the paper, in addition to meeting all four other ICJME requirements. JJ served as the mentoring author for BC and contributed all of the veterinary radiology content for the paper, in addition to meeting all four other ICJME requirements. IH contributed all of the statistical analysis content for the paper, in addition to meeting all four other ICJME requirements. LF contributed all of the veterinary anatomy content for the paper, in addition to meeting all four other ICJME requirements. BP contributed all of the veterinary sports medicine and rehabilitation content for the paper, in addition to meeting all four other ICJME requirements.

ACKNOWLEDGMENTS

The authors would like to thank the staff and veterinarians at the Daniel Holland Military Working Dog Hospital for their assistance in collecting CT and medical record data for this study.

FUNDING

Funding support for this project was provided by the West Virginia Agricultural and Forestry Experiment Station, Morgantown, WV, USA (Scientific Article No. 3251); Hatch Formula Fund, National Institute of Food and Agriculture, U.S. Department of Agriculture (award number WVA00645); and WVU Davis-Michael Endowment for Pre-Veterinary Sciences.

REFERENCES

1. Jones JC, Banfield CM, Ward DL. Association between postoperative outcome and results of magnetic resonance imaging and computed tomography in working dogs with degenerative lumbosacral stenosis. *J Am Vet Med Assoc* (2000) **216**(11):1769–74. doi:10.2460/javma.2000.216.1769
2. Linn LL, Bartels KE, Rochat MC, Payton ME, Moore GE. Lumbosacral stenosis in 29 military working dogs: epidemiologic findings and outcome after surgical intervention (1990-1999). *Vet Surg* (2003) **32**(1):21–9. doi:10.1053/jvet.2003.50001
3. Worth AJ, Thompson DJ, Hartman AC. Degenerative lumbosacral stenosis in working dogs: current concepts and review. *N Z Vet J* (2009) **57**(6):319–30. doi:10.1080/00480169.2009.64720
4. Jones JC, Tucker TJ, Tan JC, Pierce BJ, Foxworth JL, Long B, et al. Improving understanding of early behavioral indicators of lumbosacral disease in working dogs using 3D visualization of skeletal movements during working tasks: feasibility study. *J Vet Behav* (2013) **8**(5):309–15. doi:10.1016/j.jveb.2013.01.003
5. Breur GJ, Blevins WE. Traumatic injury of the iliopsoas muscle in three dogs. *J Am Vet Med Assoc* (1997) **210**(11):1631–4.
6. Breit S, Kunzel W. On biomechanical properties of the sacroiliac joint in purebred dogs. *Ann Anat* (2001) **183**(2):145–50. doi:10.1016/S0940-9602(01)80037-6
7. Gradner G, Bockstahler B, Peham C, Henninger W, Podbregar I. Kinematic study of back movement in clinically sound malinois dogs with consideration of the effect of radiographic changes in the lumbosacral junction. *Vet Surg* (2007) **36**(5):472–81. doi:10.1111/j.1532-950X.2007.00294.x
8. Seibert R, Marcellin-Little DJ, Roe SC, DePuy V, Lascelles BD. Comparison of body weight distribution, peak vertical force, and vertical impulse as measures of hip joint pain and efficacy of total hip replacement. *Vet Surg* (2012) **41**(4):443–7. doi:10.1111/j.1532-950X.2012.00957.x
9. Ghamkhar L, Kahlaee AH. Trunk muscles activation pattern during walking in subjects with and without chronic low back pain: a systematic review. *PM R* (2015) **7**(5):519–26. doi:10.1016/j.pmrj.2015.01.013
10. Marcellin-Little DJ, Levine D, Taylor R. Rehabilitation and conditioning of sporting dogs. *Vet Clin North Am Small Anim Pract* (2005) **35**(6):1427–39. doi:10.1016/j.cvsm.2005.08.002
11. Edge-Hughes L. Hip and sacroiliac disease: selected disorders and their management with physical therapy. *Clin Tech Small Anim Pract* (2007) **22**(4):183–94. doi:10.1053/j.ctsap.2007.09.007
12. Gross Saunders D. Therapeutic exercise. *Clin Tech Small Anim Pract* (2007) **22**(4):155–9. doi:10.1053/j.ctsap.2007.09.003
13. Millis D, Levine D. *Canine Rehabilitation and Physical Therapy*. Philadelphia, PA: Elsevier Health Sciences (2013).
14. Zink MC. Conditioning and retraining the canine athlete. *Canine Sports Medicine and Rehabilitation*. (2013). 176–200.
15. Nickel R, Schummer A, Seiferle E. The anatomy of the domestic animals. *The Locomotor System of the Domestic Mammals*. Vol. **1**. Hoboken, NJ: Blackwell Science (1986).
16. Evans HE, De Lahunta A. The muscular system. *Miller's Anatomy of the Dog*. Maryland Heights, MO: Elsevier Health Sciences (2013).
17. Feeney DA, Fletcher TF, Hardy RM. *Atlas of Correlative Imaging Anatomy of the Dog: Ultrasound and Computed Tomography*. Philadelphia, PA: Saunders (1991).
18. Smallwood JE, George TF. Anatomic atlas for computed tomography in the mesaticephalic dog: caudal abdomen and pelvis. *Vet Radiol Ultrasound* (1993) **34**(3):143–67. doi:10.1111/j.1740-8261.1993.tb01510.x
19. Asshauer J, Sager M. *MRI and CT Atlas of the Dog. [Magnetic Resonance Imaging and Computed Tomography]*. Hoboken, NJ: Blackwell Science (1997).
20. Done SH, Goody PC, Evans SA, Stickland NC. Color atlas of veterinary anatomy. *The Dog and Cat*. Vol. **3**. Maryland Heights, MO: Elsevier Health Sciences (2009).
21. Stokes MJ, Cooper RG, Morris G, Jayson MI. Selective changes in multifidus dimensions in patients with chronic low back pain. *Eur Spine J* (1992) **1**(1):38–42. doi:10.1007/BF00302141
22. Danneels L, Vanderstraeten G, Cambier D, Witrouw E, De Cuyper H. CT imaging of trunk muscles in chronic low back pain patients and healthy control subjects. *Eur Spine J* (2000) **9**(4):266–72. doi:10.1007/s005860000190
23. Keller A, Gunderson R, Reikeras O, Brox J. Reliability of computed tomography measurements of paraspinal muscle cross-sectional area and density in patients with chronic low back pain. *Spine* (2003) **28**(13):1455–60. doi:10.1097/01.BRS.0000048651.92777.30
24. Hu Z, He J, Zhao F, Fang X, Zhou L, Fan S. An assessment of the intra- and inter-reliability of the lumbar paraspinal muscle parameters using CT scan and magnetic resonance imaging. *Spine* (2011) **36**(13):E868–74. doi:10.1097/BRS.0b013e3181ef6b51
25. Hicks G, Simonsick E, Harris T, Newman A, Weiner D, Nevitt M, et al. Trunk muscle composition as a predictor of reduced functional capacity in the health, aging and body composition study: the moderating role of back pain. *J Gerontol A Biol Sci Med Sci* (2005) **60**(11):1420–4. doi:10.1093/gerona/60.11.1420
26. Kamaz M, Kiresi D, Oguz H, Emlik D, Levendoglu F. CT measurement of trunk muscle areas in patients with chronic low back pain. *Diagn Interv Radiol* (2007) **13**(3):144–8.
27. Kalichman L, Hodges P, Li L, Guermazi A, Hunter D. Changes in paraspinal muscles and their association with low back pain and spinal degeneration: CT study. *Eur Spine J* (2010) **19**(7):1136–44. doi:10.1007/s00586-009-1257-5
28. Fortin M, Yuan Y, Battie MC. Factors associated with paraspinal muscle asymmetry in size and composition in a general population sample of men. *Phys Ther* (2013) **93**(11):1540–50. doi:10.2522/ptj.20120457
29. Jones J, Wright J, Bartels J. Computed tomographic morphometry of the lumbosacral spine of dogs. *Am J Vet Res* (1995) **56**(9):1125–32.
30. Henderson AL, Hecht S, Millis DL. Lumbar paraspinal muscle transverse area and symmetry in dogs with and without degenerative lumbosacral stenosis. *J Small Anim Pract* (2015) **56**:618–22. doi:10.1111/jsap.12385
31. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* (1995) **57**:289–300.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

CASE REPORT
published: 08 June 2016
doi: 10.3389/fvets.2016.00045

18F-FDG Positron Emission Tomography – An Innovative Technique for the Diagnosis of a Canine Lameness

Kelly Mann[1]*, *Juliette Hart*[2] *and Felix Duerr*[2]

[1] Veterinary Diagnostic Imaging Section, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA, [2] Small Animal Sports Medicine and Rehabilitation Section, Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

Introduction: Positron emission tomography (PET) imaging with fluorine-18-fluorodeoxyglucose (18F-FDG) is widely known for its use in the diagnosis and tracking of primary and metastatic tumors *via* uptake and retention of the radiopharmaceutical by hypermetabolic cells. 18F-FDG is also used to study the normal physiology of glucose uptake, metabolism, and muscle activity during and after exercise.

Background: A pilot study adding PET imaging to the diagnostic evaluation of canine patients undergoing computed tomography (CT) for mild or intermittent thoracic and pelvic limb lameness is ongoing. Dogs with an observable (grade 1–2/5) lameness that have undergone routine radiography and complete physical examination by board-certified veterinary surgeons and sports medicine and rehabilitation specialists are enrolled. Each patient undergoes leash walking for 15 min prior to premedication and induction of general anesthesia for the PET–CT examination. 18F-FDG is injected intravenously, and a whole-body PET examination is conducted after 1 h of radiopharmaceutical uptake time. Standard algorithm, whole-body pre- and post-contrast CT examinations, and focused, standard, and bone algorithm CT scans of the thoracic or pelvic limb areas of interest are obtained concurrently. Abnormal PET–CT findings are further investigated with additional diagnostic imaging or at surgery (e.g., ultrasound, MRI, and arthroscopy).

Discussion: This case report uses a canine patient referred for thoracic limb lameness to illustrate the role of advanced imaging in a diagnostic plan and to discuss a recommended PET–CT procedure for lameness evaluation. The PET–CT imaging protocol recommended in this report was designed to significantly enhance a routine thoracic limb CT examination and to identify areas of muscle, tendon, or ligament overuse, inflammation, or injury for further diagnostic procedures or definitive treatment.

Concluding remarks: 18F-FDG PET–CT adds valuable physiologic and anatomic information to the diagnostic evaluation of patients presenting with indistinct or intermittent clinical signs of musculoskeletal inflammation or injury. In addition, tailoring the PET acquisition and radiopharmaceutical parameters allows for detailed information gathering to more closely assess normal and abnormal physiology, unlocking a new frontier in the study of canine athletic injury and optimal performance.

Keywords: positron emission tomography, computed tomography, fluorodeoxyglucose, canine, lameness

OPEN ACCESS

Edited by:
Cynthia M. Otto, University of Pennsylvania, USA

Reviewed by:
Fintan John McEvoy, University of Copenhagen, Denmark
Elizabeth LaFond, University of Minnesota, USA

***Correspondence:**
Kelly Mann
kelly.mann@colostate.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *12 February 2016*
Accepted: *18 May 2016*
Published: *08 June 2016*

Citation:
Mann K, Hart J and Duerr F (2016) 18F-FDG Positron Emission Tomography – An Innovative Technique for the Diagnosis of a Canine Lameness. Front. Vet. Sci. 3:45. doi: 10.3389/fvets.2016.00045

INTRODUCTION

An 8.5-year-old castrated male, 20.9 kg, Chow-mix dog was presented to the Small Animal Sports Medicine and Rehabilitation Service for evaluation of a mild, intermittent right thoracic limb lameness of approximately 1-year duration. The patient history includes hip dysplasia with mild degenerative joint disease of both coxofemoral joints and grade 1–2/4 left-sided systolic heart murmur. Previous radiographs of the right shoulder and elbow were normal, and routine laboratory tests of blood and urine were also within normal limits. On orthopedic examination, he had a grade 1/5 right thoracic limb lameness at the trot and intermittent discomfort on Campbell's test with normal range of motion and no joint effusion. Campbell's test is based on a study evaluating collateral ligament integrity by supinating and pronating the carpus when the elbow and carpus are flexed in a 90° position (1). Modifications also include applying gentle pressure to the medial compartment and subjectively assessing patient reaction (2). The patient also had mild thickening at the distal insertion of the right flexor carpi ulnaris (FCU) tendon.

Challenging orthopedic and sports medicine cases are often referred for advanced imaging after routine diagnostic procedures are performed by the referring veterinarian. Magnetic resonance imaging (MRI) and computed tomography (CT) are frequently the next imaging procedures of choice. At our institution, a pilot study adding positron emission tomography (PET) to the diagnostic evaluation of canine patients undergoing CT as part of the normal diagnostic plan for mild or intermittent thoracic or pelvic limb lameness is ongoing. On the morning of the PET–CT examination, the patient was leash-walked for 15 min prior to premedication and induction of general anesthesia. The fluorine-18-fluorodeoxyglucose (18F-FDG) was injected intravenously, and a whole-body PET examination was conducted after 1 h of radiopharmaceutical uptake time. Increased radiopharmaceutical uptake (IRU) was identified in the FCU muscle of the right antebrachium, and a slight increase in muscle size and density (approximately 20 HU) was noted in corresponding CT images. A faint ring of contrast enhancement corresponding to a 5-mm area of the most intense radiopharmaceutical uptake was also noted. A diagnostic ultrasound examination performed the following day revealed a hypoechoic region consistent with muscle tearing within the right FCU muscle. Patient discharge instructions included: daily stretching of the thoracic limb muscles, restricted activity within the home (e.g., no running, jumping, stairs, or rough play), leash walks of no more than 10 min duration for 6 weeks, and a NSAID (37.5 mg Carprofen *per os* every 12 h) as needed for pain relief. After 6 weeks, the length of daily walks was gradually increased 5–10 min per week until the patient could comfortably walk for 30 min without evidence of lameness. A recheck ultrasound examination was performed 6 weeks after discharge, and an injection of platelet rich plasma (PRP) was performed at that time. Additional ultrasound and physical examinations are planned at 3-month intervals.

This case report uses a canine patient referred for a subtle forelimb lameness to illustrate the role of advanced imaging in a diagnostic plan and to recommend an innovative PET–CT imaging protocol, which identifies glucose uptake abnormalities and asymmetries useful in the diagnosis of muscular overuse, inflammation, or infection, in clinically lame canine patients.

BACKGROUND

Positron emission tomography imaging with 18F-FDG is widely known for its use in the diagnosis and tracking of primary and metastatic tumors *via* uptake and retention of the radiopharmaceutical by hypermetabolic cells (3). 18F-FDG is also used to study the physiology of glucose uptake, metabolism, and muscle activity during and after exercise (4, 5) or as a result of concurrent disease conditions (6, 7). The additional physiologic data gained from PET imaging are of particular interest to the Sports Medicine and Rehabilitation Service because they may aid in the diagnosis of subtle abnormalities that are not apparent on standard radiographic or US techniques. Additionally, it is not feasible to perform whole-body scanning with US or MR modalities, and similar to its extensive use in oncology, PET–CT can provide objective morphologic lesion localization and quantifiable functional measurements to document response to therapeutic intervention in muscle groups that are difficult to evaluate through other means. Availability of integrated PET–CT equipment has increased in veterinary university and referral centers because of its universal application in cancer diagnosis and response to therapy. Although the half-life of fluorine-18 is short (110 min), commercial production for human medical procedures is common, which increases the logistic availability of the radiopharmaceutical, and the short half-life allows release of the patient on the same day. In our geographic area, the availability and cost of 18F-FDG is reasonable because of its extensive use in human and veterinary oncology. Its use in human musculoskeletal diagnostic imaging and research is also increasing. Canine patients are commonly referred to our institution for shoulder and elbow pathology but also for lumbar spine or pelvic limb pain, creating the opportunity for PET as a complementary procedure, which adds physiological data to the CT findings.

EQUIPMENT

Positron emission tomography–computed tomography imaging was performed with a Philips GEMINI TF Big Bore PET–CT system (Philips Healthcare, Koninklijke Philips Electronics N.V., Amsterdam). This system combines a 16-slice CT machine with time-of-flight (TOF) PET technology capable of 495-ps timing resolution and list mode reconstruction. The PET and CT gantries have an 85-cm inner diameter with up to 60-cm diameter field of view.

PET–CT IMAGING PROTOCOL

Immediately prior to premedication and induction of general anesthesia, the patient is leash-walked for a minimum of 15 min. Patient monitoring during the anesthetic episode includes: ECG, arterial pressure, capnography, pulse oximetry, ventilation rate and quality, and urinary catheterization. The patient is positioned on the PET–CT table in dorsal recumbency, and the table position is zeroed. 18F-FDG (5.2–6.3 MBq/kg) is injected *via* indwelling catheter in the cephalic or saphenous vein. The venous and arterial catheter locations are selected to specifically avoid the thoracic or pelvic limb area of interest noted in the patient history. Local radiation safety policies require that the 1-h radiopharmaceutical uptake time occurs in the PET–CT suite. After intravenous administration of the radiopharmaceutical, the patient's vital signs and plane of anesthesia are monitored remotely from the shielded PET–CT control room.

At 20 min prior to PET acquisition (after 40 min of uptake time), a non-contrast helical CT series focused on the thoracic or pelvic limb area of interest is performed and reconstructed with 1-mm bone and 2-mm standard filters in a 1024 × 1024 matrix. A non-contrast whole-body CT series immediately follows the focused series, and 5- and 2-mm (768 × 768 matrix) standard reconstructions are created.

After 60 min of radiopharmaceutical uptake time, a whole-body PET acquisition using consecutive 8-cm beds with 1.5-min bed times is initiated. At the conclusion of the PET acquisition, an early venous phase contrast-enhanced whole-body CT is performed using a standard algorithm, with 5- and 2-mm standard reconstructions as described in the non-contrast series above. For the contrast-enhanced CT, 2 ml/kg of a non-ionic, iodinated contrast solution [iohexol 755 mg/ml (equivalent to 350 mg of organic iodine), GE Healthcare] is administered *via* autoinjector at 2 ml/s followed by 1 ml/kg of 0.9% NaCl at 2 ml/s. The CT scan begins after a 40- to 50-s delay based on patient heart rate.

POST-SCANNING PROCEDURES

All images from each of the PET and CT acquisitions are transferred to the hospital picture archiving and communication system and fused PET–CT images are viewed on a dedicated Philips IntelliSpace Portal workstation (v6.0.2.32500, 13 June 2014, Koninklijke Philips Electronics N.V.) for clinical interpretation. In our experience, the blue palette (cardiac) lookup table (LUT) significantly increases the conspicuity of radiopharmaceutical uptake in fused PET–CT images when compared to the red palette (thermal) LUT. The patient recovers from anesthesia in a dedicated nuclear medicine ward and segregated walking area with closed circuit television monitoring after extubation. Local radiation safety policies allow the patient to return to the client or other sections of the veterinary teaching hospital after reaching a survey meter reading of ≤20 μSv/h at the skin surface of the shoulder region. Abnormal PET–CT findings are further investigated with additional diagnostic imaging or at surgery (e.g., ultrasound, MRI, and arthroscopy).

DISCUSSION

The initial clinical observations confirmed a chronic, mild right thoracic limb lameness, but failed to localize the specific cause. A PET–CT was recommended and elected. Fused PET–CT images were evaluated in multiple, preset, and adjustable Hounsfield unit window levels and widths to identify abnormal areas of uptake or areas of asymmetric uptake in the skeletal muscles of the thoracic limbs, pelvic limbs, or axial regions of the patient. The three-dimensional PET maximum intensity projection (MIP) is helpful for orientation prior to detailed review of the data acquisitions in two-dimensional transverse, sagittal, and dorsal planes or three-dimensional CT volume rendering (**Figure 1**).

Setting the PET overlay to display a narrow standardized uptake value (SUV) window width (0–5) is particularly important when searching for asymmetric radiopharmaceutical uptake in skeletal muscle because subtle areas of increased uptake must be conspicuous in comparison to the surrounding or contralateral normal tissues.

In this case, normal uptake of 18F-FDG was noted within the central nervous system, zygomatic and mandibular salivary glands, vocal folds, myocardium, and abdominal viscera, as well as normal excretion *via* the urinary tract (8–10) (**Figure 1**). On evaluation of the radionuclide uptake within the muscles of the thoracic limbs, asymmetrically IRU was identified in the FCU muscle of the right antebrachium with a maximum standardized uptake value (SUVmax) of 2.4, compared to the contralateral muscle with 1.0 SUVmax (11) (**Figures 2A–C**). This finding is related to increased cellular metabolic activity and may be the result of compensatory overuse of the muscle, inflammation, or injury. IRU in a neoplastic cell results from unregulated increase in glucose transporters and dependence on glycolysis for energy production. Inflammation, injury, or increased use of glucose by non-neoplastic muscle cells causes IRU secondary to normal physiologic glycolysis in response to cell injury and repair mechanisms. Although iodinated contrast may increase the conspicuity of soft tissue injury in CT images as a result of neovascularization or extravasation from increased vascular permeability, a change in the density, size, or enhancement may be inconspicuous or mild, as in this patient (12). Contrast enhancement and tissue density in CT images are affected by variability in the amount of neovascularization, vascular permeability and edema in the lesion itself, and the timing of contrast administration. In this case, a diagnostic ultrasound examination identified two small hypoechoic areas within the muscle of the right FCU, just proximal to the insertion on the accessory carpal bone. Corresponding irregular and hypoechoic muscle fibers were also visible on long axis images (**Figure 3**). These findings were consistent with muscle tearing within the right FCU muscle and corresponded to the most intense region of radiopharmaceutical uptake on the PET–CT images (**Figure 2B**) (13, 14). The extent of IRU identified a larger area of affected FCU muscle, when compared to the small focus of CT contrast enhancement. At the recheck ultrasound examination 6 weeks after discharge, an intralesional injection of platelet-rich plasma (PRP) was performed to promote healing. Clinical use of PRP is becoming commonplace in both human and veterinary medicine for the treatment of musculoskeletal injuries. The beneficial effects of PRP are attributed in part to growth factors released by activated platelets leading to neovascularization (15–18). Additional ultrasound and physical examinations to document the healing process are scheduled every 3 months.

There are unique challenges to overcome when conducting PET–CT imaging of veterinary patients, and attention to

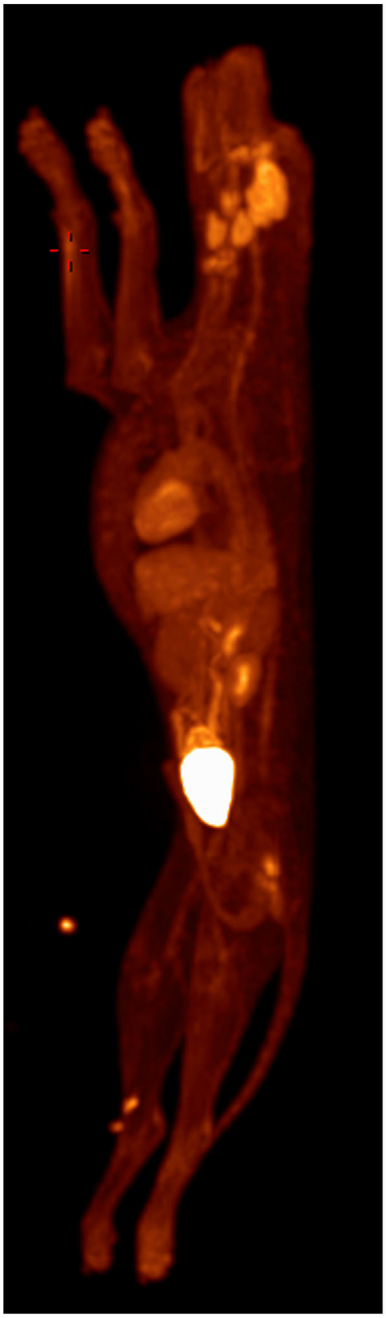


FIGURE 1 | Whole-body PET MIP with abnormal 18F-FDG uptake noted in right thoracic limb (red crosshairs). Normal radiopharmaceutical uptake in the brain, zygomatic and mandibular salivary glands, vocal folds, myocardium, abdominal viscera, and urinary tract is also present.

FIGURE 2 | PET–CT images demonstrating increased 18F-FDG uptake in the right flexor carpi ulnaris muscle. (A) PET MIP, ventral view, red palette (thermal) lookup table (LUT). Normal radionuclide uptake is also present in the brain, zygomatic and mandibular salivary glands, and vocal folds. **(B)** PET–CT, ventral view, right antebrachium, blue palette (cardiac) LUT. **(C)** PET–CT, transverse view, blue palette (cardiac) LUT. Comparison of the right and left antebrachii at the level of the right flexor carpi ulnaris muscle injury. The right antebrachium is on the right of the image. The blue palette LUT increases lesion conspicuity when viewing fused images.

certain details can maximize the quality of the data acquisition. A short period of mild muscular effort (leash walking) is recommended prior to anesthesia to increase the possibility of detecting asymmetric radiopharmaceutical uptake in individual muscles or muscle groups. However, strenuous muscle activity (e.g., focused strength training, sled pulling, or excessive physical effort) can result in IRU for 24–48 h after the event and upset the balance between detectable asymmetry versus induced normal physiologic uptake that masks underlying abnormal pathologic uptake. External monitoring instrumentation and intravenous and intra-arterial catheters are placed outside the thoracic or pelvic limb area of interest to avoid the possibility of beam hardening artifacts or IRU from inflammation or extravasation at the site of medication, iodinated contrast, or radiopharmaceutical injection. Using radiolucent, padded v-troughs, wedges, and blocks to position the patient in dorsal recumbency, with thoracic limbs moderately extended, elbows and paws parallel to one another, and pelvic limbs extended with stifles, and paws parallel to one another will help to achieve bilateral symmetry. This also extends the spine axially to avoid curvature and allow better evaluation of the cervical and lumbosacral regions. Positioning the patient with the pelvic limbs first into the gantry allows the greatest access to the endotracheal tube, long ventilation tubes, anesthesia cart, and patient monitoring display screens (remotely viewed on closed circuit or wireless monitors in the CT control

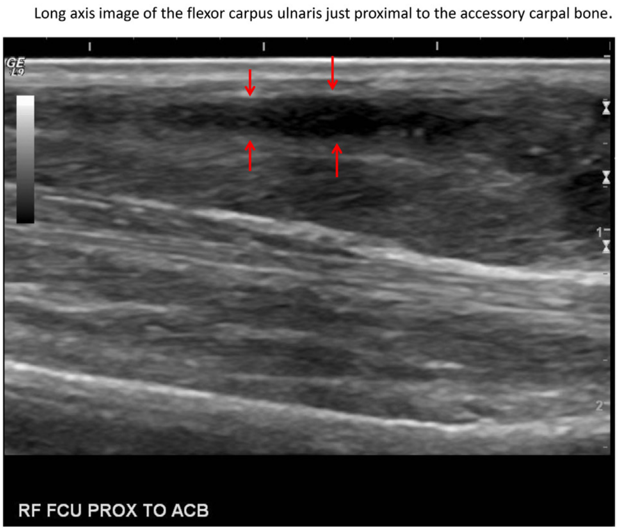


FIGURE 3 | **Sagittal ultrasound image of the muscle tear (red arrows) in the right flexor carpi ulnaris muscle just proximal to the accessory carpal bone (left side of image is distal).**

room) as the patient table travels more than 2 m in and out of the PET and CT gantries. This consistent and symmetric method of positioning also greatly assists in the assessment of ipsilateral and contralateral bone and soft tissue anatomical structures during image evaluation, improving comparison and detection of abnormalities of radiopharmaceutical uptake, iodinated contrast enhancement, density, and size. An indwelling urinary catheter minimizes urinary bladder size, improving image interpretation in the caudal abdomen, and facilitates the removal of radioactive urine, protecting against contamination of the gantry and further decreasing patient exposure.

This PET–CT protocol is also consistent with the tenets of radiation safety and published nuclear medicine dosing recommendations. Local radiation safety policies require the radionuclide uptake time to occur in the PET–CT suite. All personnel wear body and ring badges, lab coats, gloves, and closed-toe shoes. The patient achieves a stable plane of general anesthesia prior to radiopharmaceutical administration, and remote monitoring equipment minimizes contact with the patient during the PET–CT data acquisition and anesthesia recovery time periods. The veterinary patient dose is in accordance with North American and European Association of Nuclear Medicine recommendations for human pediatric patients, further ensuring that the exposure to veterinary personnel will be minimized (19).

CONCLUDING REMARKS

Integrated PET–CT is a significant diagnostic method, synergizing the localization and anatomic and physiologic data analysis tools of two advanced imaging modalities. Although primarily known for extensive use in the field of oncology, the application of 18F-FDG imaging for the clinical evaluation and research of musculoskeletal disease conditions and normal exercise physiology is increasing. The PET–CT imaging protocol recommended in this report was designed to significantly enhance a routine thoracic or pelvic limb CT examination and to identify areas of muscle, tendon, or ligament overuse, inflammation, or injury for further diagnostic procedures or definitive treatment. The 18F-FDG PET–CT added valuable physiologic and anatomic information to the diagnostic evaluation of this patient, which can also be used for objective evaluation of the healing process in serial examinations. Current efforts are aimed at the validation of this technique through demonstrated benefit to additional patients in the ongoing pilot study. PET–CT imaging is recommended after standard means of diagnosis have failed or when the specific physiologic information provided by a PET radiopharmaceutical may detect subtle metabolic abnormalities in the muscle, indicating compensatory overuse, inflammation, or injury. Continued tailoring of the PET acquisition and radiopharmaceutical parameters will allow for more detailed information gathering in the realm of exercise physiology. Future efforts will increase the objective PET–CT data available to more closely assess normal and abnormal physiology, unlocking a new frontier in the study of canine athletic injury and optimal performance.

AUTHOR CONTRIBUTIONS

This case report was designed and coordinated by KM, JH and FD to specifically introduce a novel imaging protocol and the reasoning behind its use in the diagnostic evaluation of canine patients with intermittent or subtle, undiagnosed lameness.

ACKNOWLEDGMENTS

This pilot study was inspired by the work of researchers studying asymmetric glucose uptake and premature fatigability in leg muscles of Multiple Sclerosis patients in the Integrative Neurophysiology Laboratory, Department of Health and Exercise Science, Colorado State University (http://inplab.colostate.edu/). Additional patient care and medical procedures other than PET–CT were performed by professional colleagues in the Veterinary Diagnostic Imaging, Small Animal Sports Medicine and Rehabilitation, and Small Animal Orthopedics services of the CSU Veterinary Teaching Hospital.

REFERENCES

1. Campbell JR. Nonfracture injuries to the canine elbow. *J Am Vet Med Assoc* (1969) 155(5):735–44.
2. Farrell M, Draffan D, Gemmill T, Mellor D, Carmichael S. In vitro validation of a technique for assessment of canine and feline elbow joint collateral ligament integrity and description of a new method for collateral ligament prosthetic replacement. *Vet Surg* (2007) 36:548–56. doi:10.1111/j.1532-950X.2007.00281.x
3. Alazraki N, Shumate M, Kooby D. FDG PET and PET/CT molecular imaging. In: *A Clinician's Guide to Nuclear Oncology: Practical Molecular Imaging and Radionuclide Therapies*. Reston, VA: The Society of Nuclear Medicine (2007). p. 1–6.

4. Rudroff T, Kalliokoski KK, Block DE, Gould JR, Klingensmith WC III, Enoka RM. PET/CT imaging of age- and task-associated differences in muscle activity during fatiguing contractions. *J Appl Physiol* (2013) 114:1211–9. doi:10.1152/japplphysiol.01439.2012
5. Rudroff T, Kindred JH, Kalliokoski KK. [18F]-FDG positron emission tomography – an established tool opening a new window into exercise physiology. *J Appl Physiol* (2015) 118:1181–90. doi:10.1152/japplphysiol.01070.2014
6. Aydin A, Hickeson M, Yu JQ, Zhuang H, Alavi A. Demonstration of excessive metabolic activity of thoracic and abdominal muscles on FDG-PET in patients with chronic obstructive pulmonary disease. *Clin Nucl Med* (2005) 30(3):159–64. doi:10.1097/00003072-200503000-00003
7. Rudroff T, Kindred JH, Koo PJ, Karki R, Hebert JR. Asymmetric glucose uptake in leg muscles of patients with multiple sclerosis during walking detected by [18F]-FDG PET/CT. *NeuroRehabilitation* (2014) 35(4):813–23. doi:10.3233/NRE-141179
8. LeBlanc A, Jakoby B, Townsend D, Daniel G. Thoracic and abdominal organ uptake of 2-deoxy-2-[18F]fluoro-D-glucose (18FDG) with positron emission tomography in the normal dog. *Vet Radiol Ultrasound* (2008) 49(2):182–8. doi:10.1111/j.1740-8261.2008.00348.x
9. Lee M, Lee A, Jung M, Lee I, Choi J, Chung H, et al. Characterization of physiologic 18F-FDG uptake with PET-CT in dogs. *Vet Radiol Ultrasound* (2010) 51(6):670–3. doi:10.1111/j.1740-8261.2010.01727.x
10. Randall E, Loeber S, Kraft S. Physiologic variants, benign processes, and artifacts from 106 canine and feline FDG-PET/Computed Tomography scans. *Vet Radiol Ultrasound* (2013) 55(2):213–26. doi:10.1111/vru.12138
11. Lee K. Basic science of nuclear medicine. In: Bailey DL, Townsend DW, Valk PE, Maisey MN, editors. *Positron Emission Tomography*. Reston, VA: Society of Nuclear Medicine and Molecular Imaging (2015). p. 167–88.
12. Rossmeisl JH, Rohleder JJ, Hancock R, Lanz OI. Computed tomographic features of suspected traumatic injury to the iliopsoas and pelvic limb musculature of a dog. *Vet Radiol Ultrasound* (2004) 45(5):388–92. doi:10.1111/j.1740-8261.2004.04070.x
13. Bohndorf K, Kilcoyne RF. Traumatic injuries: imaging of peripheral musculoskeletal injuries. *Eur Radiol* (2002) 12:1605–16. doi:10.1007/s00330-002-1461-8
14. Mattoon JS, Nyland TG, editors. Musculoskeletal system. 3rd ed. *Small Animal Diagnostic Ultrasound*. (Chap. 14), St. Louis, MO: Saunders (2015). p. 517–40.
15. Visser LC, Arnoczky SP, Caballero O, Kern A, Ratcliffe A, Gardner KL. Growth factor-rich plasma increases tendon cell proliferation and matrix synthesis on a synthetic scaffold: an in vitro study. *Tissue Eng* (2010) 16(3):1021–9. doi:10.1089/ten.TEA.2009.0254
16. Bosch G, Moleman M, Barneveld A, van Weeren PR, van Schei HTM. The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. *Scand J Med Sci Sports* (2011) 21:554–61. doi:10.1111/j.1600-0838.2009.01070.x
17. Visser LC, Arnoczky SP, Caballero O, Gardner KL. Evaluation of the use of an autologous platelet rich fibrin membrane to enhance tendon healing in dogs. *Am J Vet Res* (2011) 72(5):699–705. doi:10.2460/ajvr.72.5.699
18. Beitzel K, Allen D, Apostolakos J, Russell R, McCarthy MB, Gallo G, et al. US definitions, current use, and FDA stance on use of platelet-rich plasma in sports medicine. *J Knee Surg* (2015) 28:29–34. doi:10.1055/s-0034-1390030
19. Lassmann M, Treves ST. Paediatric radiopharmaceutical administration: harmonization of the 2007 EANM paediatric dosage card (version 1.5.2008) and the 2010 North American consensus guidelines. *Eur J Nucl Med Mol Imaging* (2014) 41(5):1036–41. doi:10.1007/s00259-014-2731-9

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 22 June 2016
doi: 10.3389/fvets.2016.00049

Aspects of Juvenile and Adolescent Environment Predict Aggression and Fear in 12-Month-Old Guide Dogs

James A. Serpell and Deborah L. Duffy*

Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Maturational changes in behavior, and the possible influence of the puppy-raising environment on behavioral development, were investigated in a total sample of 978 prospective guide dogs belonging to four different breeds/crosses. All dogs belonged to the same guide dog organization, and had been exposed to similar early environmental influences prior to being assigned to puppy-raising households at 7–8 weeks of age. Behavioral data were collected from puppy raisers when the dogs were 6 and 12 months old using the C-BARQ©, a standardized, validated, and widely used survey instrument that measures the frequency and/or severity of most common behavior problems in dogs. Information about the puppy-raising environment was obtained from puppy raisers using a standardized questionnaire shortly before the dogs were returned to the guide dog organization for training. Data were analyzed using both univariate and multivariate statistics (binary logistic generalized estimating equations modeling and generalized linear modeling). The findings demonstrated specific maturational changes in behavior between 6 and 12 months of age. In particular, German Shepherd dogs displayed an increase in stranger-directed aggression compared with the other breeds/crosses between 6 and 12 months of age. Several aspects of the puppy-raising environment were associated with puppies' C-BARQ scores at 12 months of age. In particular, growing up in households with more experienced puppy raisers, and in the company of at least one other dog, were both associated with positive effects on a number of puppy behaviors. By contrast, puppies that had been frightened by a person or threatened by another unfamiliar dog showed significantly worse scores for fear of strangers and dogs, respectively. Being frightened by a person, being reared by less experienced puppy raisers, and/or in households without other pets were associated with less successful training outcomes. The relevance of these findings to current guide dog breeding and husbandry practices is discussed.

Keywords: dog, behavior, development, puberty, C-BARQ

Edited by:
Erik Wilsson, Swedish Armed Forces, Sweden

Reviewed by:
Ruth Catriona Newberry, Norwegian University of Life Sciences, Norway
Mia Cobb, Working Dog Alliance Australia, Australia

****Correspondence:***
James A. Serpell
serpell@vet.upenn.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *29 January 2016*
Accepted: *07 June 2016*
Published: *22 June 2016*

Citation:
Serpell JA and Duffy DL (2016) Aspects of Juvenile and Adolescent Environment Predict Aggression and Fear in 12-Month-Old Guide Dogs. Front. Vet. Sci. 3:49. doi: 10.3389/fvets.2016.00049

INTRODUCTION

According to Scott and Fuller (1), the juvenile period in dogs runs from ~12 weeks (the postulated end of the socialization period) until 6 months of age or later, corresponding to the onset of sexual maturation (i.e., puberty). Extensive studies in rodents and both human and non-human primates have demonstrated that changes in circulating gonadal hormones during the adolescent period have dramatic effects on brain development and behavior in mammals (2, 3). Despite the likelihood of similar developmental influences in adolescent dogs (between ~6 months and 1–2 years of age depending on the individual and breed), this period is probably the most poorly studied in terms of its effects on adult behavior (4). Anecdotal evidence certainly suggests that experiences during the juvenile and adolescent periods appear exert long-term effects on behavior in dogs (5–8), and one recent study of military working dogs found a positive association between dogs' overall scores for working ability and the amount of time they were left at home alone during this period (9). In rodents, exposure to enriched environments around puberty has been found to erase the negative effects of early life stress on the development of the HPA axis (10), but whether similar effects occur in dogs is currently unknown.

Purpose-bred guide dogs provide a useful model for studying canine behavioral development during this period for several reasons. First, they usually comprise a relatively small number of different breed types – typically Labrador retriever, golden retriever, German Shepherd dog (GSD), and Labrador × golden retriever crosses – and this enables both within-breed and across-breed comparisons. Second, these dogs remain sexually intact until around 14–18 months of age so the potentially confounding effects of the loss of gonadal hormones on brain development and behavior due to surgical sterilization are effectively eliminated. Third, up to the age of 7–9 weeks, the puppies produced within any given organization will experience similar and relatively consistent early rearing environments, thus helping to reduce the effects of differential environmental influences occurring during early development. Finally, from roughly 2 to 14 months of age, guide dog puppies are reared in the households of volunteer puppy raisers (i.e., individuals and families who foster guide dog puppies) where they are exposed to variable environments, thereby providing an opportunity to explore the possible effects of this variability on their behavioral development during the juvenile and adolescent periods. Here, we present findings based on behavioral data collected from puppy raisers associated with a single guide dog organization when the dogs were 6 and 12 months of age, respectively. In addition to exploring maturational changes in behavior in dogs between 6 and 12 months (i.e., assumed to represent juvenile and adolescent dogs, respectively), we also investigate associations between behavioral outcomes at 12 months of age, and during subsequent training, and antecedent environmental factors and events occurring during the juvenile and adolescent periods.

MATERIALS AND METHODS

Sample

For the study of maturational changes in behavior, a sample 741 young guide dogs (376 males and 365 females) were used, which included a mix of four breed types ($N = 226$ GSDs, 145 golden retrievers, 210 Labrador retrievers, and 160 golden × Labrador crosses). For the analysis of environmental influences on behavioral development, the sample of dogs comprised 472 males and 506 females ($N = 978$ total) and included the same mix of breed types ($N = 276$ GSDs, 207 golden retrievers, 296 Labrador retrievers, and 219 golden × Labrador crosses). All of the dogs belonged to a single, US-based guide dog organization.

Data Collection

Behavioral data were provided by the participating guide dog organization that routinely collects behavioral information on each dog from puppy raisers when the dogs are ~6 and 12 months old using the C-BARQ©, a standardized, validated, and widely used survey instrument. The C-BARQ© allows dog owners and handlers to describe the frequency and/or severity of most common behavior problems in dogs (11, 12). The specific behavioral variables that were examined included four different types of aggression and five different types of fear/anxiety (**Table 1**).

Information on the dogs' household characteristics and environmental exposures, was obtained from the "Puppy-Raiser Report" questionnaire (see Supplementary Materials), which all puppy raisers are asked to complete routinely prior to returning their dogs to the parent organization for training at ~14 months of age. This survey provides relatively detailed information about the characteristics of the household, the experience level of the puppy raisers, and the puppy's level of exposure to a wide variety of potential environmental stressors and stimuli.

In addition, information on each dogs' eventual disposition – i.e., whether it became a successful working dog, a breeding dog, a dog that was released for health reasons, or one that was released for behavioral reasons – was also provided by the participating guide dog organization.

As the owner of both the dogs and the data, the participating guide dog organization provided written consent for the use of this information in the current study.

TABLE 1 | C-BARQ aggression and fear/anxiety variable descriptions (numbers of questionnaire items in parentheses).

C-BARQ trait	C-BARQ variable description (number of questionnaire items)
Stranger-directed aggression	Severity of threatening or aggressive responses to strangers approaching or invading the dog's or owner's personal space, territory, or home range (10)
Dog-directed aggression	Severity of threatening or aggressive responses when approached directly by unfamiliar dogs (4)
Owner-directed aggression	Severity of threatening or aggressive responses to the owner or other members of the household when challenged, manhandled, stared at, stepped over, or when approached while in possession of food or objects (8)
Dog rivalry	Severity of aggressive or threatening responses to other familiar dogs in the same household (4)
Stranger-directed fear	Severity of fearful or wary responses when approached directly by strange or unfamiliar people (4)
Dog-directed fear	Severity of fearful or wary responses when approached directly by unfamiliar dogs (4)
Non-social fear	Severity of fearful or wary responses to sudden or loud noises, traffic, and unfamiliar objects, and situations (6)
Separation-related behavior	Frequency of vocalizing and/or destructive behavior when separated from the owner, including autonomic signs of anxiety – restlessness, loss of appetite, trembling, and excessive salivation (8)
Touch sensitivity	Severity of fearful or wary responses to potentially painful or uncomfortable procedures, including bathing, grooming, nail-clipping, and veterinary examinations (4)

Statistical Analyses

For the determination of maturational changes in aggressive and fearful behavior, both 6- and 12-month C-BARQ scores were used, and the effects were analyzed with binary logistic generalized estimating equations modeling, with dichotomized C-BARQ scores as dependent variables. Because scores for all aggression and fear/anxiety variables were strongly skewed, C-BARQ scores were dichotomized such that a score of 0 indicated the absence of the behavior and all scores above 0 were recoded as indicating the presence of the behavior. Predictor variables included age at evaluation as a within-subjects variable, and breed and sex as between-subjects variables. Interactions between age at evaluation with either breed or sex were examined and retained in the final models, if significant.

To investigate the effects of environmental factors on behavior, preliminary analyses were conducted using non-parametric tests (Mann–Whitney U, Kruskal–Wallis, and Chi-square tests) to identify the main household and experiential factors associated with behavioral differences at 12 months of age and later success in training ("outcomes") (see Supplementary Tables). Generalized linear models (with logit link function) were then used to identify the key environmental variables associated with training outcomes and the presence of aggression and fear/anxiety while accounting for the effects of breed differences in behavior. A backwards elimination procedure was utilized, removing predictor variables with p-values below 0.05. Cases with missing values ($N \leq 50$) for any predictors were excluded from the analysis on a model by model basis. As before, aggression and fear/anxiety C-BARQ subscales were dichotomized, with 0 indicative of the absence of the behavior and all scores greater than 0 indicative of the presence of the behavior.

RESULTS

Maturational Changes

Comparisons of young guide dogs' C-BARQ scores at 6 and 12 months of age revealed interesting developmental changes, which sometimes varied by breed or sex. GSDs, a breed that has been selected historically for guarding or "protective" behavior, showed increases in "stranger-directed aggression" between 6 and 12, while the other three breeds showed only minimal increases or none at all (**Table 2**; **Figure 1**). During the same time frame, decreases were observed in "owner-directed aggression," "separation-related problems," and "non-social fear," though the latter was only detectable among females (**Table 2**).

Environmental Influences on Behavior

Puppy-raisers' prior experience with raising guide dogs was associated with a number of behavioral differences in guide dogs at 12 months of age, including "owner-directed aggression," "stranger-directed aggression," "dog-directed aggression," "dog-directed fear," "non-social fear," and "touch sensitivity" (**Tables 3** and **4**; **Figures 2** and **3**). Likewise, there was also a tendency for an association between the number of previous guide dog puppies raised by the puppy raiser and the likelihood that a puppy would successfully complete training (**Table 5**).

TABLE 2 | Results of binary logistic generalized estimating equations modeling to examine maturational changes in C-BARQ traits.

C-BARQ traits and predictors[a]	Wald Chi-square	df	p-value	OR	95% CI
Stranger-directed aggression					
Age at evaluation	14.13	1	0.0002[b]	–	–
Age = 12 months	2.67	1	0.102	1.45	0.93, 2.45
Sex = Female	0.21	1	0.648	0.93	0.68, 1.27
Breed	67.96	3	<0.0001[b]	–	–
German Shepherd	22.62	1	<0.0001[b]	3.70	2.16, 6.35
Golden retriever	2.83	1	0.092	1.71	0.92, 3.19
LabGolden Cross	2.13	1	0.144	1.59	0.85, 2.97
Breed × Age	10.01	3	0.018	–	–
German Shepherd at 12 months	3.21	1	0.073	1.68	0.95, 2.97
Golden retriever at 12 months	1.49	1	0.223	0.67	0.36, 1.27
Lab × Golden Cross at 12 months	0.42	1	0.838	1.07	0.56, 2.04
Owner-directed aggression					
Age = 12 months	14.31	1	0.0002[b]	0.69	0.58, 0.84
Sex = female	0.04	1	0.839	0.97	0.58, 1.30
Breed	2.43	3	0.488	–	–
German Shepherd	0.98	1	0.321	1.22	0.82, 1.80
Golden retriever	0.84	1	0.360	1.22	0.79, 1.89
Lab × Golden Cross	0.08	1	0.782	0.94	0.61, 1.45
Dog-directed aggression/fear					
Age = 12 months	0.48	1	0.489	0.92	0.71, 1.17
Sex = female	0.03	1	0.874	0.98	0.71, 1.33
Breed	9.26	3	0.026	–	–
German Shepherd	4.15	1	0.042	1.54	1.02, 2.33
Golden retriever	1.38	1	0.241	1.30	0.84, 2.03
Lab × Golden Cross	8.53	1	0.003[b]	1.96	1.25, 3.09
Dog rivalry					
Age at evaluation	0.06	1	0.804	0.97	0.78, 1.21
Sex = Female	0.08	1	0.783	1.04	0.78, 1.40
Breed	15.11	3	0.002[b]	–	–
German Shepherd	13.55	1	<0.0001[b]	2.10	1.42, 3.12
Golden retriever	1.08	1	0.299	1.28	0.81, 2.03
Lab × Golden Cross	4.59	1	0.032	1.64	1.04, 2.58
Stranger-directed fear					
Age = 12 months	0.001	1	0.981	1.00	0.74, 1.34
Sex = Female	0.002	1	0.962	0.96	0.65, 1.50
Breed	6.57	3	0.087	–	–
German Shepherd	2.02	1	0.156	1.48	0.86, 2.52
Golden retriever	0.55	1	0.458	0.77	0.38, 1.54
Lab × Golden Cross	2.71	1	0.100	1.61	0.91, 2.85
Touch sensitivity					
Age = 12 months	0.64	1	0.425	1.29	0.99, 1.67
Sex = Female	0.39	1	0.531	1.09	0.78, 1.53
Breed	18.71	3	<0.0001[b]	–	–
German Shepherd	15.87	1	<0.0001[b]	2.15	1.48, 3.13
Golden retriever	0.62	1	0.432	1.17	0.79, 1.75
Lab × Golden Cross	6.77	1	0.009	1.69	1.14, 2.50
Separation-related problems					
Age = 12 months	7.43	1	0.006[b]	0.74	0.59, 0.92
Sex = Female	0.67	1	0.412	0.88	0.66, 1.19
Breed	9.91	3	0.019	–	–
German Shepherd	7.60	1	0.006[b]	1.73	1.17, 2.55
Golden retriever	0.04	1	0.842	0.96	0.63, 1.45
Lab × Golden Cross	1.09	1	0.297	1.24	0.83, 1.85

(Continued)

TABLE 2 | **Continued**

C-BARQ traits and predictors[a]	Wald Chi-square	df	*p*-value	OR	95% CI
Non-social fear					
Age at evaluation	2.52	1	0.112	–	–
Age = 12 months	0.30	1	0.585	1.11	0.77, 1.60
Sex	0.12	1	0.728	–	–
Sex = Female	2.54	1	0.111	1.45	0.92, 2.30
Breed	10.74	3	0.013	–	–
German Shepherd	2.22	1	0.136	1.35	0.91, 2.00
Golden retriever	10.50	1	0.001[b]	2.37	1.41, 3.98
Lab × Golden Cross	2.35	1	0.125	1.41	0.91, 2.19
Sex (Females) × Age (12 months)	5.49	1	0.019	0.53	0.31, 0.90

[a]Results in italics are model effects, non-italicized results are parameter estimates. Reference groups were as follows: age = 6 months, sex = male, and breed = Labrador retriever.
[b]Statistically significant with Bonferroni correction for multiple comparisons (0.05/8 tests = 0.006).

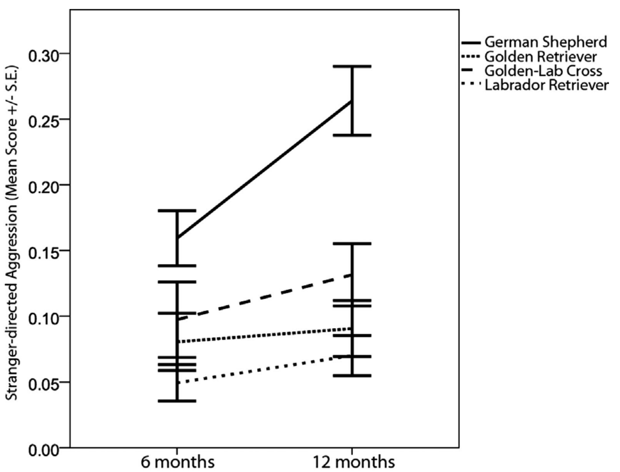


FIGURE 1 | **Maturational changes in average scores (±SE) for the C-BARQ factor "stranger-directed aggression" between 6 and 12 months in the four guide dog breeds/breed types**.

Another contributor to behavioral differences observed among dogs at 12 months of age was whether a puppy was raised in a home with other dogs. Growing up in homes with other dogs was associated with significantly lower levels of aggression directed toward household members (**Table 3**; **Figure 4**), an effect specific to other dogs in the home and not the presence of pets other than dogs. Other dogs in the household was also associated with lower levels of aggression and fear directed toward unfamiliar dogs (**Tables 3** and **4**), but these effects were not significant after corrections for multiple comparisons were made. Other pets in the household, whether dogs or pets other than dogs, were associated with improved likelihood of succeeding in training (**Table 5**).

Particular frightening or traumatic events during the puppy-raising period were associated with differences in C-BARQ scores for some behaviors. Specifically, puppies that were reported as having been attacked or threatened by another (unfamiliar) dog displayed significantly higher "dog-directed fear" and "stranger-directed aggression" at 12 months of age compared to those that had not had that experience (**Tables 4** and **5**; **Figure 5**). Likewise, puppies that were reported as having been frightened by a familiar or unfamiliar person showed significantly higher

TABLE 3 | **Results of generalized linear modeling with logit link function to examine environmental factors associated with C-BARQ aggression factors.**

C-BARQ trait and predictors	*p*-value	Odds ratio	Confidence interval	Est. marginal means
Owner-directed aggression[a]				
Number of puppies raised	<0.0001[b]			
None	–	–	–	0.41
1 or 2	0.0002[b]	0.47	0.32, 0.70	0.25
3 or 4	0.078	0.61	0.35, 1.05	0.30
5 or more	<0.0001[b]	0.29	0.16, 0.44	0.16
Other pets	<0.0001[b]			
None	–	–	–	0.34
Other dogs	<0.0001[b]	0.35	0.23, 0.54	0.015
Other pets but no dogs	0.80	1.06	0.67, 1.69	0.35
Stranger-directed aggression				
Breed	<0.0001[b]			
German Shepherd	–	–	–	0.62
Golden retriever	<0.0001[b]	0.23	0.15, 0.35	0.27
Lab × Golden Cross	<0.0001[b]	0.32	0.21, 0.48	0.34
Labrador retriever	<0.0001[b]	0.20	0.13, 0.30	0.25
Number of puppies raised	0.007			
None	–	–	–	0.41
1 or 2	0.65	0.92	0.63, 1.33	0.39
3 or 4	0.97	0.99	0.60, 1.64	0.40
5 or more	0.001[b]	0.51	0.34, 0.77	0.26
Puppy threatened by dog	0.004[b]			
No	–	–	–	0.31
Yes	0.004[b]	1.61	1.16, 2.34	0.42
Dog-directed aggression				
Breed	<0.0001[b]			
German Shepherd	–	–	–	0.60
Golden retriever	<0.0001[b]	0.23	0.14, 0.36	0.26
Lab × Golden Cross	0.004[b]	0.46	0.30, 0.071	0.41
Labrador retriever	<0.0001[b]	0.25	0.16, 0.37	0.27
Number of puppies raised	0.015			
None	–	–	–	0.46
1 or 2	0.008	0.60	0.41, 0.87	0.34
3 or 4	0.23	0.72	0.43, 1.22	0.39
5 or more	0.007	0.55	0.36, 0.85	0.32
Other dogs in the household	0.02			
No	–	–	–	0.42
Yes	0.02	0.68	0.49, 0.95	0.33
Teenagers in the household	0.03			
No	–	–	–	0.34
Yes	0.03	1.44	1.04, 2.00	0.42

[a]Breed effects were controlled for by inclusion in the model but did not reach significance.
[b]Statistically significant with Bonferroni correction (eight GLM tests with family-wise error rate of 0.05).

TABLE 4 | Results of generalized linear modeling with logit link function to examine environmental factors associated with C-BARQ fear factors.

C-BARQ trait and predictors	*p*-value	Odds ratio	Confidence interval	Est. marginal means
Stranger-directed fear[a]				
Puppy frightened by a person	<0.0001[b]			
No	–	–	–	0.08
Yes	<0.0001[b]	4.60	2.45, 8.39	0.29
Dog-directed fear[a]				
Number of puppies raised	0.05			
None	–	–	–	0.54
1 or 2	0.04	0.69	0.48, 0.99	0.45
3 or 4	0.02	0.54	0.33, 0.89	0.39
5 or more	0.07	0.68	0.47, 1.03	0.45
Puppy threatened by dog	0.003[b]			
No	–	–	–	0.40
Yes	0.003[b]	1.59	1.17, 2.18	0.51
Other dogs in household	0.06			
No	–	–	–	0.49
Yes	0.06	0.75	0.55, 1.01	0.43
Non-social fear				
Breed	0.033			
German Shepherd	–	–	–	0.78
Golden retriever	0.43	1.22	0.75, 1.97	0.81
Lab × Golden Cross	0.21	0.74	0.47, 1.18	0.72
Labrador retriever	0.04	0.65	0.43, 0.99	0.70
Number of puppies raised	0.037			
None	–	–	–	0.82
1 or 2	0.03	0.64	0.43, 0.97	0.74
3 or 4	0.06	0.60	0.35, 1.02	0.73
5 or more	0.009	0.57	0.38, 0.87	0.72
Touch sensitivity				
Breed	0.002[b]			
German Shepherd	–	–	–	0.69
Golden retriever	0.001[b]	0.48	0.31, 0.73	0.51
Lab × Golden Cross	0.35	0.81	0.52, 1.27	0.64
Labrador retriever	0.003[b]	0.55	0.37, 0.82	0.55
Number of puppies raised	<0.0001[b]			
None	–	–	–	0.73
1 or 2	0.001[b]	0.53	0.37, 0.78	0.58
3 or 4	0.005[b]	0.48	0.29, 0.80	0.56
5 or more	<0.0001[b]	0.43	0.29, 0.64	0.52
Teenagers in household	0.038			
No	–	–	–	0.56
Yes	0.04	1.39	1.02, 1.91	0.64

[a]*Breed effects were controlled for by inclusion in the model but did not reach significance.*
[b]*Statistically significant with Bonferroni correction (eight GLM tests with family-wise error rate of 0.05).*

levels of "stranger-directed fear" (**Table 4**; **Figure 6**). Having been frightened by a person was also associated with lower likelihood of successfully completing training (**Table 5**).

The presence of teenagers in the household was associated with greater tendencies for dogs to display "dog-directed aggression" and "touch sensitivity" (**Tables 4** and **5**), though these effects were no longer significant with correction for multiple comparisons.

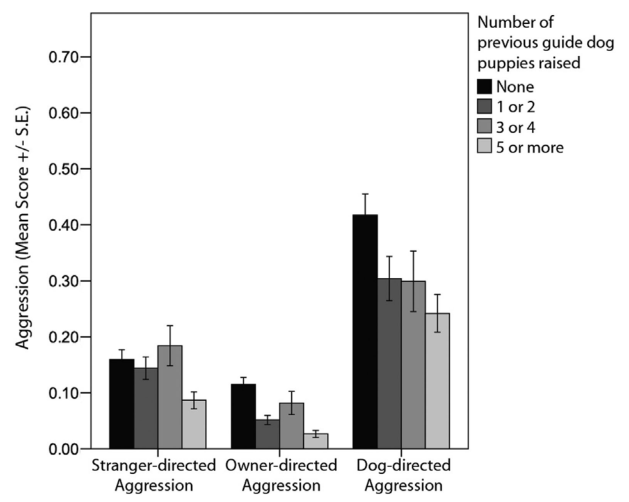


FIGURE 2 | **Comparison of average C-BARQ scores (±SE) for "stranger-directed aggression," "owner-directed aggression," and "dog-directed aggression" in relation to the number of previous guide dog puppies raised by the puppy raiser**.

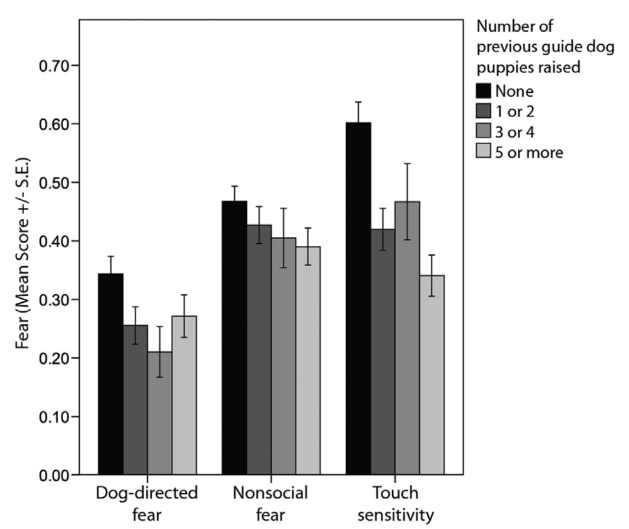


FIGURE 3 | **Comparison of average C-BARQ scores (±SE) for "dog-directed fear," "non-social fear," and "touch sensitivity" in relation to the number of previous guide dog puppies raised by the puppy raiser**.

DISCUSSION

This analysis of guide dog development has been able to partially isolate the effects of the post-socialization rearing environment from those arising from events and exposures occurring during the earlier prenatal, neonatal, and primary socialization periods. Although the conclusions that can be drawn from retrospective

TABLE 5 | Results of generalized linear modeling of environmental variables (predictors) and training outcomes.[a]

Environmental variables	*p*-value	Odds ratio	Confidence interval	Est. marginal means
Puppy ever frightened by a familiar or unfamiliar person	0.008			
No	–	–	–	0.57
Yes	0.008	0.446	0.245, 0.812	0.37
Other pets in the household	0.040			
None	–	–	–	0.38
Other dogs	0.047	1.525	1.005, 2.315	0.49
Other pets but no dogs	0.013	1.884	1.144, 3.103	0.54
Number of guide dog puppies raised[b]	0.054	1.032	0.999, 1.065	–

[a]*Training outcomes reference = released from training.*
[b]*Continuous variable.*

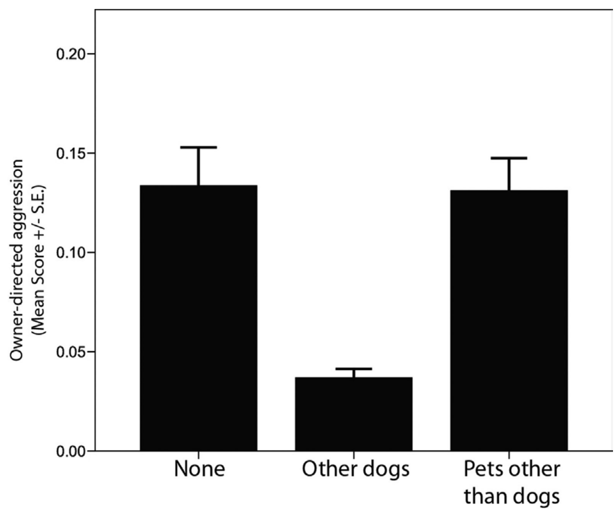


FIGURE 4 | **Comparison of average C-BARQ scores (±SE) for "owner-directed aggression" among dogs raised in households with no other pets, with other dogs, and with pets other than dogs**.

reports are inevitably somewhat limited, the findings support the view that certain events and experiences occurring during the juvenile and adolescent periods are predictive of behavioral outcomes at 12 months of age, and that different breeds appear to respond differently to these experiences. Whether such associations are restricted to particular sensitive periods, or are equally likely to occur at any time throughout this stage of development, is unknown. However, the current analysis of maturational changes suggests that some dogs are predisposed to develop aggressive reactions to unfamiliar individuals between roughly 6 and 12 months of age, and that this predisposition is stronger in the German Shepherd breed than in the other three breed types investigated. This finding is of interest in light of anecdotal reports of a phase of heightened sensitivity to territorial stimuli in wolves at around 4–5 months age [see Ref. (6–8)], and also indicates that this onset of defensive behavior is not necessarily associated with a corresponding increase in fear of strangers.

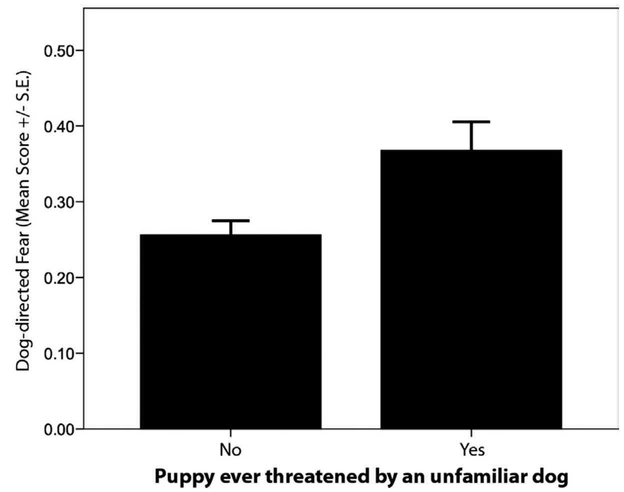


FIGURE 5 | **Comparison of average C-BARQ scores (±SE) for "dog-directed fear" in guide dogs reported to have been threatened by an unfamiliar dog**.

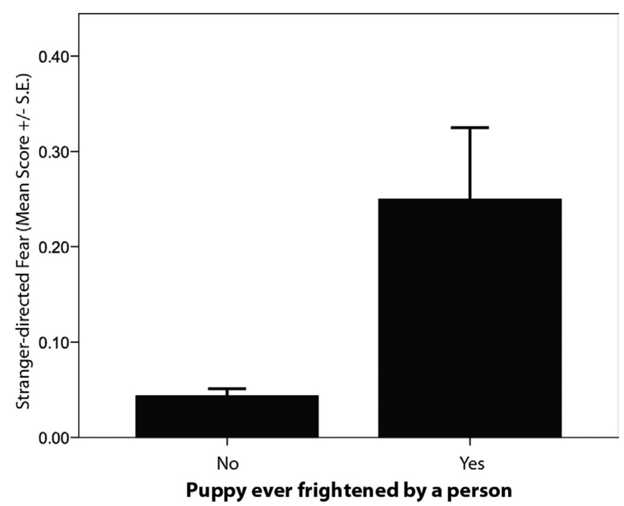


FIGURE 6 | **Comparison of average C-BARQ scores (±SE) for "stranger-directed fear" in guide dogs reported to have been frightened by a person**.

This implies that the aggressive element of defensive/territorial behavior develops separately from the fearful component, and suggests that such fears may become established earlier in life. Since owner-directed aggression decreases in all breeds over the same period, the findings further suggest that the rise in stranger-directed aggression in German Shepherds is not due to an overall increase in aggressiveness, but rather a specific response to unfamiliar persons. These results also reinforce the view that it is the *interaction* between a dog's genetic background and its developmental environment that ultimately determines the adult behavioral phenotype.

With respect to developmental effects of specific events and exposures during this period, the findings illustrate the important role that the puppy-raiser's puppy-rearing experience plays in determining behavioral outcomes, as well as the value of social exposure to other dogs in the household. Both of these variables are potentially modifiable either through education, in the case of experience, or through the deliberate recruitment of puppy raisers from among existing experienced dog owners, and such efforts or interventions would be likely to have a significant positive impact on the performance of future working dogs, especially for those breeds that are predisposed to developing problems.

The results also draw attention to the apparent sensitivity of dogs at this age to the effects of traumatic events or experiences, such as being frightened by a person, or being threatened or attacked by another dog. Such findings reinforce the view that puppies and young dogs are sensitive to aversive social encounters long after the ostensible end of the socialization period (i.e., 12 weeks), and that such encounters may have long-term negative consequences for behavior. Unfortunately, because of the correlational nature of these outcomes, it is not possible to determine whether these dogs became more fearful and/or aggressive as a direct consequence of their experiences or if they were more likely to be traumatized by an aversive encounter due to a pre-existing disposition toward fearfulness.

While some of the current results are unsurprising – for example, the reduced scores for the C-BARQ variables dog-directed fear and dog-directed aggression among puppies reared with other dogs – others are less easy to interpret. The reduction in "owner-directed aggression" in multi-dog households could perhaps be attributed to puppies developing enhanced social skills as a consequence of entering households at the bottom of established hierarchies. A study of pet dogs in Taiwan also found lower rates of owner-directed aggression among dogs living in multi-dog households (13), and an early study of guide dog puppies found that those reared in homes with other dogs were less distracted by other unfamiliar dogs when tested on walks at 6 and 12 months of age (14). By contrast, an Australian study of GSDs found that the presence of another dog in the household had a deleterious effect on dogs' scores on tests of fearfulness, social attraction and "dominance" (15), while a recent study of guide dog puppies in the UK found a positive association between puppies' ratings for energy level and distractibility and the number of dogs in the puppy-raising household (16).

With respect to puppy-raiser experience, these results are in agreement with those of previous studies that found that pet dogs belonging to first-time dog owners were more likely to display owner-directed aggression than those of more experienced owners (17, 18). One interpretation of such findings is that dog owners learn from experience how to prevent the development of some canine behavior problems, and that they get better at doing this with each successive dog owned. Similar effects may also account for the reduced energy and distractibility ratings of puppies reared by more experienced "puppy walkers" in a recent UK study of guide dogs (16). It is also possible that puppy-raising experience enhances owners'/handlers' ability to "read" and react to canine emotions. Previous research has shown, for example, that increasing levels of experience with dogs is associated with improved human ability to recognize canine emotions, such as fear (19).

Most previous studies of canine behavioral development have tended to focus exclusively on the traditional 3- to 12-week socialization period, and this has perhaps discouraged investigators from exploring the possible consequences of later exposure to biologically relevant events and stimuli. Further investigations that emphasize the precise timing and quality of experiences within this developmental period would be likely to yield valuable information regarding appropriate husbandry practices for dogs of this age.

ETHICS

The study was approved by the Privately-owned Animals Subcommittee of the University of Pennsylvania Institutional Animal Care and Use Committee. The guide dogs and the survey data that were used in this study were owned by The Seeing Eye, Inc., Morristown, NJ, USA. This organization provided written consent for the use of all survey data pertaining to these dogs.

AUTHOR CONTRIBUTIONS

JS initiated the research, proposed the idea, and drafted the paper. DD performed the analysis, wrote up the results, and edited the paper.

ACKNOWLEDGMENTS

This work was supported by a grant from the Arthur L. "Bud" Johnson Foundation. We much appreciate the assistance of the director and staff at The Seeing Eye, Inc. without whose help the study would not have been possible.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fvets.2016.00049

REFERENCES

1. Scott JP, Fuller JL. *Genetics and the Social Behavior of the Dog*. Chicago: University of Chicago Press (1965).
2. Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp Neurol* (2012) 233:102–11. doi:10.1016/j.expneurol.2011.10.032
3. Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. *Front Neuroendocrinol* (2005) 26:163–74. doi:10.1016/j.yfrne.2005.10.003
4. Miklósi A. *Dog Behaviour. Evolution, and Cognition*. Oxford: Oxford University Press (2007).
5. Dehasse J. Sensory, emotional and social development of the young dog. *Bull Vet Clin Ethol* (1994) 2:6–29.
6. Fentress JC. Observations on the behavioral development of a hand-reared male timber wolf. *Am Zool* (1967) 7:339–51. doi:10.1093/icb/7.2.339
7. Fox MW. *Behavior of Wolves, Dogs and Related Canids*. New York: Harper and Row (1971).
8. Mech LD. *The Wolf: the Ecology and Behavior of an Endangered Species*. New York: Natural History Press (1970).

9. Foyer P, Bjällerhag N, Wilsson W, Jensen P. Behaviour and experiences of dogs during the first year of life predict the outcome in a later temperament test. *Appl Anim Behav Sci* (2014) 155:93–100. doi:10.1016/j.applanim.2014.03.006
10. Francis DD, Diorio J, Plotsky PM, Meaney MJ. Environmental enrichment reverses the effects of maternal separation on stress reactivity. *J Neurosci* (2002) 22(18):7840–3.
11. Hsu Y, Serpell JA. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *J Am Vet Med Assoc* (2003) 223:1293–300. doi:10.2460/javma.2003.223.1293
12. Duffy DL, Serpell JA. Predictive validity of a method for evaluating temperament in young guide and service dogs. *Appl Anim Behav Sci* (2012) 138:99–109. doi:10.1016/j.applanim.2012.02.011
13. Hsu Y, Sun L. Factors associated with aggressive responses in pet dogs. *Appl Anim Behav Sci* (2010) 123:108–23. doi:10.1016/j.applanim.2010.01.013
14. Goddard ME, Beilharz RG. Factor analysis of fearfulness in potential guide dogs. *Appl Anim Behav Sci* (1984) 12:253–65. doi:10.1016/0168-1591(84)90118-7
15. Hoffmann G. (2002). *Puppy Tests: An Evaluation of Their Predictive Validity*. Unpublished PhD thesis, University of Queensland, Australia.
16. Harvey ND, Craigon PJ, Blythe SA, England GCW, Asher L. Social rearing environment influences dog behavioral development. *J Vet Behav* (2016). doi:10.1016/j.jveb.2016.03.004
17. Jagoe JA, Serpell JA. Owner characteristics and interactions and the prevalence of canine behavior problems. *Appl Anim Behav Sci* (1996) 47:31–42. doi:10.1016/0168-1591(95)01008-4
18. Kobelt AJ, Hemsworth PH, Barnett JL, Colemen GJ. A survey of dog ownership in suburban Australia – conditions and behavior problems. *Appl Anim Behav Sci* (2003) 82:137–48. doi:10.1016/S0168-1591(03)00062-5
19. Wan M, Bolger N, Champagne FA. Human perception of fear in dogs varies according to experience with dogs. *PLoS One* (2012) 7(12):e51775. doi:10.1371/journal.pone.0051775

Conflict of Interest Statement: The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The copyright for the C-BARQ instrument that was used as the source of behavioral information in the current study is owned jointly by the corresponding author (JS) and the University of Pennsylvania.

frontiers in Veterinary Science

HYPOTHESIS AND THEORY
published: 24 June 2016
doi: 10.3389/fvets.2016.00047

Canine Detection of the Volatilome: A Review of Implications for Pathogen and Disease Detection

Craig Angle[1], Lowell Paul Waggoner[1], Arny Ferrando[2], Pamela Haney[1] and Thomas Passler[3]*

[1] Canine Performance Sciences Program, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2] Center for Translational Research in Aging and Longevity, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [3] Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

The volatilome is the entire set of volatile organic compounds (VOC) produced by an organism. The accumulation of VOC inside and outside of the body reflects the unique metabolic state of an organism. Scientists are developing technologies to non-invasively detect VOC for the purposes of medical diagnosis, therapeutic monitoring, disease outbreak containment, and disease prevention. Detection dogs are proven to be a valuable real-time mobile detection technology for the detection of VOC related to explosives, narcotics, humans, and many other targets of interests. Little is known about what dogs are detecting when searching for biological targets. It is important to understand where biological VOC originates and how dogs might be able to detect biological targets. This review paper discusses the recent scientific literature involving VOC analysis and postulates potential biological targets for canine detection. Dogs have shown their ability to detect pathogen and disease-specific VOC. Future research will determine if dogs can be employed operationally in hospitals, on borders, in underserved areas, on farms, and in other operational environments to give real-time feedback on the presence of a biological target.

Keywords: volatilome, volatile organic compound, canine detection, disease detection

OPEN ACCESS

Edited by:
Cynthia M. Otto, University of Pennsylvania, USA

Reviewed by:
George Preti, Monell Chemical Senses Center, USA
Allen Goldblatt, Retired, formerly affiliated with Tel Aviv University, Israel

****Correspondence:***
Craig Angle
angletc@auburn.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *25 February 2016*
Accepted: *03 June 2016*
Published: *24 June 2016*

Citation:
Angle C, Waggoner LP, Ferrando A, Haney P and Passler T (2016) Canine Detection of the Volatilome: A Review of Implications for Pathogen and Disease Detection. Front. Vet. Sci. 3:47. doi: 10.3389/fvets.2016.00047

INTRODUCTION

All living things are susceptible to pathogens and diseases. Scientists have been investigating technologies that aid in early detection, therapeutic monitoring, and prevention of transmission of pathogens and diseases. Recent developments in the field of volatilomics have resulted in novel, emerging technologies that identify pathogens or disease states using characteristic chemical vapor emission patterns.

Dogs are a mobile real-time detection technology that identify targeted chemical vapor profiles. Dogs have been used in operational environments for years as real-time chemical detectors of explosives, narcotics, accelerants, people, animals, and other targets of interest. Dogs possess an extremely sensitive sense of smell, with a demonstrated lower limit of detection at concentrations of one part per trillion (ppt) (1), which is three orders of magnitude more sensitive than today's available instruments, which can reliably identify substances at concentrations as low as parts per million (ppm) or billion (ppb). To illustrate the tremendous canine olfactory sensitivity, a dog could detect the equivalent of one drop of a liquid in 20 Olympic-size (2500 ft^3) swimming pools.

Their powerful sensory system allows dogs to detect diseases, pathogens, cadavers, lost/criminal persons, and other biological targets. However, much more research needs to be conducted to understand what exactly the dogs are detecting.

Advancements in analytical chemistry in recent years have made it possible to quantify and compare volatile organic compounds (VOC) of cellular origin. VOC are low molecular weight compounds that easily evaporate at normal temperatures and pressure (2). All odorants are VOC, and advancements in VOC analysis have provided a foundation to begin to understand what dogs are detecting in regard to biological targets. Current VOC analysis requires sophisticated stationary analytical chemical instrumentation, such as liquid/gas chromatography–mass spectrometry. These instruments are valuable for the discovery of biological VOC and to identify specific VOC, but unlike dogs, instruments are typically confined to a laboratory, unable to detect VOC in real time, and do not have the capability to track the odor to its source. Diagnostic testing using laboratory instrumentation in an operational environment (e.g., mass transit, cargo, agriculture) is often impeded by the lack of cleanliness, interference by air particulates, presence of non-target VOC produced by various substances in the environment, and the constantly changing variables, such as temperature, humidity, wind, and thermal plumes. Rapid advancements will increase the analytical sensitivity of available instrumentation; however, current instruments are only capable of accurately analyzing compounds in the ppb range (3). In operational environments, there is a need for real-time detection of static and dynamic targets. Analytical instruments cannot fill this capability gap without significant improvements in portability, sensitivity, user knowledge, and the ability to trace an odor to its source.

Dogs provide many force-multiplying advantages in operational environments. Dogs can scan large areas efficiently, which is important for detecting pathogens in large herds of animals, in crowds of people, on or in objects (e.g., ships, airplanes, buildings), or around areas of land. Therefore, from an operational perspective, dogs are a superior technology offering a highly sensitive, real-time sensory system on an intelligent mobile platform without the need to collect, process, or analyze samples, which gives them significant advantages over machines in operational environments. This review paper will discuss cited literature and postulate potential targets of canine detection. Furthermore, we hope to stimulate thought about future advances and discoveries in canine detection of pathogens and diseases. The scope of this review is not to discuss the identity of specific VOC, but to discuss the concepts of VOC identification related to canine detection. Other research and review articles such as by de Lacy Costello et al. (4) have described specific VOC found in diseases and pathogens.

THE VOLATILOME

Physiological processes of all organisms produce metabolic products, including VOC (5). Production of cellular VOC occurs in millions of cells simultaneously, thus potentially releasing extracellular VOC on a detectable scale. These VOC enter the blood stream and are then released into the air around a human, animal, or plant. The mechanism of the release is through breath, urine, feces, skin emanations, and blood (6). The volatilome is the entire set of VOC produced by an organism. The volatilome is the accumulation of VOC in an organism, and the VOC reflect its unique metabolic state. Animals, humans, insects, and even plants can detect VOC that are released from an organism. For example, Bicchi and Maffei (7) stated that the plant volatilome is involved in critical processes, including plant–plant interactions, the signaling between symbiotic organisms, the attraction of pollinating insects, and a range of biological activities in mammals. Recent research demonstrated that the volatilome can be used to detect disease, pathogens, and many other unique aspects of an organism. VOC are released in concentrations of ppb to ppt in human breath, and ppm to ppb in human blood and urine (3). Dogs were demonstrated to detect *n*-amyl acetate in the ppt range (8), indicating that most of the VOC in the volatilome are within a dog's capabilities of detection.

Volatile organic compounds are emitted constantly from the human body and consist of hundreds of VOC secreted from cells as a result of metabolic processes (9). Amman et al. (10) stated that more than 1000 VOC appear in exhaled breath, skin emanations, urine, saliva, or feces. de Lacy Costello et al. (4) reported that VOC were identified and assigned in breath (872), saliva (359), blood (154), milk (256), skin secretions (532), urine (279), and feces (381) in apparently healthy individuals. Besa et al. (11) stated that VOC may be products of various inflammatory and metabolic processes, either physiological or disease related, that take place in the airways and other parts of the human body, or products of the oxidative stress that occur in disease states. Variations in these compounds between individuals are great and concentrations may depend on several factors, such as metabolism, differences in lung, or systemic physiologies (11). Schmidt and Podmore (3) stated that VOC patterns differ between individuals because of uncontrolled variables, such as genetic differences, environmental settings, diet, drug ingestion, and smoking. There are many different physiological processes that influence the detectable VOC profile. However, not all VOC released from the body are related to human metabolism. Many VOC are related to the commensal microbiota or from microbial infections. For example, bacteria present in the mouth, lungs, and digestive tract are potent producers of VOC (12). Other VOC may be from foods, drinks, medications, personal hygiene products, and pollutants that are ingested, inhaled, or absorbed through the skin. Thus, the volatilome is a complex combination of endogenous and exogenous VOC, and care should be taken not to label exogenous compounds as disease biomarkers.

The source of endogenous VOC that are part of the volatilome are diverse metabolic processes occurring simultaneously in the various cell types that shape an organism. It is estimated that the average human body contains 37.2 trillion cells (13) that simultaneously conduct metabolic processes, releasing specific VOC unique to the particular type of cell and cellular process. Differences in VOC expression patterns may result from genetic variation among individuals. Aksenov et al. (5) demonstrated that human leukocyte antigen (HLA) alleles can directly influence production of specific volatile compounds at the cellular level. The authors stated that the resulting odor fingerprint depends on the expression of specific HLA sequences, and as a result, a unique VOC pattern or "odorprint" is formed. This specific odorprint is the result of downstream intracellular metabolic pathways that have been influenced by a particular allele. This highly specific and unique VOC profile, derived by metabolic changes at the cellular level, may be useful for imprinting and reliable detection by a properly trained canine. More research is needed to determine if a canine's olfactory capability can accurately distinguish between complicated VOC profiles of similar diseases, despite the likelihood of many overlapping compounds.

Following intracellular production of the VOC, the VOC may transverse the cellular wall and enter into the blood stream. The blood stream represents the main means of communication with different parts of the body and as such collects information on the metabolic, nutritional, and immunological status of an organism (9). The secreted VOC are transported with the blood to various organs where they are off-loaded. For example, when the blood travels to the lungs, some of the VOC are removed and exhaled in the breath. Other VOC are removed in the kidneys and released in the urine, while VOC released in the feces are a direct reflection of the endpoint of many excretory and secretory processes in the organism (9). Some VOC are secreted through the skin *via* the sweat glands and released from the gut. These VOC either enter the environment directly or are altered by the microbiome and released as altered VOC. de Lacy Costello et al. (4) stated that skin is not homogeneous and the distribution of the different types of glands and bacterial flora across the body can be expected to lead to different VOC profiles. Furthermore, the odors of a single individual vary over time though diet, emotional state, menstrual cycle, age, and many others factors. Therefore, once VOC are produced by cells, they enter the blood stream or intestines and take multiple paths out of the body and into the environment. Once in the environment, the VOC surround the body and may be detected as a unique individual volatilome that allows dogs to detect the biological target. Currently, the field of volatilomics is in its infancy, and the biochemical mechanisms behind the release of disease-specific VOC is largely unknown (3, 9, 14). It is not known if unrelated diseases can produce identical VOC profiles. If disease A and disease B result in identical metabolic changes to a cell, then identical VOC profiles may be released. In this case, false-positive indication by a trained dog would reduce the diagnostic specificity and hamper operational usefulness. Therefore, canine detection research must use disease distractors that cause similar metabolic changes in infected cells (15).

BIOMEDICAL DETECTION

Recent research interest revolved around the ability of dogs to detect cancer in real time. Working dogs have been successfully used in the detection of other biomedical targets, such as hypoglycemic episodes in diabetic people. Petry et al. (16) stated that glucose-detecting dogs are potentially effective in the detection of diabetes in children. Studies demonstrated that tissues or systems infected with pathogens or affected by disease release unique VOC, which become part of detectable odor signatures (9, 14). For example, Jezierski et al. (17) stated that aberrant protein synthesis and changed metabolisms in cancer cells produce VOC that are likely to have distinctive odors detectable by highly sensitive analytical devices.

Unique Pathogenic and Disease State VOC

Multiple studies demonstrated that VOC patterns may be specific to diseases or pathogen-specific infection. Abd El Qader et al. (18) demonstrated that metabolic changes in viral and bacterial microbiological cultures are associated with significant VOC released by the pathogens, providing sample fingerprints for both its identity and existence. Schmidt and Podmore (3) stated that different patterns of VOC expression are associated with diseases, such as cancer, asthma, cystic fibrosis, diabetes, tuberculosis, chronic obstructive pulmonary disease, heart allograph rejection, and irritable bowel syndrome. The authors further described that these VOC expression patterns may be caused by pathological processes that generate new VOC not produced during normal physiological processes and/or altered VOC concentrations. Therefore, the production of new VOC or alteration of the VOC expression pattern may serve as a biomarker for the assessment or detection of disease by dogs.

In a review of 31 publications, Bos et al. (19) concluded that many pathogenic bacteria have distinct metabolisms that produce species-specific VOC and suggested that the presence of these VOC in patients indicated infection. In a cell culture model, Schivo et al. (20) demonstrated different VOC expression patterns in primary human tracheobronchial cells infected or uninfected with human rhinovirus. Aksenov et al. (21) determined that VOC produced by B lymphoblastoid cells following infection with three live influenza virus subtypes were unique for each virus subtype. In addition, Abd El Qader et al. (18) examined the VOC released from cultures of five viruses (influenza A, influenza B, adenovirus, respiratory syncitial virus, and parainfluenza 1 virus) and four bacteria (*Moraxella catarrhalis*, *Haemophilus influenza*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*). The researchers detected 12 and 6 VOC that were associated with bacterial and viral growth, respectively, and identified 2 VOC that differentiated bacterial and viral infection (18). Lastly, Mashir et al. (22) administered live attenuated H1N1 vaccine (FluMist®) to humans and demonstrated that, in exhaled breath, VOC increased for 7 days after the vaccination. If pathological processes, such as infections, neoplasia, and metabolic disorders, influence the type, ratio, and strength of VOC emitted from an organism, then unique VOC patterns may create a specific signature odor (23) for the dog to detect.

Dogs as a Pathogen and Disease Sensor

Detection dogs are able to detect cancer by sampling breath, feces, urine, blood, and tissue. In some cases, dogs were able to detect disease states in exhaled breath, which contains the lowest known VOC concentrations of the volatilome (3). Sonoda et al. (24) trained a dog to examine human patients with colon cancer using samples of exhaled breath and watery stool. This dog's sensitivity and specificity for cancer detection in breath samples was 0.91 and 0.99, respectively. The sensitivity and specificity for detection in stool samples was 0.97 and 0.99, respectively. Another study demonstrated that the overall sensitivity of canine scent detection of lung cancer utilizing exhaled breath samples was 0.99, with a specificity of 0.99 (25). In the same report utilizing trained dogs to evaluate breath samples from breast cancer patients and controls, the sensitivity of detection was 0.88 and specificity was 0.98 (25). In addition, Angle et al. (15) demonstrated that dogs could detect bovine viral diarrhea virus and discriminate it from bovine herpesvirus 1 and bovine parainfluenza virus 3, in cell cultures. The diagnostic sensitivity and specificity in this study was 85 and 96% and 98 and 99% for each of the two dogs, respectively. These reports suggest that VOC or similar compounds from diseased internal tissues and cell cultures are released externally and may be detected in the volatilome with trained dogs with a high degree of diagnostic accuracy.

Purpose-bred detection dogs have a demonstrated ability to search for unique odor patterns and identify specific targets in field conditions amidst substantial "odor noise" (i.e., varied and/or strong odors). Although at least 381 unique VOC are emitted from human feces (6), a trained dog was able to detect *Clostridium difficile* in human stool samples (26). The dog detected *C. difficile* with high diagnostic sensitivity and specificity in stool samples and hospitalized patients, correctly identifying 25 of the 30 *C. difficile* cases and 265 of 270 control cases (26). This study emphasizes a dog's ability to detect a specific odor pattern among the myriad of odors from other bacteria, fungi, and viruses, naturally present in feces, and emphasis its ability to function operationally, as the dog was working in a hospital room and successfully found *C. difficile*. In another article, Alasaad et al. (27) trained two detector dogs to follow the scent of sarcoptic-infected live animals and to find carcasses in the Italian Alps. The authors concluded that properly trained disease-detector dogs are an efficient and straightforward tool for surveillance and control of sarcoptic mange in affected wild animal populations. These two studies show that dogs have the capabilities to detect disease targets in an operational setting among environmental odor noise (i.e., not in an odor sterile laboratory environment).

Most of the studies mentioned in this section only used one or two dogs in simple target distractor arrangements. Moser and McCulloch (28) reviewed the methods and accuracy of studies of canine scent detection of human cancers. The authors noted that variability and inadequacies in reported training methods, target and control sample preparation and presentation, utilized dog numbers, and a paucity of peer-reviewed articles made it difficult to assess the general capability of trained dogs as a diagnostic tool for the detection of cancer. Further research with more dogs and more complex targets and distractor arrangements is crucial to determine the feasibility of dogs as operational biological detectors.

Disease Detection by Free-ranging Predators

From a comparative biology standpoint, there may be much that can be learned from the detection dog model about the behavior of free-ranging predators. With the knowledge that disease and pathogen detection is within the canine olfaction capability and that diseases can be detected outside the body, the question arises in which other ways animals might utilize this extraordinary sensory capability in the wild. Do animals learn to hunt prey that is diseased or stressed based on VOC biomarkers? Animals that hunt herd animals often stalk their prey and select slow and diseased animals, prompting the question: are they using their olfaction capabilities to find potentially diseased animals? This is not a farfetched idea as the dogs cited in the research papers, in this review, were rewarded with a toy or food for identifying the target, in the wild they are rewarded with the satisfaction of a meal. Considering an animal might feed daily or weekly, there is considerable opportunity to learn patterns (olfaction, auditory, and vision) of successful kills. Therefore, it is possible that animals learn with experience from the prey that they kill and that there are particular odors associated with animals they can catch and do not catch. Future research utilizing detection dog models could give a better understanding of how animals might use their sense of smell to identify the volatilome of weak and diseased animals for hunting.

VOC MOVEMENT OUTSIDE THE BODY

There is no published literature on the movement of the odorous VOC. The human thermal plume and human aerodynamic wake may serve as a model for the movement of the detectable odorous volatilome. Craven and Settles (29) stated that knowledge of the behavior and underlying physics of the human thermal plume is essential to the study of contaminant transport from people and the understanding of the entrainment of respirable particles into the human breathing zone. It is important to determine if the detectable odorous volatilome moves around the body and away from the body, as future discoveries may show that dogs could sense biological VOC better in certain regions around the body for the purposes of non-invasive sampling.

When a human body and an environment differ in temperature, a temperature gradient forms in the surrounding air that drives buoyant convection about the human. Experiments and computational fluid dynamics simulations demonstrated that the maximum plume velocity is about 0.2–0.3 m/s and plume flow rates are in the 20–80 L/s range, depending on activity levels, height above the human subject, and characteristics of the indoor environment (30). Entrapped in these convective currents are VOC, pathogens, and even skin cells. For example, Craven and Settles (29) described that exfoliated human skin is the most prevalent and transported particulate in the thermal plume. On average, a complete layer of skin is shed every 1–2 days, releasing a million skin cells per minute. Most inhaled air comes from the human boundary layer that contains these particles, from which 6000 to 50,000 particles/L of air enter the human nose. Jia et al. (31) demonstrated that the thermal plume was capable of dispersing solid particles (5 μm in diameter) deposited on the floor by the legs. The particles traveled up the body, over the head, and into the area above the head toward the ceiling of a small room. Therefore, transportation of VOC by the thermal plume, which moves VOC around and away from the body, is plausible and likely. This hypothesized movement of VOC should be considered and verified, as it could lead to concentrated sampling positions for non-invasive canine detection.

Craven and Settles (29) described the human thermal plume as a potential whole-body chemical trace sampling system. "If the plume is collected and sampled, its contaminant burden may be analyzed for various purposes, including medical diagnosis and substance detection." That study referred to the thermal plume of static subjects in a sitting position and not dynamic subjects walking about. Walking subjects change the aerodynamics of the volatilome and, ultimately, the search technique and sampling positions of the dog. As a person walks, air streams behind them in their wake. Craven et al. (30) described that the overall effect of free convection about the body is diminished and the human thermal plume bends over at an angle with respect to the flow direction. As free-stream velocity increases (as a person walks faster), the flow about the body becomes dominated by horizontal forced convection and the temperature difference between the body and the air ceases to be relevant. At this point, the term "human thermal plume" is dropped and the flow now is called the "human aerodynamic wake." The authors described the human aerodynamic wake as the unsteady airflow downstream of a walking person (or a motionless person standing in a breeze). This is an important concept, as, in theory, the volatilome may stream behind the body in this wake and be available for detection by the dog. More research into static and dynamic subjects needs to be conducted in order to understand VOC concentration zones within the volatilome for the purposes of identifying specific canine search patterns, which target optimal VOC zones for non-invasive detection.

Dogs search the volatilome in two main ways, as static objects in containers (e.g., urine sample head space) in a laboratory environment or by actively searching for a target in an operational environment (e.g., trailing a human subject). It is important to understand the movement of the detectable odorous volatilome in order to maximize a dog's capability to detect the desired target. The fluid dynamics of the human thermal plume and aerodynamic wake of both organisms and inanimate objects in conjunction with wind currents dictate optimal micro- and macro-search patterns for canine detection. Micro air currents occur immediately around the target, while macro currents occur downstream from the target. The dog uses odor pools and air currents (which contain odor) to follow target odor back to its source. The air currents in conjunction with the geometry and surface texture of surrounding objects influence the behavioral characteristics of the target odor. The behavioral characteristics of the target odor directly influence the search pattern of the dog and, ultimately, the training of the dog to enhance its macro- and micro-search patterns in the target odor zone. Much more research needs to be conducted in order to understand the movement of biological VOC within the thermal plume (e.g., micro currents) and in the aerodynamic wake/wind currents in order to develop search patterns to optimize biomedical detection.

CONCLUSION

Dogs and analytical chemical instrumentation have verified that pathogens and diseases produce unique VOC signals, which can be detected outside the body. There are many uses for dogs in the detection of pathogens and diseases because of their outstanding mobility and sensory capacity. Increasing knowledge about the detectable odorous volatilome will aid to train dogs to detect specific biomedical targets. By uncovering volatilome characteristics and behaviors, productive search areas and targets will be identified. This will help increase the portfolio of operational uses for detection dogs. Conversely, studying the VOC signatures that control the detection responses of dogs to particular pathogens may inform the design of detection instrumentation.

There are many potential operational uses for detection dogs to detect biological targets. Further understanding of the volatilome will lead to discoveries of how to utilize the dog's highly sensitive detection capabilities. Below is a list of potential uses of detection dogs and areas that need further research:

- Dogs could be used in fields such as analytical chemistry to identify trace materials in compounds that currently cannot be quantified due to a lack of instrument sensitivity. This could lead to new discoveries and challenges in chemical analysis.
- Dogs could be utilized as a model for sensor development for mobile real-time sensors in operational environments.
- Dogs could be used in underserved areas to identify crop, livestock, and human diseases. These applications would enhance food security, biosecurity, and prevention and containment of bio- and agro-terrorism.
- Dogs could be used in hospitals to identify pathogens and diseases (e.g., methicillin-resistant *Staphylococcus aureus*) that could spread throughout the hospital.
- Dogs could be used as point-of-care diagnostics for plants, humans, and animals to detect pathogens and diseases that currently do not have an available real-time diagnostic test.
- Dogs could be used to establish disease outbreak containment zones where they could screen "things" (i.e., people, vehicles, items) coming out of the containment zone.

The dog's sensory system affords it the ability to detect biological targets. The biological targets are very complex and their detection is intricate. There are challenges faced by the canine industry because of the wide range of performance capabilities by the dogs in biological detection. One of the main challenges is how to employ the appropriately trained canines for the task. Researchers should use purpose-bred dogs for detection and select dogs with highly sensitive olfactory capabilities, innate search techniques optimal for biological detection, and calculated methodical skill sets. Researchers should utilize highly skilled professional trainers with an in-depth understanding of detection dog behaviors. There are intricacies in training dogs on biological targets that are complex and unlike any skill set utilized in other aspects of dog training. A detection dog is a highly complex sensory technology, and not understanding its full capabilities and its influences could skew study results to not reflect the true potential of a dog. Lastly, researchers should carefully consider biological target research methodologies to reduce specificity issues and the inflation of sensitivities that are not related to the target odor. Future research should address the above issues.

Studies demonstrated that the VOC present in the volatilome are well within the detection capability of the dog. Dogs have shown their ability to detect pathogen and disease-specific VOC. Future research will determine if dogs can be employed operationally in hospitals, on borders, in underserved areas, and in counter-terrorism operations to give real-time feedback on the presence of biological targets.

AUTHOR CONTRIBUTIONS

CA, LW, AF, PH, and TP developed the literature review idea and need for this knowledge within the industry. All authors co-wrote the manuscript, and read and approved the final manuscript.

FUNDING

Financial support for this review article was provided by the Richard G. and Dorothy A. Metcalf Endowment at the Auburn University College of Veterinary Medicine.

REFERENCES

1. Walker DB, Walker JC, Cavnar PJ, Taylor JL, Pickel DH, Hall SB, et al. Naturalistic quantification of canine olfactory sensitivity. *Appl Anim Behav Sci* (2006) 97(2):241–54. doi:10.1016/j.applanim.2005.07.009
2. Hung R, Lee S, Bennett JW. Fungal volatile organic compounds and their role in ecosystems. *Appl Microbiol Biotechnol* (2015) 99(8):3395–405. doi:10.1007/s00253-015-6494-4
3. Schmidt K, Podmore I. Current challenges in volatile organic compounds analysis as potential biomarkers of cancer. *J Biomark* (2015) 2015:981458. doi:10.1155/2015/981458
4. de Lacy Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, et al. A review of the volatiles from the healthy human body. *J Breath Res* (2014) 8(1):014001. doi:10.1088/1752-7155/8/1/014001
5. Aksenov AA, Gojova A, Zhao W, Morgan JT, Sankaran S, Sandrock CE, et al. Characterization of volatile organic compounds in human leukocyte antigen heterologous expression systems: a cell's "chemical odor fingerprint". *Chembiochem* (2012) 13(7):1053–9. doi:10.1002/cbic.201200011
6. Amann A, Costello B, Miekisch W, Schubert J, Buszewski B, Pleil J, et al. The human volatilome: volatile organic compounds (VOC) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res* (2014) 8:034001. doi:10.1088/1752-7155/8/3/034001
7. Bicchi C, Maffei M. The plant volatilome: methods of analysis. *High-Throughput Phenotyping in Plants Methods in Molecular Biology*. (2012). p. 289–310.
8. Walker DB, Walker JC, Cavnar PJ, Taylor TL, Pickel DH, Hall SB, et al. Naturalistic quantification of canine olfactory sensitivity. *Appl Anim Behav Sci* (2006) 97(2):241–54. doi:10.1016/j.applanim.2005.07.009
9. Buljubasic F, Buchbauer G. The scent of human diseases: a review on specific volatile organic compounds as diagnostic biomarkers. *Flavour Fragrance J* (2015) 30:5–25. doi:10.1002/ffj.3219
10. Amann A, Costello B, Miekisch W, Schubert J, Buszewski B, Pleil J, et al. The human volatilome: volatile organic compounds (VOC) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res* (2014) 8:034001. doi:10.1088/1752-7155/8/3/034001
11. Besa V, Teschler H, Kurth I, Khan AM, Zarogoulidis P, Baumbach JI, et al. Exhaled volatile organic compounds discriminate patients with chronic obstructive pulmonary disease from healthy subjects. *Int J Chron Obstruct Pulmon Dis* (2015) 10:399–406.
12. Boots AW, Bos LD, van der Schee MP, van Schooten FJ, Sterk PJ. Exhaled molecular fingerprinting in diagnosis and monitoring: validating volatile promises. *Trends Mol Med* (2015) 21(10):633–44. doi:10.1016/j.molmed.2015.08.001
13. Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol* (2013) 40(6):463–71. doi:10.3109/03014460.2013.807878
14. Wilson AD. Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath. *Metabolites* (2015) 5:140–63. doi:10.3390/metabo5010140
15. Angle TC, Passler T, Waggoner PL, Fischer TD, Rogers B, Galik PK, et al. Real-time detection of a virus using detection dogs. *Front Vet Sci* (2016) 2:79. doi:10.3389/fvets.2015.00079
16. Petry NM, Wagner JA, Rash CJ, Hood KK. Perceptions about professionally and non-professionally trained hypoglycemia detection dogs. *Diabetes Res Clin Pract* (2015) 109(2):389–96. doi:10.1016/j.diabres.2015.05.023
17. Jezierski T, Walczak M, Ligor T, Rudnicka J, Buszewski B. Study of the art: canine olfaction used for cancer detection on the basis of breath odour. Perspectives and limitations. *J Breath Res* (2015) 9(2):027001. doi:10.1088/1752-7155/9/2/027001
18. Abd El Qader A, Lieberman D, Shemer Avni Y, Svobodin N, Lazarovitch T, Sagi O, et al. Volatile organic compounds generated by cultures of bacteria and viruses associated with respiratory infections. *Biomed Chromatogr* (2015) 29(12):1783–90. doi:10.1002/bmc.3494
19. Bos LDJ, Sterk PJ, Schultz MJ. Volatile metabolites of pathogens: a systematic review. *PLoS Pathog* (2013) 9:e1003311. doi:10.1371/journal.ppat.1003311
20. Schivo M, Aksenov AA, Linderholm AL, McCartney MM, Simmons J, Harper RW, et al. Volatile emanations from *in vitro* airway cells infected with human rhinovirus. *J Breath Res* (2014) 8:037110. doi:10.1088/1752-7155/8/3/037110
21. Aksenov AA, Sandrock CE, Zhao WX, Sankaran S, Schivo M, Harper R, et al. Cellular scent of influenza virus infection. *Chembiochem* (2014) 15:1040–8. doi:10.1002/cbic.201300695
22. Mashir A, Paschke KM, van Duin D, Shrestha NK, Laskowski D, Storer MK, et al. Effect of the influenza A (H1N1) live attenuated intranasal vaccine on nitric oxide (FENO) and other volatiles in exhaled breath. *J Breath Res* (2011) 5(3):037107. doi:10.1088/1752-7155/5/3/037107
23. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. *J Biochem* (2011) 150(3):257–66. doi:10.1093/jb/mvr090
24. Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, et al. Colorectal cancer screening with odour material by canine scent detection. *Gut* (2011) 60:814–9. doi:10.1136/gut.2010.218305
25. McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K, Janecki T. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. *Integr Cancer Ther* (2006) 5:30–9. doi:10.1177/1534735405285096
26. Bomers MK, van Agtmael MA, Luik H, van Veen MC, Vandenbroucke-Grauls CMJE, Smulders YM. Using a dog's superior olfactory sensitivity to identify *Clostridium difficile* in stools and patients: proof of principle study. *Br Med J* (2012) 345:e7396. doi:10.1136/bmj.e7396
27. Alasaad S, Permunian R, Gakuya F, Mutinda M, Soriguer RC, Rossi L. Sarcoptic-mange detector dogs used to identify infected animals during outbreaks in wildlife. *BMC Vet Res* (2012) 8:110. doi:10.1186/1746-6148-8-110
28. Moser E, McCulloch M. Canine scent detection of human cancers: a review of methods and accuracy. *J Vet Behav* (2010) 5:145–52. doi:10.1016/j.jveb.2010.01.002
29. Craven BA, Settles GS. A computational and experimental investigation of the human thermal plume. *J Fluid Eng* (2006) 128(6):1251–8.
30. Craven BA, Hargather MJ, Volpe JA, Frymire SP, Settles GS. Design of a high-throughput chemical trace detection portal that samples the aerodynamic wake of a walking person. *IEEE Sensors J* (2014) 14(6):1852–66. doi:10.1109/JSEN.2014.2304538

31. Jia X, McLaughlin JB, Derksen J, Ahmadi G. Simulation of a mannequin's thermal plume in a small room. *Comput Math Appl* (2013) 65(2):287–95. doi:10.1016/j.camwa.2011.06.056

Conflict of Interest Statement: The authors declare that they have no conflicts of interest. There are no MTAs, patents, or patent applications that apply to reagents, methods, or data in the review paper.

ORIGINAL RESEARCH
published: 30 June 2016
doi: 10.3389/fvets.2016.00053

Influence of the Environment on Body Temperature of Racing Greyhounds

Jane McNicholl, Gordon S. Howarth and Susan J. Hazel*

School of Animal and Veterinary Sciences, University of Adelaide, Adelaide, SA, Australia

Heat strain is a potential risk factor for racing greyhounds in hot climates. However, there have been limited studies into the incidence of heat strain (when excess heat causes physiological or pathological effects) in racing greyhounds. The aim of this study was to determine if heat strain occurs in racing greyhounds, and, if so, whether environmental factors (e.g., ambient temperature and relative humidity) or dog-related factors (e.g., sex, bodyweight, color) are associated with the risk of heat strain. A total of 229 greyhounds were included in over 46 race meetings and seven different race venues in South Australia, Australia. Rectal temperatures of dogs were measured pre- and postrace and urine samples collected for analysis of myoglobinuria. Ambient temperature at race times ranged between 11.0 and 40.8°C and relative humidity ranged from 17 to 92%. There was a mean increase in greyhound rectal temperature of 2.1°C (range 1.1–3.1°C). A small but significant association was present between ambient temperature and increase in rectal temperature (r^2 = 0.033, P = 0.007). *The mean ambient temperature at race time, of dogs with postrace rectal temperature of or exceeding 41.5°C, was significantly greater than at race time of dogs with a postrace rectal temperature ≤41.5*°C *(31.2 vs. 27.3°C, respectively, P = 0.004).* When the ambient temperature reached 38°C, over one-third (39%) of dogs had a rectal temperature >41.5°C. Over half of postrace urine samples were positive by Dipstick reading for hemoglobin/myoglobin, and of 77 urine samples positive for Dipstick readings, 95% were positive for myoglobin. However, urinary myoglobin levels were not associated with ambient temperature or postrace rectal temperatures. The mean increase in rectal temperature was greater in dark (black, blue, brindle) than light (fawn and white) colored greyhounds. The results suggest heat strain occurs in racing greyhounds, evidenced by postrace rectal temperatures over 41.5°C and postrace myoglobinuria. Risk of heat strain may be increased in higher ambient temperatures and in darker colored greyhounds. Further research into the incidence of heat strain in racing greyhounds, and longer term physiological responses to heat strain, are warranted.

Keywords: greyhound, heat strain, heat stress, animal welfare, myoglobin, sport

OPEN ACCESS

Edited by:
Mia Cobb,
Working Dog Alliance Australia, Australia

Reviewed by:
Howard H. Erickson,
Kansas State University, USA
Nicholas Julian Branson,
Deakin University, Australia

****Correspondence:***
Susan J. Hazel
susan.hazel@adelaide.edu.au

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *12 April 2016*
Accepted: *17 June 2016*
Published: *30 June 2016*

Citation:
McNicholl J, Howarth GS and Hazel SJ (2016) Influence of the Environment on Body Temperature of Racing Greyhounds. Front. Vet. Sci. 3:53. doi: 10.3389/fvets.2016.00053

INTRODUCTION

Regulation of body temperature is essential for maintenance of life. Vertebrates regulate body temperature by both behavioral and physiological means. In mammals, cutaneous thermal sensors measure surface temperature, while core temperature is measured in the spinal cord and areas of the brain (1, 2). Heat stress describes the environmental or metabolic factors impacting on the body when its thermoregulatory mechanisms are challenged, due either to excessive ambient temperatures or extreme heat production whereas heat strain is the resultant physiological or pathological effects (3). Heat stroke occurs when the body's heat dissipating mechanisms are overwhelmed due to exposure to an environmental temperature exceeding body temperature (classic or environmental heat stroke) or when metabolic heat accumulates due to strenuous exercise (exertional heat stroke) (4). Heat stroke entails major organ failure and is life threatening (5, 6). In climates with high ambient temperatures, such as in Australia, heat stress and heat stroke are potential risk factors for dogs used in work and recreation, including the racing greyhound.

Dogs are able to maintain their temperature over a broad range of environmental and climatic conditions. Different thermoneutral zones have been estimated using a variety of methods and types of dog. A range of 23–27°C has been suggested as thermoneutral for three mixed breed dogs with bodyweights 8.5–10.5 kg (7), while for Innuit dogs it is −25 to 10°C (8). In greyhounds, the thermoneutral zone has been estimated to be 16–24°C (9). Symptoms of heat illness in dogs include panting, dry mucous membranes, prolonged capillary refill time, ataxia, and elevated body temperature (10, 11).

As greyhounds have been subject to intense selection for athletic performance over several centuries (12, 13), and 60% of the body mass is muscle (14), the large locomotor muscles may contain high mitochondrial and capillary volumes as found in some athletic marsupials (15). It could therefore be expected greyhounds would generate heat at a high rate and be particularly susceptible to exertional hyperthermia. Muscular activity generates heat as a by-product of ATP production and utilization. When the ambient temperature nears or exceeds body temperature, heat can only be lost by evaporation, which in dogs is achieved *via* the respiratory tract (16). During strenuous exercise, the respiratory rate increases, thus facilitating heat transfer, however, high levels of humidity may restrict the amount of heat lost. Although the milder manifestations of heat illness described in humans, such as heat rash and heat edema, have not been described in dogs, more significant symptoms such as cramps and fatigue are commonly exhibited by greyhounds following even short periods of strenuous exercise (17, 18). The elevation in rectal and muscle temperature resulting from prolonged exercise by dogs is associated with reduced levels of high energy phosphates (ATP and CrP) and increased levels of muscle lactate, pyruvate, and AMP, which may contribute to fatigue (19). Although greyhounds racing in environmental temperatures of 42°C are thought to be at risk of heat stroke (20), there has been little research into the effects on greyhounds of running in high ambient temperatures.

Heat stroke has been recognized for centuries, but until relatively recently the mechanisms were poorly understood. Shapiro et al. (21) was able to demonstrate, using dogs (which do not sweat), that heat stroke was due to tissue damage resulting from elevated body temperature and not cessation of sweating as had been previously believed. A subsequent study in which anesthetized dogs were heated to a rectal temperature of 44.5°C, revealed increased levels of serum enzymes such as glutamic-pyruvic glutaminase (SGPT) and alkaline phosphatase in the terminal stages of hyperthermia, indicative of tissue damage (22). The authors also noted necrosis of liver and intestinal epithelium and turbid, brown urine, indicative of impaired renal function. Current understanding is that heat stroke involves impairment of cellular function, denaturing of proteins (both structural and enzymatic) and disruption of lipid membranes, similar to systemic inflammatory response syndrome (SIRS) (23). Hyperthermia induces intestinal ischemia and increased intestinal wall permeability, which permits leakage of endotoxins (10, 24).

Rhabdomyolysis (muscle breakdown) may be a consequence of both strenuous exercise (25, 26) and heat strain (27). Exercise-induced muscle fiber damage has been reported in humans (28) and animals (29–32). Following rhabdomyolysis, there is rapid release of cell breakdown products, such as the enzymes creatine kinase and lactate dehydrogenase; ions such as potassium and phosphate and muscle proteins such as myoglobin (25, 33). Muscle fiber damage may be detected by the presence of myoglobin in urine (myoglobinuria) (33). Myoglobin is nephrotoxic and in humans, elevated levels of myoglobin in serum or urine are associated with a risk of acute renal failure and subsequent mortality. Myoglobinuria has been reported in greyhounds (34) but the incidence is unknown and the levels of myoglobin excreted have not been quantified.

Dehydration has been identified by many authors as a precursor or precipitating factor for heat stroke in humans (35, 36). It has been widely accepted that dehydration of 2–3% is a major risk factor for heat illness and that a fluid deficit of as little as 1.5–2% may have a negative effect on performance (37). A decrease in plasma volume of $6 \pm 2\%$ increases the rate of heat accumulation in dogs exposed to high external heat load (38); furthermore, a significant elevation in rectal temperature occurs in dehydrated versus non-dehydrated dogs exercising on a treadmill at 25°C (39). Racing greyhounds may lose up to 6% of their bodyweight, due to dehydration, in the prerace kenneling period (40). Such losses might lead to increased risk of hyperthermia and increased risk of renal damage from myoglobinuria.

Phenotypical factors, such as sex and bodyweight, may also influence the response of greyhounds to environmental conditions. Sex-based differences in response to elevated temperatures and exercise occur in humans (41) and mice (42). Differences exist in the susceptibility of male and female rats to disruption of the sarcolemma following exercise (43), and there are apparent protective effects of estrogen against exercise-induced muscle damage in rats and humans (44, 45). Sex differences in susceptibility to exertional rhabdomyolysis in dogs have not been reported. Body weight is also important, as the basal metabolic rate of mammals and resultant heat production increases with bodyweight (46). With an increase in body mass there is a reduction in the ratio of body surface area to body mass and an increase in the distance from core to surface: both of these factors reduce the ability of an animal to dissipate heat and in exercising animals, may lead to greater heat accumulation (47). Finally, coat color is important as there is a widely held lay opinion that black greyhounds are more stressed by high ambient temperatures than other colored greyhounds, a belief which might be supported by studies in other species. The white winter coats of arctic species have greater reflectivity than darker summer coats (48) and in cattle, white coat color increases heat tolerance over brown or black (49).

Since this study is performed in Australia, it is important to provide some background on greyhound racing in this country. Greyhound racing is conducted in all Australian states and territories, each of which has a governing body, which administers and regulates racing. The population of racing greyhounds in South Australia is approximately 60% male and 40% female (T. Hayles, GRSA personal communication, June 2013). Sex limited races for greyhounds are seldom programed so the majority of races include animals of both sexes. Greyhounds commence racing at or above 16 months of age and generally have a racing career of 18–24 months, thus racing greyhounds have a relatively narrow age span of approximately 2–4 years of age. Five basic coat colors are recognized: black, blue (a dilute of black which can be pale to dark gray), brindle (dark stripes over a base color producing black brindle, blue brindle, red brindle, dun brindle, fawn brindle, dark brindle, light brindle), fawn (dark fawn, light fawn, red fawn, blue fawn, or dun fawn), and dun which may range from a light blue fawn, through a rich red fawn, to a deep rich chocolate color, with the dominating factor being a pink to brown colored nose leather. Dun is extremely rare. Any of these colors may be distributed over the body in patches over a white base, and such greyhounds are described as parti-colored.

No studies to date to our knowledge have investigated body temperature changes in greyhounds during racing in Australia. Furthermore, it is not known if potential risk factors, such as sex, body weight, or coat color, may alter the risk of heat strain. The aims of this study were to determine if:

1) body temperature increases more in greyhounds raced during hot and during humid days;
2) body temperature is influenced by dog sex, body weight, coat color, or cooling vest use;
3) myoglobin is present in urine in greyhounds following racing, and;
4) if myoglobin is present, if levels are affected by ambient temperature, race distance, dogs' level of fitness, sex, body weight, or postexercise rectal temperature.

MATERIALS AND METHODS

An observational study was commenced in 2010 to record temperature and humidity at racing venues around South Australia and to record body temperature changes of greyhounds competing in races. The climate of the more populated districts of South Australia is described as Mediterranean with cool wet winters and hot dry summers (50). In summer, mean maximum daily temperature for the capital city, Adelaide (Latitude 34° 50′S–Longitude 138° 30′E) is 29°C (51). Animal ethics approval was provided for this study by the University of Adelaide Animal Ethics Committee.

Ethic Statement

Owners or trainers gave informed signed consent at the racetrack to participate in the study.

Venues

The pool of racing greyhounds in South Australia fluctuates, as dogs commence or terminate their racing careers and move between states. Across Australia in 2011, there were 43,259 races organized in 4068 race meetings and over $82,000,000 (AUS) prize money was distributed: the greyhound industry had 12,280 greyhounds registered for racing for a total of 330,429 starters (Greyhounds Australasia 2011). In South Australia, greyhound racing is controlled by Greyhound RacingSA (GRSA) and the industry represents over 16% of Totalizer Agency Board (TAB) market share and distribution, which in 2010–2011 was $10 m (AUS) (52).

Racing is conducted throughout the year. At the time of commencement of this study, there were eight racetrack venues, three of which (Barmera, Port Augusta, and Virginia) did not conduct race meetings during January and February. All racetracks with the exception of Virginia were oval tracks. The Virginia track was straight and only held lure coursing events. Race meetings were conducted three times per week at both Angle Park and Gawler but at approximately fortnightly intervals at other tracks.

On oval tracks, 7–12 races are programed per meeting and eight greyhounds (plus two reserves) are drawn to compete in each race. At lure coursing meetings, 6–10 events are held, each of which include 4–32 runners drawn to run off in pairs. Winners of each course proceed to the next round until the surviving pair contested a final. At the Virginia straight track, lure coursing meetings are only held between April and October and meetings commenced at 9.30 a.m. and finished by 2 p.m. At other tracks, races are scheduled between 12 p.m. and 11 p.m. and all greyhounds engaged at a meeting are presented for inspection prior to kenneling and kenneled at least 30 min prior to the first race on the program.

Pre-kenneling inspection entails an identity check of each greyhound by color, markings, ear tattoo, and/or microchip number. Every greyhound is then weighed and undergoes a brief veterinary inspection for health and racing suitability. Following inspection, each greyhound is confined in an allocated kennel until approximately 10 min prior to its race start time. At the Virginia racetrack, greyhounds are confined in their transport vehicles for the duration of the meeting.

Forty-six race meetings were attended at seven different venues and at different times of the year (**Table 1**). One track was straight (Virginia), and the other six were oval with various radii and circumferences. Two race tracks (Port Augusta and Virginia) have grass surfaces and five (Angle Park, Barmera, Gawler, Port Pirie, and Strathalbyn) have sand/loam surfaces. Races were conducted over distances of 300–731 m.

TABLE 1 | Racetrack venues attended during 2011.

Track	January	February	March	April	May	June	July	August	September	October	November	December
Angle Park	1	3	2					1	1	1		4
Gawler	7	3	3		1			1	2		1	2
Strathalbyn	2	2										
Barmera			1	1				1	1	1		
Port Pirie		1										
Port Augusta			1									
Virginia				1	1							

Environmental Monitoring

Ambient conditions inside the kennel houses and trackside were monitored with a weather station (La Crosse Technology, wireless 433-MHz Weather Station). Kennel house conditions were measured by the monitor placed approximately 1.2 m above ground level in the kennel area. Trackside conditions were measured by the outdoor monitor placed 1.2–1.8 m above ground level adjacent to the track, at a readily accessible location. Temperature and relative humidity were manually recorded at the start time of each race in which a selected greyhound was competing. Cloud cover was recorded in oktas on a scale 0–8 on which 0 = nil cloud and 8 = complete cloud cover (53). Some races were conducted after dusk, which was recorded as 10.

Thermometers

Temperatures were measured using a rectal clinical veterinary thermometer (Vicks Speed Read digital thermometer).

Cooling Jackets

The use of cooling jackets on greyhounds at racetracks, in temperatures >30°C, has been encouraged by GRSA for over 4 years (P. Marks, personal communication, January 2014). The jackets used during this study (Cool Champions, Silver Eagle Outfitters http://www.coolweave.com.au) were soaked in iced water for 30 min prior to initial use and also between uses.

Animals

The population of racing greyhounds in South Australia is approximately 1200 animals (G. Barber, GRSA personal communication, January 2010). On the day prior to a race meeting, the fields were accessed online at http://sa.thedogs.com.au and greyhounds were selected by random draw using Excel RANDBETWEEN function. If two greyhounds in the care of one trainer at one meeting were selected, a draw was repeated to select an alternative greyhound. Details of the age, sex, color, sire, dam, and previous race history of each greyhound were recorded from the published race fields.

For the purpose of this study, greyhounds were assigned a fitness score expressed in meters from 300 to 700 m in 100-m increments. The fitness score was based on the mode of the greyhound's last three races or trials to the nearest 100 m. From 246 races, 238 greyhounds were selected to participate in the study (134 males, 104 females) aged 18 months to 5 years (mean 2.6 years). In the greyhound industry, the generally accepted desirable weight range for racing dogs is 26–34 kg, for the purpose of analysis, the greyhounds were divided by bodyweight into four groups commonly used in the industry: <26, 26–30, >30–34, >34 kg. Any dog in with more than 50% white coat color was classified as white, thus creating five color groups (**Figure 1**). One greyhound was selected three times, and six greyhounds were selected twice. For the purpose of analysis, each of these greyhound's race starts was treated as a separate data point.

Procedure

At each race meeting, approximately 30 min prior to the commencement of greyhound admission, a list of selected greyhounds was provided to the stewards and a copy was posted in a prominent position at the kennel house entrance. As the selected greyhounds were presented for identification checks, the trainer was advised of the selection and permission sought for inclusion in the study. Some trainers declined to include some of the preselected greyhounds because of perceived temperamental unsuitability. Each participating greyhound was weighed and then had its temperature recorded as arrival temperature. Greyhounds were subsequently confined in their allocated kennels (or at lure coursing meetings, in trailers) up until approximately 10 min prior to their race start time.

Each greyhound was then collected from its allocated kennel and had a racing vest fitted, underwent identity check by stewards and was then taken outdoors to relieve itself. Urine samples were collected by voluntary voiding from 182 greyhounds. Rectal temperature was then recorded (prerace temperature). After completion of a race, greyhounds were collected by their handlers and returned to the kennel house and each greyhound's temperature was again recorded (postrace temperature). This time point was between 2 and 3 min after greyhounds ceased to run. All greyhounds then underwent hosing and were allowed to drink from a hose prior to being returned to their allocated kennels. The greyhounds, which competed in lure coursing events, were assessed after two courses, which were conducted at least 30 min apart, during which time the greyhounds were lightly hosed with cold water, offered cold water to drink, and confined in well ventilated trailers, thus permitting cooling. An attempt was made to follow up each greyhound to collect a second urine sample before the greyhound left the racetrack. Postrace urine samples were collected from 203 greyhounds. Rectal temperature before and after racing was obtained for 229 dogs (131 males, 98 females).

Urinalysis

Matched pre- and postrace urine samples were collected by voluntary voiding from 177 greyhounds, 104 males, 73 females:

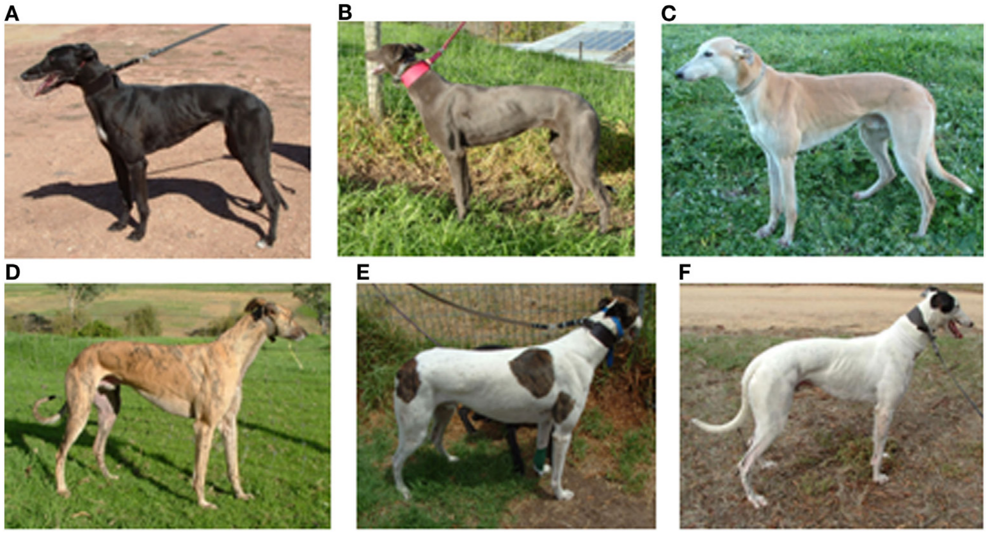


FIGURE 1 | **Greyhound colors: (A) black, (B) blue, (C) fawn, (D) brindle, (E) parti-colored white and brindle, (F) parti-colored white and black**.

(a) in the immediate prerace exercise period, and (b) as the greyhounds were leaving the track. Postrace samples were obtained between 30 min and 3 h after racing, dependent on the trainer's schedule. Urine samples were transferred from the collecting ladle into specimen containers immediately after collection and placed on ice for transport, then refrigerated up to 12 h prior to screening. Urine samples were centrifuged at 3000 rpm for 5 min to remove red blood cells. Reagent strips (Siemens Multistix 10 SG) were then immersed in the supernatant urine and read following the manufacturer's protocol. Results for blood/hemoglobin are expressed as trace, 1+, 2+, 3+ (1+ equivalent to 0.030–0.065 mg/dl). The manufacturer states the test is equally sensitive to myoglobin and hemoglobin. Samples were then stored frozen at −20°C for up to 12 months. Subsequently, 87 urine samples (77 which tested positive for blood/hemoglobin, 7 which tested negative and 3 unknown) were thawed and subjected to enzyme linked immunosorbent assay (ELISA) using Dog Myoglobin (Life Diagnostics, Inc.) and read at 450 nm (Benchmark Plus BIO-RAD).

Data Analysis

Data for the analysis of rectal temperature changes and associations with ambient temperature and humidity was analyzed with GraphPad Prism 6. Linear regression analysis was conducted to determine the association between shade temperature and relative humidity on rectal temperature at three time points: (a) on arrival; (b) pre-race; and (c) postrace. Linear regression analysis was also used to determine any association between ambient temperature, race distance, dogs' level of fitness, bodyweight, and postexercise body temperature and postexercise urine levels of myoglobin. Data were inspected for normality in distribution using the D'Agostino–Pearson test. Unpaired *t*-tests with Welches correction were conducted to determine sex-based differences in urine myoglobin, and the effects of cooling jackets worn postrace.

Statistical analyses to analyze effects of dog sex, weight, and color on rectal temperature changes were conducted using SPSS version 21. Data were inspected for normality in distribution using the D'Agostino–Pearson test. A mixed model which included the fixed effects of sex and color and covariate of weight and sire as a random term was fitted to the increase in rectal temperature and the postrace rectal temperature data. There was no sire variance thus a general linear model was fitted to the data with the above fixed effects and covariate. Any significant two-way interactions were retained in the model. A level of significance of $P < 0.05$ was used throughout.

RESULTS

Environmental Conditions

Ambient (shade) temperature at each dog race start ranged from 11.0 to 40.8°C. Relative humidity ranged between 17 and 92%. As ambient temperature increased, relative humidity decreased (**Figure 2**, $r^2 = 0.64$, $P = 0.0001$).

Body Temperature

Mean rectal temperature of 229 greyhounds on arrival at the race meetings was 39.2°C ± 0.5°C (range 38.2–40.5°C). Postrace, there was an increase in rectal temperature in all dogs. Mean postrace temperature was 41.0± 0.5°C (range 39.7–42.1°C) with a mean increase of 2.1°C (SD 0.4°C, range 1.1–3.1°C; **Figure 3**).

There was no significant effect of shade (ambient) temperature on rectal temperature on arrival ($r^2 = 4.5 \times 10^{-7}$, $P = 0.99$). Postrace there was a small but significant relationship between shade temperature and both rectal temperature ($r^2 = 0.023$, $P = 0.03$) and increase in rectal temperature ($r^2 = 0.033$, $P = 0.007$; **Figure 4**). A significant inverse relationship between prerace rectal temperature and the increase in rectal temperature after racing was determined ($r^2 = -0.15$, $P = 0.001$).

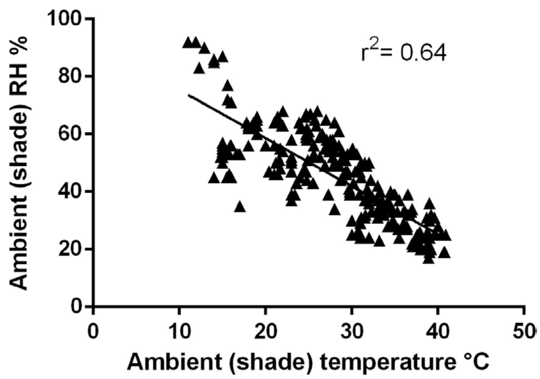


FIGURE 2 | **Relationship between ambient temperature and relative humidity recorded at racetracks (r^2 = 0.64, p = 0.0001)**.

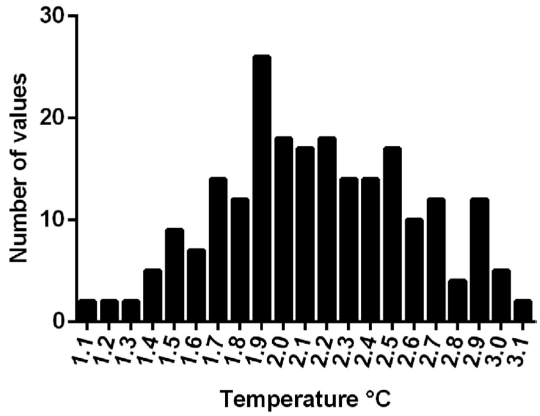


FIGURE 3 | **Increase in rectal temperature from pre- to postrace in 229 greyhounds**.

Effect of Relative Humidity

There was no significant association between relative humidity and postrace rectal temperature ($r^2 = 0.02$, $P = 0.06$).

Effect of Race Distance

No association between race distances and levels of postrace rectal temperature was found ($r^2 = 0.004$, $P = 0.4$).

Effect of Dog Fitness

No association was detected between dogs' levels of fitness and levels of postrace rectal temperatures ($r^2 = 0.001$, $P = 0.7$).

Cooling Jackets

Dogs that wore cooling jackets ($N = 41$) had a significantly higher rectal temperature postrace compared to those that did not ($N = 80$; mean 41.19 ± SEM 0.06°C versus 41.01 ± SEM 0.06°C, respectively, $P = 0.04$; unpaired t-test with Welch's correction).

Critical Temperatures

As 41.5°C has been suggested as a critical body temperature for precipitating heat illness in dogs (11, 54), animals were allocated into two groups using a postrace rectal temperature delimiter of >41.5°C. The mean ambient temperature at race time of dogs with postrace rectal temperature >41.5°C was significantly greater (31.2°C ± SEM 1.0°C, $N = 40$) than at race time of dogs recording a rectal temperature ≤41.5 (27.3°C ± SEM 0.5°C, $N = 189$, unpaired t-test, $t = 2.9$, df = 227, $P = 0.004$).

The percentage of greyhounds with postrace rectal temperatures of >41.5°C was plotted against ambient temperature (**Figure 5**). When the ambient temperature was 38°C, 39% of dogs had a rectal temperature of >41.5°C.

Urinalysis

In both males and females, over half (100/177, 57%) of postrace urine samples provided positive dipstick readings for hemoglobin/myoglobin (**Tables 2** and **3**). There were more positive dipstick results for males versus females.

Myoglobin levels detected in postrace urine samples ranged from 3 to 402 ng/ml (mean 93.3 ± 8.6 ng/ml). A significant association between dog bodyweight and myoglobin levels was detected ($r^2 = 0.05$, $P = 0.05$). As variances of male and female myoglobin levels differed ($P = 0.0003$) an unpaired t-test with Welches correction was conducted. A significant difference in myoglobin levels between males and females was detected (male 73.17 ± SEM 7.76 ng/ml, female 128.20 ± SEM 20.15 ng/ml, $P = 0.01$). Linear regression analysis showed no significant associations between urinary myoglobin levels and ambient temperature, race distance, level of fitness, or postrace rectal temperature.

Effect of Greyhound Color

The most common coat color of the included greyhounds was black ($N = 115$), followed by white ($N = 37$), with similar numbers of blue ($N = 23$), Brindle ($N = 28$), and Fawn ($N = 26$) colors. There was no significant difference in arrival or prerace rectal temperature of the five color groups ($P = 0.5$). However, mean postrace temperatures of the black, blue, and brindle greyhounds were 41.1 ± 0.4°C, 41.1 ± 0.5°C, and 41.1 ± 0.4°C, respectively, which were significantly higher than the fawn (40.9 ± 0.5°C) and white (40.8 ± 0.5°C; all $P < 0.05$) greyhounds. When the dogs were grouped into dark (black, blue, brindle) and light (fawn and white), the mean increase in temperature of the dark-colored dogs (2.2 ± 0.4°C) was significantly greater than the mean increase in temperature of the light-colored dogs (2.0 ± 0.4°C, $P = 0.005$). Postrace rectal temperatures of greyhounds racing in fully overcast/dark conditions ($N = 31$), or sunlight ($N = 131$) showed no significant difference ($P = 0.5$).

Effect of Sex

There was no significant sex-related difference in arrival rectal temperature for 128 males and 101 females ($P = 0.897$). Prerace temperatures for males ($N = 132$) and females ($N = 100$) also did not differ (38.9 ± 0.5°C versus 38.8°± 0.3°C respectively, $P = 0.46$). A significant difference was found in postrace rectal temperature of male ($N = 131$) and female ($N = 98$) greyhounds ($P = 0.004$). Mean male postrace temperature was 41.1 ± 0.5°C, and mean female postrace temperature was 40.9 ± 0.4°C.

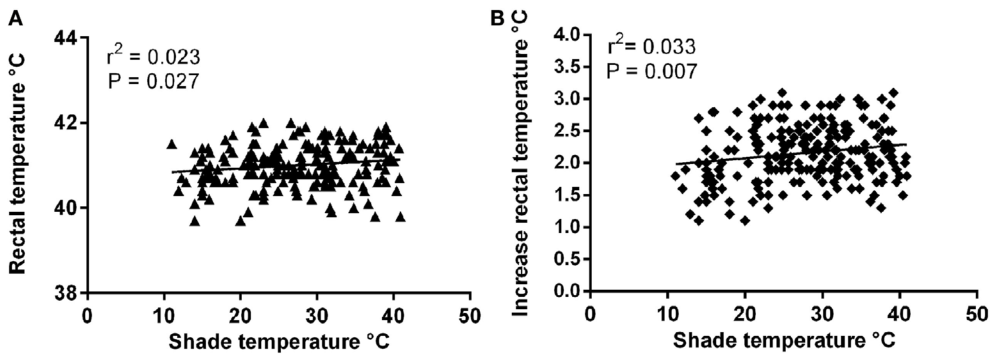


FIGURE 4 | **Relationship between ambient temperature and: (A) postrace rectal temperature and (B) increase in rectal temperature**.

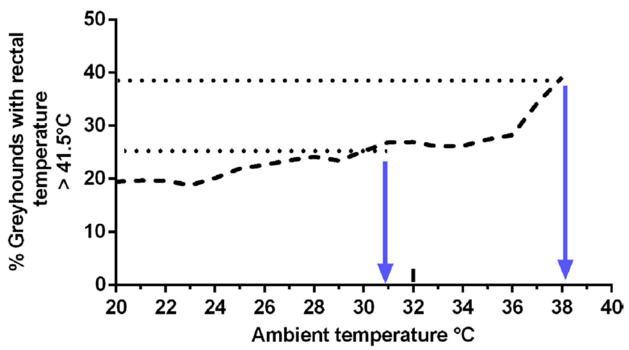


FIGURE 5 | **Relationship between ambient temperature and the percentage of greyhounds with postrace rectal temperature >41.5°C**.

TABLE 2 | **Results of dipstick test for blood, hemoglobin, or myoglobin in postrace urine samples.**

	Screened	Total positive hemoglobin	$\geq 2^6$ ng/ml hemoglobin
Male	104	70 (67%)	47 (45%)
Female	73	30 (41%)	24 (33%)

TABLE 3 | **Myoglobin results from 87 greyhound urine samples.**

Dipstick test result		Positive myoglobin[a]	Negative myoglobin
Positive blood/hemoglobin	77	73 (95%)	4 (5%)
Negative blood/hemoglobin	7	3 (43%)	4 (57%)
Unknown blood/hemoglobin	3	2 (67%)	1 (33%)

[a]*The lowest positive value was 5.8 ng/ml.*

Effect of Bodyweight

In the greyhound industry, the generally accepted desirable weight range for racing dogs is 26–34 kg: 73% of the selected greyhounds were within this range. The mean bodyweight was 30.2 ± 3.4 kg. Mean male bodyweight was 32.5 kg (median 32.0 kg), and mean female bodyweight was 27.2 kg (median 27.0 kg). There were no males in the <26 kg weight group, and no females in the >34 kg weight group (**Table 4**).

TABLE 4 | **Sex distribution in four body weight groups of selected greyhounds.**

	<26 kg		26–30 kg		>30–34 kg		>34 kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of dogs	0	23	10	68	82	7	39	0

A significant effect of bodyweight was noted on both actual rectal temperature (r^2 = 0.043, P = 0.009) and the increase in rectal temperature (r^2 = 0.05, P = 0.006) following racing (**Figure 6**).

DISCUSSION

The aims of the current study were to determine the body temperature responses to racing in greyhounds in South Australia. The study was designed to determine changes in rectal temperature following racing, and if any associations were present between increase in rectal temperature and environment factors, such as ambient temperature and relative humidity, and dog-related factors, such as sex, bodyweight, and color. Urinary myoglobin was measured postrace to determine if any pathological changes occurred and were related to heat strain.

In the current study, greyhounds competed in temperatures between 11 and 40°C. The mean increase in rectal temperature of 2.1°C was remarkable in view of the short duration of the periods of exercise. Although, as greyhounds expend almost as much energy in the first 7.5 s of a race as in the subsequent 22 s (55), it is not surprising that body temperature increases markedly in a short period of time. Although differences of up to 2°C between muscle and rectal temperatures have been measured in horses after 50-min exercise (56), the difference was less than 1°C after 10 min exercise as completed by the greyhounds in this study. Indeed in dogs, postexercise rectal temperatures higher than core temperature may result from the heat generated by the major muscles of the hind quarters (57). Most of the studies on hyperthermia in human and equine

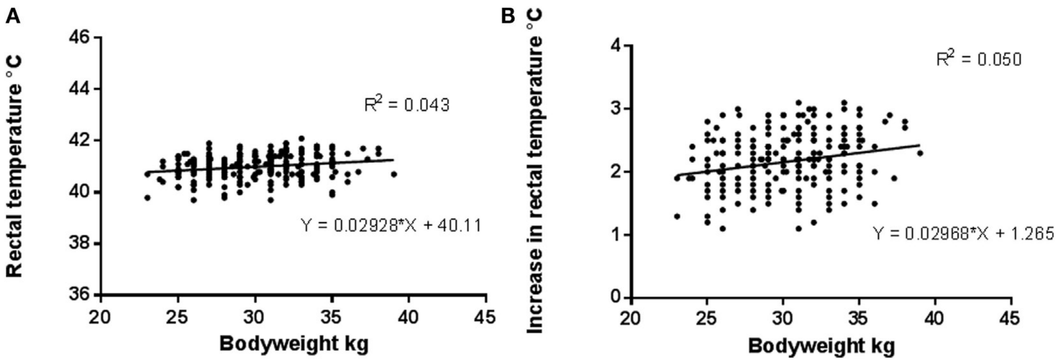


FIGURE 6 | **Relationship between bodyweight and (A) postrace rectal temperature (r^2 = 0.043, P = 0.009) and (B) increase in rectal temperature (r^2 = 0.05, P = 0.006) after racing.**

athletes have focused on hyperthermia as a result of prolonged periods of exercise. However, Drobatz and Macintyre (58) in their review of 42 clinical cases of heatstroke in dogs, remarked on the degree of morbidity after relatively short (20–30 min) periods of exercise, suggesting a high degree of susceptibility for dogs, compared to other species. As heat storage has been shown to be the principle limiting factor to intense exercise in cheetahs (59), it is probable that heat storage might similarly be a limiting factor to sprinting performance in greyhounds.

In the current study, the period of strenuous exercise was between 15 and 45 s for distances from 300 to 730 m. Additional activity was low intensity and was restricted to a total period of <15 min during which greyhounds were removed from holding kennels, approximately 10 min prior to scheduled race start time and walked to the starting boxes, 2 min prior to the race. Greyhounds exhibited varying levels of excitement in the prerace period, demonstrated by fine tremors or vigorous activity such as pulling or bouncing. The muscular activity involved in such behavior would generate heat and may have contributed to the increases in rectal temperature recorded. Greyhounds, bred and trained for racing, may develop an increase in rectal temperature (from resting levels) due to anticipation of activity (60). The negative association between prerace rectal temperature and increase in rectal temperature found in the current study illustrates the effectiveness of the thermoregulatory system, even under significant challenge.

Environmental Conditions and Body Temperature

The current study revealed a small but positive association between ambient temperature and postexercise body temperature. These findings are in accord with those of Bjotvedt et al. (20) where greyhounds performing in temperatures above 107°F (42.0°C) were at risk of heat stroke. Although intense exercise is generally estimated to cause an increase in metabolic rate of 10–14 times the basal metabolic rate (61), increases in metabolic rate of up to 25 times BMR have been recorded in some canine species (8, 62). Dissipation of the heat generated may pose a particular challenge. Susceptibility to heat illness may vary between breeds of dog as ambient temperature has not been shown to affect rectal temperature in exercising Labrador retrievers (63), although the Labradors were only exercised in a temperature range of 11–28°C. In contrast, a significant association between ambient temperature and rectal temperatures is present in sled dogs working in ambient temperatures between −9 and 25°C (64).

No significant effect of relative humidity on rectal temperature was demonstrated in the current study. However, as the climate of South Australia is described as Mediterranean (50), with an inverse relationship between temperature and humidity, days with concurrent elevation of both factors are rare. In areas with a tropical climate, humidity might impose greater challenges. In exercising horses, the rate of increase in temperature of blood, measured in the pulmonary artery, is significantly higher in hot, humid conditions than in either hot or cold, dry conditions (65).

It may be concluded that racing, or undertaking equivalent intense exercise, in hot weather carries an increased risk of greyhounds developing heat illness. The risk increases notably in ambient temperatures ≥38°C. However, under current management systems in South Australia, no racing greyhounds were found to suffer from heat stroke. The greyhound racing industry in Australia has a number of "Heat" or "Hot Weather" policies, which vary between states and lack a consistent threshold temperature. It would be prudent to set the threshold for "Hot Weather Policies" at 38°C, at which temperature, changes to race programing should be made and stringent management procedures be implemented for greyhounds participating in races or trials.

Critical Temperature

Many authors consider a rectal temperature ≥41.5°C to be a critical level for initiation of heat illness in dogs (11, 54, 58, 66, 67). During the current study, 45 greyhounds recorded a postrace rectal temperature >41.5°C which if not reduced, would place them at risk of heat illness. The mean ambient temperature at the time of these races was 31.2°C, which was 4°C greater than the mean ambient temperature at race time for greyhounds recording a rectal temperature <41.5°C. Therefore, 31°C might represent a threshold for risk estimation for heat stress in racing greyhounds. Such a threshold might be broadly accepted by participants in the greyhound racing industry, as there is a common perception amongst trainers of greyhounds that, at ambient temperatures >30°C, the animals show signs of thermal stress such as panting, which concurs with evidence from experimental settings (68). However, as in South Australia there are more than 80 days in summer with maximum daily temperature >30°C (51) setting 31°C as a threshold for canceling race meetings would represent major disruption to the industry. It has been suggested 36°C is the temperature at which thermal equilibrium can only just be maintained by dogs (69). In the current study, the percentage of greyhounds recording postrace rectal temperatures of ≥41.5° increased in gradual linear fashion up to 36°C and with a sharp rise when ambient temperatures reached 38°C. As 38°C is within the normal range of body temperature for dogs, the sharp increase in the number of greyhounds with temperature >41.5°C when ambient temperatures neared 38°C is in accord with the widely accepted view that, in environments at or above body temperature, thermoregulation is difficult.

Cooling Jackets

The use of cooling jackets on greyhounds at racetracks has been fairly limited. During the current study, there appeared to be reluctance by some trainers to use them, either because of a belief that they were uncomfortable for the dogs or because of a perception the time taken to don the jackets was wasted. Results of this study revealed the unexpected finding that the mean rectal temperature of dogs wearing jackets postrace was slightly higher than those dogs which did not. Cooling jackets of a different type have been demonstrated to be effective in reducing the duration of postexercise hyperthermia in military dogs (70). Precompetition use of ice jackets has been effective in reducing the degree of body heating in human athletes (71), but postexercise use of ice jackets is not advantageous in hyperthermic athletes (72). Further research into their use in greyhounds during hot conditions is warranted.

Alternative methods of estimating thermal stress in racing greyhounds might include panting score such as used for sheep (73) and cattle (49). However, in dogs panting is utilized not only to maintain homeothermy (74) but also as a result of exercise (19, 75), arousal (60), or anxiety (76). As this study was conducted at racetracks, all of the above factors could have influenced panting rate, and it was not practical to utilize panting rate as an indicator of heat stress.

Urinalysis

Rhabdomyolysis has been recorded as a result of strenuous exercise in greyhounds (77, 78) and rhabdomyolysis may also result from hyperthermia (79). It could therefore be expected that greyhounds undertaking strenuous exercise in hot conditions would be at increased risk of developing rhabdomyolysis and myoglobinuria. Myoglobin is a small heme protein, which is released into plasma after muscle fiber rupture; plasma levels fall rapidly, as it is excreted into urine (79). As myoglobin is recognized as being nephrotoxic (33), it is possible that repeated exposure to significant levels would have a cumulative effect and that such exposure might contribute to the high incidence of renal disease seen in greyhounds (D. Fegan, personal communication, 2013).

Effect of Phenotypic Factors

This study showed that postexercise temperature was influenced by several phenotypic factors. A significant though weak relationship was found between bodyweight and postexercise rectal temperature and also between bodyweight and the increase in rectal temperature. Coat color was also found to have a significant association with postexercise temperature, with greyhounds of dark colors developing higher rectal temperatures than light colored greyhounds. Many greyhound trainers believe that black greyhounds are more susceptible to heat stress than other colored dogs, as the trainers can feel temperature differences on the surface of their greyhounds. The finding that dark colored (black, blue, brindle) greyhounds develop higher temperatures than light colored (fawn and predominantly white) greyhounds is in keeping with findings in other species. Three naturally occurring color morphs of antelope have differences in core temperature (80) and McManus et al. (82) who examined the tolerance of different breeds and colors of sheep, to heat stress in Brazil, concluded that breed, coat type (wool/hair) and coat color were important modifiers. A number of studies of production animals have revealed that, under heat stress, white coated animals can maintain lower body temperatures than dark colored conspecifics and that coat color influences heat tolerance (81, 82). This has been attributed to the greater reflectivity of the white coats, leading to less heat accumulation. In the current study, it was anticipated that dark coated greyhounds, racing in sunlight, might develop higher body temperatures than those racing in shaded or dark conditions, however no significant effect of sunlight was detected. It seems therefore, that direct solar radiation was not a significant contributor to the temperatures recorded. However, thermal radiation from the track surface and surroundings may have contributed to the higher temperatures recorded in dark coated dogs.

Sex

No significant differences between sexes were recorded for rectal temperatures either on arrival or prerace. However, males had higher postrace and mean increases in rectal temperature than females. Sex based differences in body temperature could be a result of sex hormones, body proportions, or thermoregulatory mechanisms. Gender differences in response to thermal and exercise challenges have been reported in humans (41, 83). The principle mechanism of heat loss in humans is sweating and considerable efforts have been directed at examining differences in sweating and sudomotor responses (84, 85). However, as sweating is not utilized as a heat loss mechanism in canines, it is not valid to attempt to extrapolate from such studies. The influence of estrogen and progesterone on core temperature during the menstrual cycle of women has long been recognized (86). Complex interactions between norepinephrine and estrogen occur in the brain of women, whereby estrogen raises the sweating threshold and norepinephrine narrows the thermoneutral zone by initiating heat dissipation (87). Hanada et al. (42) identified receptor activator of necrosis factor kB ligand (RANKL) and its tumor necrosis factor receptor (RANK) as key factors of central control of thermoregulation in female but not male mice and suggested that, in murine species, female thermoregulation is, in part, regulated by ovarian sex hormones (42).

The higher levels of myoglobinuria found in female greyhounds was unexpected, as it has been reported that female animals suffer less muscle damage than males (88). However, female horses are reported to be more frequently affected by exertional rhabdomyolysis than males (89, 90). Female rats are less susceptible to exercise-induced muscle damage than males (43, 91), likely due to a protective effect of estrogen (92). However, similar protection is unlikely to have occurred in greyhounds during the current study as testosterone proprionate was permitted to prevent estrous in female greyhounds at that time (93). In Australia, female greyhounds are not permitted to race whilst in estrous or for 28 days post estrous (93) and reduced performance in the diestrous period of up to 91 days has been reported (94). Therefore, racing female greyhounds can be assumed to be in late diestrous or anestrous with relatively low levels of circulating estrogen and progesterone (95). Alternatively, there may be a species related difference in response to exercise or even a breed specific response, as greyhounds exhibit other physiological and hematological variations from other breeds of dog (96–98). During the current study, no data were collected on the use of testosterone or other permitted hormones nor on the natural hormonal status of the females, so further studies on the responses to exercise of male and female greyhounds are required.

Bodyweight

Many of the studies on thermoregulation and exercise in humans have investigated the differences, which might be attributable to anthropometric features such as body proportions and fat distribution (69, 99, 100). Lean animals may dissipate heat more readily than obese animals, as in the latter, sub-cutaneous fat impedes heat transfer to the environment (101). However, during exercise, both muscle and core temperature increase more in lean than obese rats (102). Almost all of the greyhounds in this study had a body condition score of 2 and variations in body fat are unlikely to have affected the results.

The positive associations between bodyweight and both postrace and increase in rectal temperature may be attributed to the amount of energy utilized during activity. As the energy requirements to move a body increase with an increase in bodyweight (103), metabolic heat production also rises. In both birds and mammals, the energetic cost of exercise is related to both body mass and speed (104, 105). In humans, metabolic heat production resultant from muscle contraction creates an internal heat load proportional to exercise intensity (106, 107). Although the periods of exercise of the greyhounds were limited to <45 s at maximum effort, as greyhounds have a high proportion of muscle (14) and the rate of heat accumulation in muscle increases with intensity of work (47), it is apparent that greyhounds exercising at maximum effort generate a very high heat load. In a recent study, rats selected for high capacity running, exhibited high levels of energy expenditure and muscle heat dissipation (108). The authors suggested that these effects might be due to intrinsic aerobic capacity and that similar expression of skeletal muscle proteins might be found in other species. Greyhound muscle exhibits a high rate of anaerobic glycogenolysis (109); further research is warranted in the area of greyhound muscle energetics and heat production.

CONCLUSION

It may be concluded that racing, or undertaking equivalent intense exercise, in hot weather carries an increased risk of greyhounds developing heat illness. The risk increases notably in ambient temperatures ≥38°C. Large, dark-colored greyhounds are at greater risk of developing high body temperature than small, light-colored greyhounds, when undertaking strenuous exercise in hot conditions. Pre- and postexercise cooling should therefore be applied with particular care to large black, blue, or brindle greyhounds to prevent development of heat strain. The greyhound racing industry in Australia has a number of "Heat" or "Hot Weather" policies which vary between states and lack a consistent threshold temperature. It would therefore be prudent to set the threshold for "Hot Weather Policies" at 38°C, at which temperature, changes to race programing should be made and stringent management procedures be implemented for greyhounds participating in races or trials. Further research is required to investigate environmental effects on greyhounds racing in tropical climates.

AUTHOR CONTRIBUTIONS

JM was responsible for conception and design of the work, the acquisition, analysis and interpretation of data, drafting and revising, and gave final approval for it to be published. GH was responsible for help in interpretation of data and revising, and gave final approval for it to be published. SH assisted in the conception and design of the work, the acquisition, analysis and interpretation of data, drafting and revising, and gave final approval for it to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGMENTS

The authors would like to acknowledge the assistance of the racing tracks in permitting the study to be performed, and all of the greyhound owners and trainers who agreed for their greyhounds to be included in the study.

FUNDING

Financial support for this PhD project was provided by Greyhound Racing South Australia and the Australian Greyhound Veterinarians Special Interest Group of the Australian Greyhound Veterinary Association.

REFERENCES

1. Boulant JA. Hypothalamic neurons: mechanisms of sensitivity to temperature. *Ann N Y Acad Sci* (1998) 856(1):108–15. doi:10.1111/j.1749-6632.1998.tb08319.x
2. Morrison SF, Nakamura K. Central neural pathways for thermoregulation. *Front Biosci (Landmark Ed)* (2011) 16:74–104. doi:10.2741/3677
3. Tikuisis P, McLellan TM, Selkirk G. Perceptual versus physiological heat strain during exercise-heat stress. *Med Sci Sports Exerc* (2002) 34(9):1454–61. doi:10.1097/00005768-200209000-00009
4. Bouchama A, Knochel JP. Medical progress – heat stroke. *N Engl J Med* (2002) 346(25):1978–88. doi:10.1056/NEJMra011089
5. Leon LR, Helwig BG. Heat stroke: role of the systemic inflammatory response. *J Appl Physiol* (2010) 109(6):1980–8. doi:10.1152/japplphysiol.00301.2010
6. Yan Y-E, Zhao Y-Q, Wang H, Fan M. Pathophysiological factors underlying heatstroke. *Med Hypotheses* (2006) 67(3):609–17. doi:10.1016/j.mehy.2005.12.048
7. Hammel HT, Wyndham CH, Hardy JD. Heat production and heat loss in the dog at 8-36°C environmental temperature. *Am J Physiol* (1958) 194(1):99–108.
8. Gerth N, Redman P, Speakman J, Jackson S, Starck JM. Energy metabolism of Inuit sled dogs. *J Comp Physiol B* (2010) 180(4):577–89. doi:10.1007/s00360-009-0432-7
9. Hales JRS, Dampney RAL. The redistribution of cardiac output in the dog during heat stress. *J Therm Biol* (1975) 1(1):29–34. doi:10.1016/0306-4565(75)90008-X
10. Flournoy W, Wohl JS, Macintire DK. Heatstroke in dogs: pathophysiology and predisposing factors. *Compend Contin Educ Vet* (2003) 25(6):410–8.
11. Johnson SI, McMichael M, White G. Heatstroke in small animal medicine: a clinical practice review. *J Vet Emerg Crit Care* (2006) 16(2):112–9. doi:10.1111/j.1476-4431.2006.00191.x
12. Banks NJ. *History of the Greyhound. Australian Greyhound Stud Book*. (Vol. 27). Melbourne: The Australian and New Zealand Greyhound Association (1978). p. 22–6.
13. Burnell RB. *Racing Greyhounds*. NSW: Murray Book Publishers (1973).
14. Gunn HM. The proportions of muscle, bone and fat in two different types of dog. *Res Vet Sci* (1978) 24(3):277–82.
15. Webster KN, Dawson TJ. The high aerobic capacity of a small, marsupial rat-kangaroo (*Bettongia penicillata*) is matched by the mitochondrial and capillary morphology of its skeletal muscles. *J Exp Biol* (2012) 215(18):3223–30. doi:10.1242/jeb.071365
16. Schmidt-Nielsen K, Bretz WL, Taylor CR. Panting in dogs: unidirectional airflow over evaporative surfaces. *Science* (1970) 169:1102–4. doi:10.1126/science.169.3950.1102
17. Blythe LL, Gannon JR, Craig AM. *Metabolic Disorders of Racing Greyhounds. Care of the Racing Greyhound*. Portland, Oregon, USA: American Greyhound Council (1994).
18. Pemberton PL, editor. *Azoturia in the Greyhound. Refresher Course on Greyhounds*. Sydney, Australia: The Post Graduate Committee in Veterianry Science, University of Sydney (1983).
19. Kozlowski S, Brzezinska Z, Kruk B, Kaciuba-Uscilko H, Greenleaf JE, Nazar K. Exercise hyperthermia as a factor limiting physical performance: temperature effect on muscle metabolism. *J Appl Physiol* (1985) 59(3):766–73.
20. Bjotvedt G, Weems CW, Foley K. Strenuous exercise may cause health-hazards for racing greyhounds. *Vet Med Small Anim Clin* (1984) 79(12):1481–7.
21. Shapiro Y, Rosenthal T, Sohar E. Experimental heatstroke – model in dogs. *Arch Intern Med* (1973) 131(5):688–92. doi:10.1001/archinte.1973.00320110072010
22. Bynum G, Patton J, Bowers W, Leav I, Hamlet M, Marsili M, et al. Peritoneal lavage cooling in an anesthetized dog heatstroke model. *Aviat Space Environ Med* (1978) 49(6):779–84.
23. Lugo-Amador NM, Rothenhaus T, Moyer P. Heat-related illness. *Emerg Med Clin North Am* (2004) 22(2):315–27. doi:10.1016/j.emc.2004.01.004
24. Liu Z, Sun X, Tang J, Tang Y, Tong H, Wen Q, et al. Intestinal inflammation and tissue injury in response to heat stress and cooling treatment in mice. *Mol Med Rep* (2011) 4(3):437–43. doi:10.3892/mmr.2011.461
25. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. *Clin Chem Lab Med* (2010) 48(6):757–67. doi:10.1515/CCLM.2010.179
26. Chatzizisis YS, Misirli G, Hatzitolios AI, Giannoglou GD. The syndrome of rhabdomyolysis: complications and treatment. *Eur J Intern Med* (2008) 19(8):568–74. doi:10.1016/j.ejim.2007.06.037
27. Nichols A. Heat-related illness in sports and exercise. *Curr Rev Musculoskelet Med* (2014) 7(4):355–65. doi:10.1007/s12178-014-9240-0
28. Knochel JP. Catastrophic medical events with exhaustive exercise-White Collar Rhabdomyolysis. *Kidney Int* (1990) 38(4):709–19.
29. Freestone JF, Carlson GP. Muscle disorders in the horse – a retrospective study. *Equine Vet J* (1991) 23(2):86–90. doi:10.1111/j.2042-3306.1991.tb02726.x
30. Lopez-Rivero L. Exertional rhabdomyolysis. *J Equine Vet Sci* (2000) 20(9):571. doi:10.1016/S0737-0806(00)70261-9
31. Piercy RJ, Hinchcliff KW, Morley PS, DiSilvestro RA, Reinhart GA, Nelson SL Jr, et al. Vitamin E and exertional rhabdomyolysis during endurance sled dog racing. *Neuromuscul Disord* (2001) 11(3):278–86. doi:10.1016/S0960-8966(00)00199-1
32. Wilberger MS, McKenzie EC, Payton ME, Rigas JD, Valberg SJ. Prevalence of exertional rhabdomyolysis in endurance horses in the Pacific Northwestern United States. *Equine Vet J* (2015) 47(2):165–70. doi:10.1111/evj.12255
33. Cervellin G, Comelli I, Lippi G. Rhabdomyolysis: historical background, clinical, diagnostic and therapeutic features. *Clin Chem Lab Med* (2010) 48(6):749–56. doi:10.1515/CCLM.2010.151
34. Ferguson R, Boemo C. Genitourinary diseases in the canine athlete. In: Bloomberg M, Dee J, Taylor R, Gannon J, editors. *Canine Sports Medicine and Surgery*. Philadelphia: Saunders (1998). p. 44–8.
35. Coris EE, Ramirez AM, Van Durme DJ. Heat illness in athletes – the dangerous combination of heat, humidity and exercise. *Sports Med* (2004) 34(1):9–16. doi:10.2165/00007256-200434010-00002
36. Howe AS, Boden BP. Heat-related illness in athletes. *Am J Sports Med* (2007) 35(8):1384–95. doi:10.1177/0363546507305013
37. Maughan R, Shirreffs S. Exercise in the heat: challenges and opportunities. *J Sports Sci* (2004) 22(10):917–27. doi:10.1080/02640410400005909
38. Assia E, Epstein Y, Magazanik A, Shapiro Y, Sohar E. Plasma-cortisol levels in experimental heatstroke in dogs. *Int J Biometeorol* (1989) 33(2):85–8. doi:10.1007/BF01686283
39. Baker MA. Cardiovascular and respiratory responses to heat in dehydrated dogs. *Am J Physiol* (1984) 246(3):R369–74.
40. Blythe LL, Hansen DE. Factors affecting prerace dehydration and performance of racing greyhounds. *J Am Vet Med Assoc* (1986) 189(12):1572–4.
41. Mehnert P, Bröde P, Griefahn B. Gender-related difference in sweat loss and its impact on exposure limits to heat stress. *Int J Ind Ergon* (2002) 29(6):343–51. doi:10.1016/S0169-8141(02)00073-2
42. Hanada R, Leibbrandt A, Hanada T, Kitaoka S, Furuyashiki T, Fujihara H, et al. Central control of fever and female body temperature by RANKL/RANK. *Nature* (2009) 462(7272):505–9. doi:10.1038/nature08596
43. Komulainen J, Koskinen S, Kalliokoski R, Takala T, Vihko V. Gender differences in skeletal muscle fibre damage after eccentrically biased downhill running in rats. *Acta Physiol Scand* (1999) 165:57–64. doi:10.1046/j.1365-201x.1999.00481.x
44. Amelink GJ, Bar PR. Exercise-induced muscle protein leakage in the rat – effects of hormonal manipulation. *J Neurol Sci* (1986) 76(1):61–8. doi:10.1016/0022-510X(86)90142-5
45. Tiidus PM, Bombardier E. Oestrogen attenuates post-exercise myeloperoxidase activity in skeletal muscle of male rats. *Acta Physiol Scand* (1999) 166(2):85–90. doi:10.1046/j.1365-201x.1999.00550.x
46. Hill RW, Wyse GA, Anderson M. *Thermal Relations. Animal Physiology*. 2nd ed. Sunderland, MA: Sinauer Associates (2008). 232 p.
47. Hodgson DR, McCutcheon LJ, Byrd SK, Brown WS, Bayly WM, Brengelmann GL, et al. Dissipation of metabolic heat in the horse during exercise. *J Appl Physiol* (1993) 74(3):1161–70.
48. Walsberg GE. Thermal effects of seasonal coat change in 3 sub-arctic mammals. *J Therm Biol* (1991) 16(5):291–6. doi:10.1016/0306-4565(91)90020-3
49. Gaughan JB, Mader TL, Holt SM, Lisle A. A new heat load index for feedlot cattle. *J Anim Sci* (2008) 86(1):226–34. doi:10.2527/jas.2007-0305
50. Atlas of South Australia. *Government of South Australia*. (2015). Available from: http://www.atlas.sa.gov.au/resources/atlas-of-south-australia-1986/environment-resources/climate-and-weather

51. Australian Government Bureau of Meteorology. *Climate Data Online: Australan Government Bureau of Meteorology*. (2013). Available from: http://www.bom.gov.au/climate/data/
52. Greyhound Racing South Australia. *The Greyhound Industry – A Code of Practice for Greyhound Establishments*. (2011). Available from: http://www.grsa.com.au/resources/1/Code%20of%20Practice%202011.pdf
53. Rakesh V, Stallings JD, Reifman J. A virtual rat for simulating environmental and exertional heat stress. *J Appl Physiol* (2014) 117(11):1278–86. doi:10.1152/japplphysiol.00614.2014
54. Aroch I, Segev G, Loeb E, Bruchim Y. Peripheral nucleated red blood cells as a prognostic indicator in heatstroke in dogs. *J Vet Intern Med* (2009) 23(3):544–51. doi:10.1111/j.1939-1676.2009.0305.x
55. Staaden R. *The Exercise Physiology of the Racing Greyhound*. Murdock, WA: Murdock University (1984).
56. Taylor LE, Kronfeld DS, Ferrante PL, Wilson JA, Tiegs W. Blood-gas measurements adjusted for temperature at three sites during incremental exercise in the horse. *J Appl Physiol (1985)* (1998) 85(3):1030–6.
57. Robertson S, Cooke K. 'Turning up the Heat': effects of work on physiologic variables and body temperature in working dogs. *Canine Sports Medicine Symposium*. Florida, USA: (2012).
58. Drobatz KJ, Macintire DK. Heat-induced illness in dogs: 42 cases (1976-1993). *J Am Vet Med Assoc* (1996) 209(11):1894–9.
59. Taylor CR, Rowntree VJ. Temperature regulation and heat balance in running cheetahs: a strategy for sprinters? *Am J Physiol* (1973) 224(4):848–51.
60. Gillette RL, Angle TC, Sanders JS, DeGraves FJ. An evaluation of the physiological affects of anticipation, activity arousal and recovery in sprinting greyhounds. *Appl Anim Behav Sci* (2011) 130(3–4):101–6. doi:10.1016/j.applanim.2010.12.010
61. Schutz Y. Energy balance. 2nd ed. In: Caballero B, editor. *Encyclopedia of Human Nutrition*. Oxford: Elsevier (2005). p. 115–25.
62. Gorman ML, Mills MG, Raath JP, Speakman JR. High hunting costs make African wild dogs vulnerable to kleptoparasitism by hyaenas. *Nature* (1998) 391(6666):479–81. doi:10.1038/35131
63. Matwichuk CL, Taylor SM, Shmon CL, Kass PH, Shelton GD. Changes in rectal temperature and hematologic, biochemical, blood gas, and acid-base values in healthy Labrador Retrievers before and after strenuous exercise. *Am J Vet Res* (1999) 60(1):88–92.
64. Phillips CJ, Coppinger RP, Schimel DS. Hyperthermia in running sled dogs. *J Appl Physiol* (1981) 51(1):135–42.
65. Geor RJ, McCutcheon LJ, Ecker GL, Lindinger MI. Thermal and cardiorespiratory responses of horses to submaximal exercise under hot and humid conditions. *Equine Vet J Suppl* (1995) 27:125–32.
66. Bruchim Y, Klement E, Saragusty J, Finkeilstein E, Kass P, Aroch I. Heat stroke in dogs: a retrospective study of 54 cases (1999–2004) and analysis of risk factors for death. *J Vet Intern Med* (2006) 20(1):38–46. doi:10.1111/j.1939-1676.2006.tb02821.x
67. Flournoy WS, Wohl J, McIntyre DK. Heatstroke in dogs: clinical signs, treatment, prognosis, and prevention. *Compend Contin Educ Vet* (2003) 25(6):422–31.
68. Goldberg MB, Langman VA, Taylor CR. Panting in dogs – paths of airflow in response to heat and exercise. *Respir Physiol* (1981) 43(3):327–38. doi:10.1016/0034-5687(81)90113-4
69. Thiele P, Albers C. Die Wasserdampfabgabe durch die Atemwege und der Wirkungsgrad des Wärmehechelns beim wachen Hund. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere* (1963) 278(3):316–24. doi:10.1007/BF00363495
70. Robertson S, Cooke K. Turning up the heat. *28th International Canine Sports Medicine Symposium*. Orlando, FL: (2012).
71. Hunter I, Hopkins JT, Casa DJ. Warming up with an ice vest: core body temperature before and after cross-country racing. *J Athl Train* (2006) 41(4):371–4.
72. Lopez RM, Cleary MA, Jones LC, Zuri RE. Thermoregulatory influence of a cooling vest on hyperthermic athletes. *J Athl Train* (2008) 43(1):55–61. doi:10.4085/1062-6050-43.1.55
73. Hales JRS, Hutchins JC. Metabolic, respiratory and vasomotor responses to heating scrotum of ram. *J Physiol* (1971) 212(2):353–75. doi:10.1113/jphysiol.1971.sp009329
74. Baker MA, Turlejska E. Thermal panting in dehydrated dogs – effects of plasma-volume expansion and drinking. *Pflugers Arch* (1989) 413(5):511–5. doi:10.1007/BF00594182
75. Flandrois R, Lacour J, Osman H. Control of breathing in the exercising dog. *Respir Physiol* (1971) 13(3):361–71. doi:10.1016/0034-5687(71)90040-5
76. Dreschel NA, Granger DA. Methods of collection for salivary cortisol measurement in dogs. *Horm Behav* (2009) 55(1):163–8. doi:10.1016/j.yhbeh.2008.09.010
77. Bjotvedt G, Hendricks GM, Weems CW. Exertional rhabdomyolysis in a racing greyhound – a case-report. *Vet Med Small Anim Clin* (1983) 78(8):1215–20.
78. Lording PM. *Clinical Pathology Profiles. Greyhound Medicine and Surgery; 25-29 September 1989*. Sydney: Post Graduate Committee in Veterinary Science, University of Sydney (1989). p. 369–91.
79. Bosch X, Poch E, Grau JM. Rhabdomyolysis and acute kidney injury. *N Engl J Med* (2009) 361(1):62–72. doi:10.1056/NEJMra0801327
80. Hetem RS, de Witt BA, Fick LG, Fuller A, Kerley GIH, Meyer LCR, et al. Body temperature, thermoregulatory behaviour and pelt characteristics of three colour morphs of springbok (*Antidorcas marsupialis*). *Comp Biochem Physiol A Mol Integr Physiol* (2009) 152(3):379–88. doi:10.1016/j.cbpa.2008.11.011
81. Brown-Brandl TM, Eigenberg RA, Nienaber JA. Heat stress risk factors of feedlot heifers. *Livest Sci* (2006) 105(1–3):57–68. doi:10.1016/j.livsci.2006.04.025
82. McManus C, Paludo GR, Louvandini H, Gugel R, Sasaki LC, Paiva SR. Heat tolerance in Brazilian sheep: physiological and blood parameters. *Trop Anim Health Prod* (2009) 41:95–101. doi:10.1007/s11250-008-9162-1
83. Wyndham CH, Morrison JF, Williams CG. Heat reactions of male and female Caucasians. *J Appl Physiol* (1965) 20(3):357–64.
84. Frye A, Kamon E. Sweating efficiency in acclimated men and women exercising in humid and dry heat. *J Appl Physiol* (1983) 54(4):972–7.
85. Gagnon D, Kenny GP. Sex modulates whole-body sudomotor thermosensitivity during exercise. *J Physiol* (2011) 589(24):6205–17. doi:10.1113/jphysiol.2011.219220
86. Webb P. 24-hour energy expenditure and the menstrual cycle. *Am J Clin Nutr* (1986) 44(5):614–9.
87. Freedman RR. Menopausal hot flashes: mechanisms, endocrinology, treatment. *J Steroid Biochem Mol Biol* (2014) 142(0):115–20. doi:10.1016/j.jsbmb.2013.08.010
88. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* (2002) 81(11):S52–69. doi:10.1097/00002060-200211001-00007
89. MacLeay JM, Sorum SA, Valberg SJ, Marsh WE, Sorum MD. Epidemiologic analysis of factors influencing exertional rhabdomyolysis in Thoroughbreds. *Am J Vet Res* (1999) 60(12):1562–6.
90. Upjohn M, Archer R, Christley R, McGowan C. Incidence and risk factors associated with exertional rhabdomyolysis syndrome in National Hunt racehorses in Great Britain. *Vet Rec* (2005) 156(24):763–6. doi:10.1136/vr.156.24.763
91. Enns DL, Tiidus PM. Estrogen influences satellite cell activation and proliferation following downhill running in rats. *J Appl Physiol* (2008) 104(2):347–53. doi:10.1152/japplphysiol.00128.2007
92. Tiidus PM. Estrogen and gender effects on muscle damage, inflammation, and oxidative stress. *Can J Appl Physiol* (2000) 25(4):274–87. doi:10.1139/h00-022
93. *Greyhounds Australasia 2013, 'Rule 83 (6) Greyhound to be free of prohibited substances (6).Greyhound Racing Rules of Greyhound RacingSA'*. Greyhound Racing South Australia (2015). Available from: http://sa.thedogs.com.au/Uploads/Rule%20Book%20New%20Edition%2001-09-2015.pdf
94. Payne RM. The effect of dioestrus on the racing performance of Greyhounds. *Vet J* (2013) 197(3):670–4. doi:10.1016/j.tvjl.2013.05.029
95. Noakes DE, Arthur GH, Parkinson TJ, England GCW. *Arthur's Veterinary Reproduction and Obstetrics*. Philadelphia, PA: The Curtis Center (2001).
96. Campora C, Freeman KP, Lewis FI, Gibson G, Sacchini F, Sanchez-Vazquez MJ. Determination of haematological reference intervals in healthy adult greyhounds. *J Small Anim Pract* (2011) 52(6):301–9. doi:10.1111/j.1748-5827.2011.01070.x

97. Shiel RE, Brennan SF, O'Rourke LG, McCullough M, Mooney CT. Hematologic values in young pretraining healthy greyhounds. *Vet Clin Pathol* (2007) 36(3):274–7.
98. Zaldivar-Lopez S, Marin LM, Iazbik MC, Westendorf-Stingle N, Hensley S, Couto CG. Clinical pathology of greyhounds and other sighthounds. *Vet Clin Pathol* (2011) 40(4):414–25. doi:10.1111/j.1939-165X.2011.00360.x
99. Havenith G, van Middendorp H. The relative influence of physical fitness, acclimatization state, anthropometric measures and gender on individual reactions to heat stress. *Eur J Appl Physiol Occup Physiol* (1990) 61(5–6):419–27. doi:10.1007/BF00236062
100. van Rosendal SP, Osborne MA, Fassett RG, Coombes JS. Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. *Sports Med* (2010) 40(2):113–29. doi:10.2165/11530760-000000000-00000
101. Christopherson RJ, Young BA. Heat flow between large terrestrial animals and the cold environment. *Can J Chem Eng* (1981) 59(2):181–8. doi:10.1002/cjce.5450590208
102. Ardevol A, Adan C, Remesar X, Fernandez-Lopez JA, Alemany M. Hind leg heat balance in obese Zucker rats during exercise. *Pflugers Arch* (1998) 435(4):454–64. doi:10.1007/s004240050539
103. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Engl J Med* (1995) 332(10):621–8. doi:10.1056/NEJM199503093321001
104. Buresh R, Berg K, Noble J. Heat production and storage are positively correlated with measures of body size/composition and heart rate drift during vigorous running. *Res Q Exerc Sport* (2005) 76(3):267–74. doi:10.1080/02701367.2005.10599298
105. Taylor CR, Schmidt-Nielsen K, Raab JL. Scaling of energetic cost of running to body size in mammals. *Am J Physiol* (1970) 219(4):1104–7.
106. Cheuvront SN, Haymes EM. Thermoregulation and marathon running. *Sports Med* (2001) 31(10):743–62. doi:10.2165/00007256-200131100-00004
107. Duffield R, Coutts AJ, Quinn J. Core temperature responses and match running performance during intermittent-sprint exercise competition in warm conditions. *J Strength Cond Res* (2009) 23(4):1238–44. doi:10.1519/JSC.0b013e318194e0b1
108. Gavini CK, Mukherjee S, Shukla C, Britton SL, Koch LG, Shi H, et al. Leanness and heightened nonresting energy expenditure: Role of skeletal muscle activity thermogenesis. *Am J Physiol Endocrinol Metab* (2014) 306(6):E635–47. doi:10.1152/ajpendo.00555.2013
109. Dobson GP, Parkhouse WS, Weber JM, Stuttard E, Harman J, Snow DH, et al. Metabolic changes in skeletal-muscle and blood of greyhounds during 800-m track sprint. *Am J Physiol* (1988) 255(3):R513–9.

Conflict of Interest Statement: Funding for this study was provided by Greyhound Racing South Australia and JM is employed as a racetrack veterinarian for greyhound races in South Australia. The authors believe that neither of these perceived conflicts influenced the design, analysis and interpretation of the data presented.

ORIGINAL RESEARCH
published: 02 August 2016
doi: 10.3389/fvets.2016.00059

The Effects of Varying Concentrations of Dietary Protein and Fat on Blood Gas, Hematologic Serum Chemistry, and Body Temperature Before and After Exercise in Labrador Retrievers

*John Ober[1], Robert L. Gillette[2], Thomas Craig Angle[2], Pamela Haney[2], Daniel J. Fletcher[1] and Joseph J. Wakshlag[1]**

[1] Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2] Animal Health and Performance Center, Auburn University College of Veterinary Medicine, Auburn, AL, USA

OPEN ACCESS

Edited by:
Cynthia M. Otto, University of Pennsylvania, USA

Reviewed by:
Brian Michael Zanghi, Nestlé Purina PetCare Basic Research Group, USA
Michael Davis, Oklahoma State University, USA

***Correspondence:**
Joseph J. Wakshlag
dr.joesh@gmail.com

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: 24 February 2016
Accepted: 12 July 2016
Published: 02 August 2016

Citation:
Ober J, Gillette RL, Angle TC, Haney P, Fletcher DJ and Wakshlag JJ (2016) The Effects of Varying Concentrations of Dietary Protein and Fat on Blood Gas, Hematologic Serum Chemistry, and Body Temperature Before and After Exercise in Labrador Retrievers. Front. Vet. Sci. 3:59. doi: 10.3389/fvets.2016.00059

Optimal dietary protocols for the athletic canine are often defined by requirements for endurance athletes that do not always translate into optimal dietary interventions for all canine athletes. Prior research studying detection dogs suggests that dietary fat sources can influence olfaction; however, as fat is added to the diet the protein calories can be diminished potentially resulting in decreased red blood cell counts or albumin status. Optimal macronutrient profile for detection dogs may be different considering the unique work they engage in. To study a calorically low protein: high fat (18:57% ME), high protein: high fat (27:57% ME), and high protein: low fat (27:32% ME) approach to feeding, 17 dogs were provided various diets in a 3 × 3 cross over design. Dogs were exercised on a treadmill and blood was taken pre-exercise, immediately post-exercise, 10- and 20-min post-exercise to assess complete blood count, serum chemistry, blood gases, and cortisol; as well as rectal and core body temperature. Exercise induced a decrease in serum phosphorus, potassium, and increases in non-esterified fatty acids and cortisol typical of moderate exercise bouts. A complete and balanced high protein: high-fat diet (27:57% ME) induced decreases in serum cortisol and alkaline phosphatase. Corn oil top dressed low protein: high-fat diet (18:57% ME) induced a slightly better thermal recovery than a complete and balanced high protein: high fat diet and a high protein: low fat (27%:32% ME) diet suggesting some mild advantages when using the low protein: high fat diet that warrant further investigation regarding optimal protein and fat calories and thermal recovery.

Keywords: dietary fat, lactate, protein, fat, performance, body temperature

Abbreviations: BCS, body condition score; ME, metabolic energy.

INTRODUCTION

Research surrounding optimal feeding patterns for the canine athlete has been investigated since the early 1930s with significant advances in feeding endurance canine athletes (1–3). Considerable debate regarding the optimal amount of fat and protein needed to fuel the canine athlete continues; however, Kronfeld and colleagues suggest that carbohydrate is not necessary in the endurance dog diet (4). Downey further clarified this idea studying beagles on treadmills showing that stamina improved when utilizing higher fat diets with approximately 69% metabolizable energy (ME) as fat versus 27% ME (5). Later observations showed that protein needed to be approximately 32% ME for endurance huskies to maintain their red blood cell volume for the duration of a training and racing season (6) with follow up studies examining sprint racing huskies suggesting that approximately 24% ME from protein was required to maintain optimal performance (7). Greyhound studies are less clear with consensus being that protein should be around 24% ME or higher (8). This has led to firm recommendations that performance dogs be fed over 24% ME as protein for all performance parameters to maintain red cell mass, maintain serum albumin status and promote less musculoskeletal injury; while the remaining caloric intake come from fat or carbohydrate depending on the athletic endeavor (1–3).

Even less is known about the needs of hunting and detection dogs whose primary function is olfactory activity. Many trainers and hunters provide high quality commercial dog foods with a higher fat content adopting much of the nutritional dogma of endurance sled dogs. An interesting study examined performance of plantation hunting pointers on two dog foods with similar nutrient profiles, differing minimally in protein (24–26% ME) and more from fat (10% ME difference), which showed that finds per hour were significantly lower when dogs were on the lower fat diet suggesting that a change in commercial diet can influence detection (9). Further work examining fat intake and olfaction showed that polyunsaturated fatty acids improved or maintained the efficiency of olfaction in trained pointers, while dogs fed coconut oil showed a loss of olfactory acuity (10). These findings are similar to rodent studies which suggested that polyunsaturated fatty acids may affect olfaction positively by altering the olfactory bulb cellular constitution, thereby enhancing neuronal signaling (11, 12). More recently, our lab reported that the use of corn oil improved the olfactory acuity at threshold concentrations in trained detection dogs showing that dietary fat and fat source can play a role in olfaction and/or cognition, yet the physiology of such improvements can only be speculated (13). Theoretically, dietary fat may offer an advantage of running with more thermodynamic efficiency compared to a higher protein, higher carbohydrate diets since there is less of a need for gluconeogenesis during exercise eliminating protein as a source of energy which is thermodynamically less efficient than fat or carbohydrate (6). Contradictory results suggest that glucose helps decrease thermal load by improving respiratory dynamics and loss of heat through panting (14). Based on contradictory theories, our objectives were to utilize the same Labrador Retrievers used in our previous olfaction study, to examine the effects of diet and exercise on serum lactate, blood gases, cortisol response, complete blood counts, serum biochemistry, and body temperature (rectal and core body temperature), to better understand the physiology that might explain enhanced olfaction when fed a corn oil-supplemented diet (13). Seventeen detection dogs on two standard commercial dog foods that consisted of either low fat (LF – 27% ME protein, 32% ME fat) or high-fat performance diet (HF – 27% ME protein, 57% ME fat); as well as a low protein food with high fat made through top dressing a typical adult maintenance diet with corn oil (CO – 18% ME protein, 57% ME fat) in a cross-over design before and after exercise.

MATERIALS AND METHODS

Animal Housing and Feeding

The use of animals in this study was approved by the Auburn University Animal Care and Use Committee. All dogs were housed in a 1.3 m × 2.6 m enclosure, which had an attached 1.3 m × 4 m outdoor facility that was available to the dogs daily. Seventeen sexually intact Labrador Retrievers (11 males and 7 females) between the ages of 18 and 24 months were enrolled in the study, with one male dog being discontinued within 4 weeks of starting the study due to an acute cranial cruciate tear. Throughout each 12-week "arm" of each treatment dogs were fed either a normal maintenance diet[1] [Royal Canin Maintenance; 27% ME protein, 32% ME as fat, 41% ME as carbohydrate (LF)], a performance diet[2] [27% ME protein, 57% ME fat, 14% ME carbohydrate (HF)], or a maintenance diet (see text footnote 1) with additional corn oil[3] [14.5 g of corn oil per cup of kibble fed at 269 kcals/cup; 18% ME protein, 57% ME fat, 25% ME carbohydrate (CO)]. Grams of protein intake per kg body weight for each dietary group median and range was 4.5 g/kg (3.8–6.2 g/kg) in the LF feeding, 4.4 g/kg (3.7–6.0 g/kg) when consuming the HF diet and 3.1 g/kg (2.7–4.4 g/kg) when consuming the CO diet. Each dog was fed to maintain body condition between a body condition score of 4 or 5 out of 9 and to maintain body weight from the beginning of the feeding trial until the end with kilocalorie consumption never varying more than ±15% of the caloric intake at the beginning of the dietary trial. Dog weights were recorded each week, and diets were adjusted to ensure that that the dogs did not lose or gain more than 5% of their body weight.

Conditioning Protocol

All dogs were trained and conditioned for detection performance activities by road training (30 min on the end of a rod attached to a golf cart) and 30 min of scent-detection training (smokeless powder, ammonium nitrate, and trinitrotoluene in decreasing concentrations) five times per week. All dogs were acclimated to treadmill by running 15–20 min once a week during their training and a conditioning regimen that was approximately 6 months prior to the dietary study. All dogs were between 2

[1]Low Fat (LF) diet: Royal Canin 2500, Armitage, France
[2]High Fat (HF) diet: Royal Canin 4800. Armitage, France
[3]Corn oil, ACH Food Companies, Cordova, TN, USA

and 3 years of age and had been trained for detection activity in the urban and rural field settings. Three months prior to the study, all dogs were fed a typical maintenance ration (see text footnote 1). Dogs were then randomly drawn from a hat and partitioned into three dietary groups. Conditioning and training protocols remained the same for all dogs during the dietary trials each of which lasted 12 weeks; thus, each dog served as its own control during the dietary trials.

Exercise Test

To examine the effects of diet and exercise, at the end of each 12-week arm, dogs ran on a stationary treadmill for 30 min daily at a 2.5% incline at 12.5 km/h on three consecutive days.[4] Standard commercial fans were mounted at eye level with the dogs, just to the left of the treadmills and put on medium settings (3000 CFU) to simulate wind rushing across the nasal and oral cavities. The rooms where the dogs ran were kept at 30% humidity and 72°F at all times. All data and sample collection occurred on day 3 of the 3 days of treadmill testing on week 12, while olfaction data were obtained on the other 2 days of exercise (13).

Rectal and Core Temperature Monitoring

Before running, the dogs' body temperature was assessed through core body temperature and rectal temperature monitoring. Core body temperature was assessed by administering a small thermistor pill[5] 20 min before the exercise bout and temperatures were taken immediately before exercise and then every 5 min during exercise, and immediately after exercise as well as 10 and 20 min intervals during recovery in tandem with rectal temperature acquisition. Rectal temperatures were assessed using the Becton Dickenson digital thermometer.[6] All readings were made approximately 1 min prior to treadmill exercise, immediately after coming off of the treadmill, and then again 10 and 20 min into recovery post-exercise.

Blood and Serum Collection and Analysis

Jugular venipuncture was performed on the third day at the aforementioned time intervals (pre, post, 10 min after, and 20 min after) for assessment of blood gases, complete blood count (including: red blood cells, hematocrit, hemoglobin, white blood cells, neutrophils, and lymphocytes), serum biochemistry [including; sodium, potassium, phosphorus, calcium, magnesium, chloride, albumin, globulin, urea nitrogen, creatinine, alanine amino transferase (ALT), aspartate amino transferase (AST), creatine kinase, non-esterified fatty acid, glucose], and cortisol concentrations. Venous blood gas analysis was performed on iSTAT analyzer[7] using the CG8+ cartridge[8] within 2 min of sample collection. The remaining sample was aliquoted into a coagulation tube or EDTA tube and refrigerated for approximately 30 min until centrifuged at 4,000 *g* for 10 min for serum collection. EDTA collected blood was shipped overnight on ice packs to Antech laboratory and analyzed using an Advia 120 cell counter.[9] Separated serum was frozen at −20°C and within a week all samples were shipped on dry ice overnight to the Animal Health Diagnostic Laboratory at Cornell University for analysis on a Hitachi 911 analyzer.[10] Serum cortisol concentrations were analyzed using validated canine radioimmunoassay at the Cornell University Diagnostic Laboratory Endocrinology Unit.

Statistical Analysis

To account for the study design, a mixed model analysis of variance with the fixed effects of time, diet, period of treatment, and sequence of treatment was chosen. The interaction of time and diet was forced into every model. Random effects in the model were ID and period nested within ID. Residuals were examined for normality. In cases were residuals were non-normally distributed, a log transformation of the dependent variable was successful to satisfy the model assumptions. *P*-values are reported for the fixed effects of time, diet, and diet × time. If the *P*-value for the effect of time or diet reached significance (*P* smaller 0.05), a Dunnett's *post hoc* analysis was performed to examine the strength of association from pre-exercise or the low-fat diet, controlling for multiple comparisons. Significance was set at an alpha of 0.05. JMP 12.0[11] was used for all statistical analyses, and Graph pad Prism 6.0[12] was used for graphing.

RESULTS

Complete Blood Count

There were no significant interactions of diet and exercise for red blood cells ($P = 0.96$), hematocrit ($P = 0.99$), hemoglobin ($P = 0.97$), white blood cells ($P = 0.61$), neutrophils ($P = 0.97$), or lymphocytes ($P = 0.71$) (**Table 1**).

Dietary differences were not observed for HCT ($P = 0.16$) or lymphocytes ($P = 0.24$). There were dietary decreases observed for the CO group compared to the LF and HF groups for red blood cells ($P < 0.01$), hemoglobin ($P = 0.04$), white blood cells ($P < 0.01$), and neutrophils ($P = 0.01$).

There were significant differences observed across time for hematocrit ($P < 0.01$), red blood cells ($P < 0.01$), hemoglobin ($P < 0.01$), white blood cells ($P = 0.04$), and lymphocytes ($P < 0.01$). Numerically, red blood cell and HCT concentrations increased post-exercise due to mild hemoconcentration; while lymphocytes decreased post-exercise and remained lower during recovery ($P < 0.01$). This was also reflected in a lower total white blood cell count at 20 min of recovery ($P = 0.04$).

Venous Blood Gas Analysis

There were no interactions between diet and exercise observed for base excess ($P = 0.17$), $pvCO_2$ ($P = 0.10$), TCO_2 ($P = 0.16$), bicarbonate ($P = 0.19$), and pH ($P = 0.17$) (**Table 2**).

[4] Jog a dog Treadmill, Jog a Dog LLC, Ottawa Lake, MI, USA
[5] Core Temp Sensor, HQ Inc., Palmetto, FL, USA
[6] Digital Rectal Thermometer, Becton Dickenson, Franklin Lakes, NJ, USA
[7] iSTAT, Abbott Care Inc., Princeton, NJ, USA
[8] CG8 cartridge, Abbott Care Inc., Princeton, NJ, USA
[9] Advia 120, Siemens Corp., Washington, DC, USA
[10] Hitachi 911, Roche Diagnostics, Risch-Rotkreuz, Switzerland
[11] JMP 12.0, SAS Institute, Cary, NC, USA
[12] Graph pad Prism, LaJolla, CA, USA

TABLE 1 | Mean and SD for complete blood count pre and post exercise; 10-min post exercise and 20-min post exercise for three dietary groups (*n* – 17) during feeding a high-fat high-protein diet (HF), high corn oil fat low-protein diet (CO), and a low-fat high-protein diet (LF).

CBC (ref. range)	Diet	Pre	Post	Post 10 Min	Post 20 Min	Time	Diet	Time × Diet
RBC (millions/mL) (4.8–9.3)	HF	6.6 ± 0.4	**7.1 ± 0.3**	6.6 ± 0.4	6.5 ± 0.4	$P < 0.01$	$P < 0.01$	$P = 0.96$
	CO[a]	6.5 ± 0.6	**6.9 ± 0.6**	6.5 ± 0.5	6.4 ± 0.5			
	LF	6.6 ± 0.4	**7.1 ± 0.5**	6.7 ± 0.5	6.5 ± 0.5			
HCT (%) (36–60)	HF	49.1 ± 3.0	**52.1 ± 2.4**	49.2 ± 3.6	48.8 ± 3.1	$P < 0.01$	$P = 0.16$	$P = 0.99$
	CO	48.5 ± 3.8	**50.9 ± 3.0**	47.8 ± 4.1	47.3 ± 3.9			
	LF	48.4 ± 3.9	**51.5 ± 3.3**	48.8 ± 3.9	47.9 ± 4.6			
Hemoglobin (g/L) (12.1–20.3)	HF	15.6 ± 0.8	**16.5 ± 0.8**	15.4 ± 1.0	15.2 ± 1.0	$P < 0.01$	$P = 0.04$	$P = 0.97$
	CO[a]	15.3 ± 1.2	**16.0 ± 1.1**	15.0 ± 1.1	15.0 ± 1.1			
	LF	15.3 ± 0.9	**16.3 ± 1.2**	15.4 ± 1.1	15.1 ± 1.1			
WBC (thousands/mL) (4–15.5)	HF	9.5 ± 2.4	9.2 ± 2.6	9.2 ± 2.4	**9.0 ± 2.4**	$P = 0.04$	$P < 0.01$	$P = 0.61$
	CO[a]	8.8 ± 1.9	8.7 ± 2.1	8.9 ± 1.7	**8.4 ± 2.0**			
	LF	9.7 ± 1.7	8.7 ± 1.9	8.9 ± 1.7	**9.0 ± 1.8**			
Neutrophils (2060–10600)	HF	6003 ± 1972	6292 ± 2086	6103 ± 1988	6026 ± 1954	$P < 0.48$	$P = 0.01$	$P = 0.97$
	CO[a]	5557 ± 1280	5831 ± 1580	5839 ± 1336	5477 ± 1428			
	LF	5949 ± 1296	5823 ± 1363	5826 ± 1284	5694 ± 1297			
Lymphocytes (690–4500)	HF	2406 ± 718	**2028 ± 622**	**2167 ± 603**	**2099 ± 675**	$P < 0.01$	$P = 0.24$	$P = 0.71$
	CO	2368 ± 841	**2039 ± 724**	**2150 ± 588**	**2118 ± 710**			
	LF	2611 ± 510	**1967 ± 537**	**2221 ± 476**	**2311 ± 520**			

Bolded text indicates a difference from pre-exercise over time; [a]In dietary groups represents a significant difference between groups (P < 0.05).

TABLE 2 | Mean and SD for blood gases pre and post exercise; 10-min post exercise and 20-min post exercise for three dietary groups (*n* – 17) during feeding a high-fat high-protein diet (HF), high corn oil fat low protein diet (CO), and a low-fat high-protein diet (LF).

	Diet	Pre	Post	Post 10 Min	Post 20 Min	Time	Diet	Diet × time
Base Exc. (mmol/L)	HF	−2.5 ± 1.5	**−4.5 ± 1.3**	**−5.4 ± 1.5**	**−5.2 ± 1.7**	$P < 0.01$	$P < 0.16$	$P = 0.17$
	CO	−2.2 ± 1.7	**−4.0 ± 1.7**	**−4.5 ± 1.5**	**−4.3 ± 1.8**			
	LF	−1.6 ± 1.6	**−4.1 ± 1.9**	**−5.0 ± 2.0**	**−4.8 ± 2.1**			
pCO_2 (mmHg)	HF	33.9 ± 3.4	**22.3 ± 6.0**	**24.9 ± 6.1**[b]	**28.4 ± 5.8**	$P < 0.01$	$P < 0.01$	$P < 0.10$
	CO[a]	35.0 ± 3.3	**21.7 ± 4.9**	**27.7 ± 4.6**[a]	**30.3 ± 3.8**			
	LF	35.1 ± 5.0	**20.3 ± 7.7**	**23.8 ± 6.5**[b]	**29.0 ± 5.0**			
TCO_2 (mmHg)	HF	22.9 ± 1.9	**18.8 ± 2.3**	**18.7 ± 2.4**	**19.7 ± 2.4**	$P < 0.01$	$P < 0.28$	$P < 0.16$
	CO	23.4 ± 2.1	**19.1 ± 2.0**	**20.2 ± 1.9**	**20.8 ± 2.0**			
	LF	23.8 ± 2.0	**18.6 ± 3.5**	**19.0 ± 3.1**	**20.2 ± 2.4**			
HCO3 (mmol/L)	HF	22.0 ± 1.7	**18.1 ± 2.1**	**18.1 ± 2.2**	**18.9 ± 2.1**	$P < 0.01$	$P = 0.22$	$P = 0.19$
	CO	22.5 ± 1.9	**18.4 ± 1.9**	**19.4 ± 1.8**	**19.9 ± 1.8**			
	LF	22.8 ± 1.8	**17.9 ± 3.3**	**18.2 ± 2.9**	**19.4 ± 2.2**			
pH	HF	7.42 ± 0.02	**7.53 ± 0.09**	**7.48 ± 0.07**	7.44 ± 0.06	$P < 0.01$	$P = 0.11$	$P = 0.17$
	CO	7.42 ± 0.02	**7.55 ± 0.08**	**7.46 ± 0.05**	7.43 ± 0.02			
	LF	7.43 ± 0.04	**7.58 ± 0.12**	**7.50 ± 0.06**	7.44 ± 0.05			

Bolded text indicates a difference from pre-exercise over time; [a]In dietary groups represents a significant difference between groups (P < 0.05). [a,b]Across time points represent significant differences (P < 0.05) between groups at individual times with similar or no superscripts signifying no differences.

No effects of diet were observed on blood gases other than an overall increased $pvCO_2$ ($P = 0.01$). Dogs consuming the CO diet had significantly higher $pvCO_2$ 10-min post-exercise than dogs consuming the LF diet or HF diet ($P = 0.02$).

Immediately after exercise, the $pvCO_2$ was lower immediately post-exercise, and 10-min post exercise, and returned to pre-exercise tensions at 20-min post exercise ($P < 0.01$). Blood base excess and bicarbonate concentrations were also lower immediately post-exercise and remained lower than pre-exercise values at 10- and 20-min post-exercise ($P < 0.05$). Following the exercise bout, all groups had significantly higher blood pH ($P < 0.05$), which recovered over time, approaching pre-exercise values by 20-min post-exercise.

Serum Chemistry

There were no significant diet or exercise interactions observed (all values $P > 0.1$) (**Table 3**).

There were significant diet effects on small number of chemistry parameters. The CO diet resulted in overall lower serum urea nitrogen concentrations ($P = 0.01$), while the HF diet resulted in lower alkaline phosphatase concentrations ($P < 0.01$). The HF dietary treatment also resulted in significantly higher phosphorus

TABLE 3 | Mean and SD for serum biochemistry pre and post exercise; 10-min post-exercise and 20-min post-exercise for three dietary groups (*n* – 17) during feeding a high-fat high-protein diet (HF), high corn oil fat low protein diet (CO), and a low-fat high-protein diet (LF).

Serum Biochem (Ref. range)	Diet	Pre	Post	Post 10 Min	Post 20 Min	Time	Diet	Diet × time
Sodium (mEq/L) (142–151)	HF	147 ± 2	148 ± 2	148 ± 2	**149 ± 2**	$P < 0.01$	$P = 0.67$	$P = 0.91$
	CO	148 ± 3	147 ± 2	148 ± 2	**149 ± 2**			
	LF	147 ± 2	148 ± 2	148 ± 2	**149 ± 2**			
Chloride (mEq/L) (107–117)	HF[a]	113 ± 2	114 ± 2	**115 ± 2**	**115 ± 2**	$P < 0.01$	$P < 0.01$	$P = 0.36$
	CO	112 ± 2	114 ± 2	**115 ± 2**	**115 ± 2**			
	LF	112 ± 2	114 ± 2	**114 ± 2**	**115 ± 2**			
Potassium (mEq/L) (2.9–5.3)	HF	4.3 ± 0.2	**4.5 ± 0.2**	**4.1 ± 0.2**	**4.0 ± 0.3**	$P < 0.01$	$P = 0.24$	$P = 0.21$
	CO	4.2 ± 0.3	**4.5 ± 0.2**	**4.0 ± 0.2**	**4.0 ± 0.1**			
	LF	4.3 ± 0.3	**4.5 ± 0.2**	**4.0 ± 0.3**	**4.1 ± 0.3**			
Phosphorus (mg/dL) (2.8–5.3)	HF[a]	4.7 ± 0.6	**4.2 ± 0.3**	**3.8 ± 0.5**	**3.7 ± 0.7**[a]	$P < 0.01$	$P = 0.01$	$P = 0.99$
	CO	4.4 ± 0.5	**3.9 ± 0.6**	**3.5 ± 0.7**	**3.3 ± 0.8**[b]			
	LF	4.3 ± 0.5	**3.9 ± 0.5**	**3.4 ± 0.7**	**3.2 ± 0.9**[b]			
Calcium (mg/dL) (9.3–11)	HF	10.4 ± 0.3	**10.2 ± 0.2**	10.2 ± 0.4	**10.1 ± 0.4**	$P < 0.01$	$P = 0.17$	$P = 0.67$
	CO	10.5 ± 0.3	**10.3 ± 0.3**	10.3 ± 0.3	**10.3 ± 0.3**			
	LF	10.4 ± 0.3	**10.2 ± 0.2**	10.2 ± 0.3	**10.2 ± 0.3**			
Magnesium (mg/dL) (1.4–2.0)	HF	1.6 ± 0.1	**1.5 ± 0.1**	**1.5 ± 0.1**	**1.5 ± 0.1**	$P < 0.01$	$P = 0.07$	$P = 0.33$
	CO	1.7 ± 0.1	**1.6 ± 0.1**	**1.5 ± 0.1**	**1.5 ± 0.1**			
	LF	1.6 ± 0.1	**1.5 ± 0.1**	**1.5 ± 0.1**	**1.5 ± 0.1**			
Alk phos (U/L) (12–122)	HF[a]	28 ± 13[a]	**30 ± 12**[a]	28 ± 12[a]	28 ± 12[a]	$P = 0.04$	$P < 0.01$	$P = 0.93$
	CO	48 ± 26[b]	**49 ± 26**[b]	46 ± 25[b]	47 ± 25[b]			
	LF	54 ± 24[b]	**56 ± 24**[b]	53 ± 24[b]	52 ± 22[b]			
AST (U/L) (16–50)	HF	41 ± 13	43 ± 13	**45 ± 15**	44 ± 14	$P < 0.01$	$P = 0.10$	$P = 0.98$
	CO	35 ± 8	38 ± 9	**41 ± 14**	36 ± 7			
	LF	39 ± 9	42 ± 10	**44 ± 14**	41 ± 8			
ALT (U/L) (25–106)	HF	53 ± 17	55 ± 17	55 ± 17	53 ± 17	$P = 0.07$	$P = 0.09$	$P = 0.13$
	CO	51 ± 10	54 ± 11	53 ± 11	52 ± 10			
	LF	58 ± 16	61 ± 18	60 ± 17	59 ± 16			
Urea (mg/dL) (8–30)	HF	21 ± 3[b]	22 ± 3[b]	22 ± 3[b]	22 ± 3[b]	$P = 0.13$	$P = 0.01$	$P = 0.99$
	CO[a]	15 ± 3[a]	16 ± 3[a]	16 ± 3[a]	16 ± 3[a]			
	LF	20 ± 3[b]	20 ± 3[b]	21 ± 3[b]	20 ± 4[b]			
Creatinine (mg/dL) (0.5–1.3)	HF	1.1 ± 0.1	**1.2 ± 0.2**	**1.2 ± 0.2**	1.2 ± 0.2	$P = 0.01$	$P = 0.18$	$P = 0.84$
	CO	1.1 ± 0.1	**1.2 ± 0.1**	**1.2 ± 0.2**	1.2 ± 0.2			
	LF	1.1 ± 0.2	**1.2 ± 0.2**	**1.2 ± 0.2**	1.2 ± 0.2			
Creat. kinase (U/L) (58–241)	HF	123 ± 46	**135 ± 42**	**134 ± 42**	132 ± 54	$P = 0.05$	$P = 0.37$	$P = 0.68$
	CO	106 ± 35	**116 ± 35**	**124 ± 62**	99 ± 35			
	LF	128 ± 64	**140 ± 90**	**144 ± 111**	141 ± 78			
Albumin (g/dL) (3.1–4.1)	HF	3.6 ± 0.2	**3.7 ± 0.2**	**3.7 ± 0.2**	3.6 ± 0.2	$P < 0.01$	$P = 0.08$	$P = 0.72$
	CO	3.7 ± 0.2	**3.8 ± 0.2**	**3.7 ± 0.2**	3.7 ± 0.2			
	LF	3.6 ± 0.1	**3.7 ± 0.2**	**3.7 ± 0.2**	3.6 ± 0.2			
Globulin (g/dL) (1.9–3.6)	HF	2.1 ± 0.3	**2.2 ± 0.3**	2.2 ± 0.4	2.2 ± 0.3	$P < 0.01$	$P = 0.65$	$P = 0.87$
	CO	2.1 ± 0.3	**2.1 ± 0.2**	2.1 ± 0.3	2.1 ± 0.2			
	LF	2.1 ± 0.2	**2.2 ± 0.2**	2.2 ± 0.3	2.1 ± 0.2			

Bolded text indicates a difference from pre-exercise over time; [a]In dietary groups represents a significant difference between groups (P < 0.05). [a,b]Across time points represent significant differences (P < 0.05) between groups at individual times with similar or no superscripts signifying no differences.

($P = 0.01$) and chloride concentrations ($P < 0.01$) than the CO or LF dietary treatments. At 20-min post-exercise, serum phosphorus was significantly higher in dogs that had consumed the HF diet ($P = 0.04$).

There were modest effects of exercise observed with serum phosphorus concentrations being significantly lower in all diet groups 10-min post-exercise and at 20-min post-exercise ($P < 0.01$). Concurrently, serum magnesium showed a similar decrease with significantly lower concentrations at immediately post, 10 and 20 min of recovery compared to pre-exercise concentrations ($P < 0.05$). Creatine kinase and creatinine also showed similar more transient increased in serum concentrations post-exercise, which returned to pre-exercising concentrations by 20 min of recovery ($P < 0.01$). Albumin and globulin also showed transient increases immediately post and 10 min after exercise when compared to pre-exercise values ($P < 0.01$).

Lactate, Glucose, Non-Esterified Fatty Acids, and Serum Cortisol

There were no diet and exercise interactions or significances observed for glucose ($P = 0.05$), lactate ($P = 0.26$), non-esterified fatty acids ($P = 0.76$), or cortisol ($P = 0.26$) (**Table 4**).

TABLE 4 | Mean and SD for glucose, non-esterified fatty acid, and cortisol; pre-exercise, post-exercise, 10-min post exercise and 20-min post exercise for three dietary groups (*n* – 17) during feeding a high-fat high-protein diet (HF), high corn oil fat low protein diet (CO), and a low-fat high-protein diet (LF).

Metab/hormones	Diet	Pre	Post	Post 10 min	Post 20 min	Time	Diet	Diet × time
Glucose (mg/dL) (60–120)	HF	98 ± 9	100 ± 10	99 ± 9	100 ± 10[b]	$P < 0.01$	$P = 0.05$	$P = 0.16$
	CO[a]	96 ± 10	103 ± 11	**105 ± 10**	**108 ± 7**[a]			
	LF	96 ± 10	103 ± 10	**103 ± 11**	102 ± 9[b]			
Lactate (mmol/L) (0.5–2)	HF	0.50 ± 0.26	**1.35 ± 0.82**	**1.36 ± 0.83**	**1.17 ± 0.62**	$P < 0.01$	$P < 0.01$	$P = 0.26$
	CO	0.51 ± 0.21	**1.42 ± 1.25**	**1.37 ± 1.16**	**1.10 ± 0.84**			
	LF[a]	0.56 ± 0.16	**1.98 ± 1.08**[a]	**1.93 ± 1.06**	**1.63 ± 0.87**			
NEFA (mg/dL) (0–1)	HF	0.48 ± 0.16	**1.15 ± 0.39**	**1.14 ± 0.47**	**0.73 ± 0.35**	$P < 0.01$	$P = 0.72$	$P = 0.76$
	CO	0.52 ± 0.23	**1.21 ± 0.41**	**1.10 ± 0.37**	**0.83 ± 0.35**			
	LF	0.38 ± 0.24	**1.21 ± 0.42**	**1.11 ± 0.44**	**0.74 ± 0.39**			
Cortisol (μg/mL) (1–4)	HF[a]	1.22 ± 0.52	**2.93 ± 2.31**[a]	**2.54 ± 1.89**[a]	**1.94 ± 1.52**[a]	$P < 0.01$	$P < 0.01$	$P = 0.26$
	CO	1.63 ± 1.15	**3.52 ± 2.21**[a,b]	**2.91 ± 1.79**[a,b]	**2.25 ± 1.40**[a,b]			
	LF	1.25 ± 0.37	**4.58 ± 2.42**[b]	**4.30 ± 2.67**[b]	**3.56 ± 2.36**[b]			

Bolded text indicates a difference from pre-exercise over time; [a]In dietary groups represents a significant difference between groups (P < 0.05). [a,b]Across time points represent significant differences (P < 0.05) between groups at individual times with similar or no superscripts signifying no differences.

There were significant effects of diet observed for glucose ($P = 0.05$) with mean concentrations being higher for the CO diet compared to LF and HF. Dogs consuming the CO diet showed a mild, yet significant increase in serum glucose at 20-min post exercise ($P = 0.01$). Mean serum lactate ($P < 0.01$) was significantly higher for dogs in the LF treatments compared to the HF and CO treatments. Lactate in dogs consuming the LF diet was higher than either the CO or HF diets post-exercise ($P < 0.05$). Serum cortisol concentrations were lower for dogs on the HF diet ($P < 0.01$). Dogs consuming the HF diet had significantly lower serum cortisol concentrations at all post-exercise time points than the LF-fed dogs ($P < 0.01$).

The effects of exercise over time showed significances across all four parameters tested. All test subjects had increased serum lactate concentration ($P < 0.01$) immediately post-exercise and thereafter when compared to pre-exercise ($P < 0.01$). Serum non-esterified fatty acids were increased in all dogs regardless of dietary group immediately post-exercise and remained elevated compared to the pre-exercise values ($P < 0.01$). Serum cortisol concentrations were higher immediately post-exercise in all groups ($P < 0.01$). They remained significantly increased at 10- and 20-min post-exercise, though gradually returning toward baseline values.

Core and Rectal Body Temperatures

There were no diet and exercise interactions observed for core body temperature ($P = 0.32$); however, there was an effect of diet observed with overall lower core temperatures ($P < 0.01$) in the dogs on the CO diet. Similarly, dogs consuming the CO diet had significantly ($P < 0.05$) lower core temperature 10 and 20 min after exercise when compared to the LF ration (**Figure 1**). Pre-exercise the core body temperatures were not significantly different (HF – 101.2 ± 0.8°F; LF – 101.2 ± 0.6°F; CO – 101.1 ± 0.6°F) with significant rises post-exercise ($P < 0.01$) across all groups (HF – 105.0 ± 1.0°F; LF – 105.2 ± 1.3°F; 104.8 ± 1.2°F). At 10-min post-exercise and 20-min post-exercise, core body temperatures were still elevated compared to pre-exercise (10-min post: HF – 104.3 ± 1.1°F; LF – 104.7 ± 1.2°F; CO – 104.0 ± 0.8°F and 20-min post: HF 103.2 ± 0.5°F; LF – 103.6 ± 1.0°F; CO –102.9 ± 0.5°F).

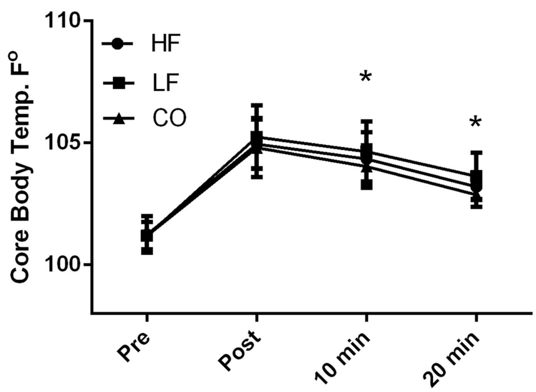


FIGURE 1 | Mean and SD of core thermister readings before immediately after, 10 min of recovery and 20 min of recovery in high-fat (HF), low-fat (LF), and corn oil (CO) treatments. Significances between dietary groups represented astericks showing CO treatment having lower temperatures than the LF group (10 min $P = 0.04$; 20 min $P = 0.02$). Time was significant between each time point for all groups ($P < 0.01$).

There were no diet and exercise interactions observed for rectal body temperature ($P = 0.45$); however, there was an effect of diet observed with overall lower rectal temperatures ($P < 0.01$) in the dogs on the CO diet. Dogs consuming the CO diet had significantly ($P < 0.05$) lower rectal temperature 20-min post-exercise than dogs consuming the HF ration (**Figure 2**). Rectal temperature was significantly ($P < 0.01$) higher immediately post-exercise in all groups, relative to baseline (Pre: HF – 101 ± 0.7°F; LF – 101.5 ± 0.7°F; CO – 101.3 ± 0.7°F and Post: HF 105.1 ± 1.4°F; LF – 104.7 ± 1.3°F; CO – 104.3 ± 1.3°F). This parameter remained significantly elevated 10-min post-exercise (HF – 102.6 ± 0.8°F; LF – 103.4 ± 1.1°F; CO – 102.8 ± 0.8°F). By 20-min post-exercise, temperatures approached baseline values and were no longer statistically significant from baseline (HF – 102.6 + 0.8°F; LF – 102.5 + 0.6°F; CO – 102.1 + 0.6°F).

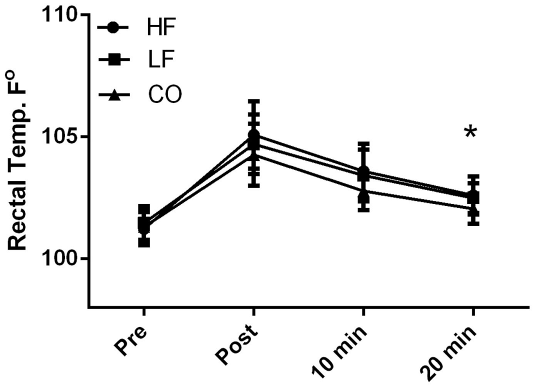


FIGURE 2 | Mean and SD of rectal thermometer readings before immediately after, 10 min of recovery and 20 min of recovery in high-fat (HF), low-fat (LF), and corn oil (CO) treatments. Significances between dietary groups represented asterisks showing CO treatment having lower temperatures than the HF group ($P = 0.03$). Time was significant between each time point for all groups ($P < 0.01$).

DISCUSSION

The effects of diet and exercise and complete blood count, serum biochemistry, blood gases, and vital parameters appear to be subtle in our study, but relevant, considering dietary change and exercise in conditioned Labradors trained for detection activities has never been assessed in a controlled manner. Our leading hypothesis was that low-protein diets would result in alterations in serum protein biochemistry, since dietary protein may play a role in maintaining appropriate red blood cell indices and it has been noted that performance dogs require 24% of their ME as high quality dietary protein to maintain performance capabilities (1–3, 7). In all of the diets used in our study, the protein base was from poultry and varied between 18 and 27% ME proving to be adequate for the dogs enrolled in this study under this type of daily exercise pattern. Our results suggest that the red blood cell counts and volumes were slightly lower universally during CO treatment with universal mild hemoconcentration occurring during exercise that was significant with modest lymphocyte decreases due to exercise (15, 16). This slightly lower red cell count with CO treatment warrants trepidation regarding feeding a lower protein diet (18% ME) due to potential long-term physiological consequences, although in this 12-week trial on each of the diets the mild alterations did not seem to affect performance according to handlers.

Serum albumin is possibly a more sensitive marker of protein deficiency than red blood cell counts. Reynolds and colleagues suggested that albumin was highest in dogs fed 32% ME as protein, while lowest in dogs fed 18% ME protein during a 16-week dietary trial (7). Serum albumin is considered a long-term marker of protein sufficiency in normal healthy dogs and we expected that serum albumin would decrease in the dogs being fed the 18% ME protein diet; however, it did not decrease over the 12-week time period. This suggests that the protein in the diet was adequate at 18% of the ME for this group of conditioned dogs; yet this feeding trial was relatively short. Longer periods of feeding may be necessary to see decreases in albumin.

Furthermore, unlike Reynolds who observed more musculoskeletal injuries when dogs were fed 18% ME (7), no musculoskeletal injuries or performance deficits during detection or conditioning activities were noted in the health records among handlers who were blinded to diet. The dogs in our study could have been consuming more protein per kilogram body weight when on the 18% ME diet than dogs in the Reynolds study; however, when examining the median and range of caloric intake from both studies, they were similar based on kilograms of body weight. Hence, we postulate that 18% ME may be adequate for low-intensity long-duration activity, but longer term feeding trials are warranted.

Further examination of the blood chemistry parameters showed a distinct change in urea concentrations due to protein consumption. Dogs fed 18 versus 27% had globally decreased serum urea nitrogen concentrations suggesting less protein metabolism in the 18% ME group. One limitation of our study was the lack of lean body mass measurement, which may have changed during the feeding of 18% ME protein, but based on handler observations this lower protein consumption did not alter performance and may actually enhance olfactory performance when tested during the same time period (13).

Much like the changes observed metabolically due to the protein differences in the diet there were some changes observed due to the fat and carbohydrate differences in ME. For the LF group, we observed a rise in serum lactate during exercise when compared to the two groups being fed 57% ME as fat. This is consistent with observations in other exercise models where dogs being fed a high-fat diet tended to have lower serum lactate (17). Consistent with the metabolic demands of exertion in the well-conditioned endurance athlete (4, 18), serum non-esterified fatty acid concentration was significantly different immediately post-exercise relative to pre-exercise values, and gradually recovered toward pre-exercise values during the ensuing 20 min of recovery for all groups.

Probably, the most interesting results from our study revolve around the vital signs of core temperature and rectal temperature. Subjects consuming the corn oil top-dressed diet showed globally lower core and rectal temperatures post-exercise and quicker thermal recovery with a significant difference at specific time points during recovery. The true impact of this in clinical/field situations remains to be fully elucidated; however, reduction of thermal stress by diet formulation represents a simple and inexpensive opportunity to potentially improve the performance of detection dogs. Formulation of rations for detection dogs for further study suggest that protein and fat (as well of fat sourcing) may be worthwhile and begs for further field exploration.

The thermal changes do not seem to be reflected in the venous blood gas changes; however, arterial blood gases would have been more appropriate for interpretation of gas exchange. It is clear that the venous pCO_2 was low in all dogs prior to exercise, which was likely from panting due to the excitement associated with the activity, further confounding any changes associated with exercise and diet (19). Corn oil consumption resulted in modestly higher venous pCO_2 than the low fat group at 10-min post-exercise. It may be that dogs consuming corn-oil experience less thermal stress during exertion, recover from it more efficiently, and may be less driven to alter respiratory pattern (panting) to recover normal body temperature retaining slightly more CO_2; but this is highly speculative base on our venous blood gas results.

The significant reduction of serum phosphorus concentration in the low-fat diet group appears perplexing; however, again this group was less conditioned for fat metabolism, hence more carbohydrate metabolism as an energy source was utilized. Glucose utilization as a major fuel to meet metabolic demands during this bout of exercise requires phosphorus during glycogenolysis to form glucose-1-phosphate and glucose-6-phosphate potentially leading to an intracellular phosphorus flux during exercise as observed in other exercising canine studies (20, 21). The reason for no dietary differences in phosphorus influx is likely due to the exercise bout being well within the range the time range for carbohydrate oxidation, which relies on either glycogenolysis and to a lesser degree protein or fat metabolism (22). In addition, the CO group had similarly lower serum phosphorus to the LF group, which might be a reflection of overall lower phosphorus in the diet due to corn oil dilution of overall phosphorus intake in this group.

Cortisol has been used as an indicator of stress and is often elevated in acute exercise stress models (15, 23). The dogs consuming 57% of ME as fat in a complete diet had a statistically and physiologically significant reduction in serum cortisol concentration. We cannot explain why this did not happen when the dogs consumed kibble top-dressed with corn oil, with the possible exception that the latter diet was obviously not properly balanced following the addition of the corn oil, with the HF diet often having higher concentrations of many vitamin and mineral; or possibly that the high polyunsaturated fat in the corn oil diet can influence serum cortisol (13). Whether these modest differences in cortisol influence cellular proteolysis or glycogenesis/glycogenolysis remain to be elucidated, yet there were no changes in rudimentary serum glucose or non-esterified fatty acids that could be explained by the modest alterations in serum cortisol other than a mild elevation in serum glucose in the CO group at 20 min of recovery that was not seen in the LF or the HF group. Further study of nutritional influences on cortisol secretion and synthesis are needed to better understand these findings. Interestingly, serum alkaline phosphatase was significantly lower in dogs consuming the HF diet. We posit that the lower alkaline phosphatase of dogs consuming a nutritionally complete and balanced high-fat diet is consistent with their lower serum cortisol concentration, representing a basis for reduced metabolic stress.

CONCLUSION

For working dogs, the lifetime cost of feed, even if specially formulated, represents a trivial fraction of the monetary investment in training and purchase. Formulation of a nutritionally complete and balance ration with 18% of ME as protein and polyunsaturated fat as the primary fat source may aid in post-exercise thermoregulation, support maintenance of body weight, albumin, and hemogram profiles; and offer potential improvements in challenging work situations during short-term feeding (12 weeks). These data suggest that diet can affect baseline cortisol secretion and ameliorate dissipation of body heat and further study and optimization of this dietary strategy may be worthwhile for detection dogs.

AUTHOR CONTRIBUTIONS

JW, TA, and RG postulated the experimental design. PH, JW, RG, TA, and JO performed work associated with this study. RG, JW, JO, and DF performed statistical analysis and prepared manuscript. All authors reviewed manuscript upon submission.

FUNDING

This study was funded by a Bureau of Justice Assistance: BJA FY 09 Congressionally Selected Grant 2009-D1-BX-0060. All food for the trial was supplied by Royal Canin Pet Foods.

REFERENCES

1. Hill RC. The nutritional requirements of exercising dogs. *J Nutr* (1998) 128(12 Suppl):2686S–90S.
2. Toll PW, Gillette RL, Hand MS. CH: 9 feeding working and sporting dogs. In: Hand MS, Thatcher CD, Remillard RL, Roudebusch P, Novotny TL, editors. 5th ed. *Small Animal Clinical Nutrition*. Topeka, KS: Mark Morris Institute (2010). p. 321–58.
3. Wakshlag J, Shmalberg J. Nutrition for working and service dogs. *Vet Clin North Am Small Anim Pract* (2014) 44(4):719–40. doi:10.1016/j.cvsm.2014.03.008
4. Kronfeld DS, Hammel EP, Ramberg CF, Dunlap HL. Hematological and metabolic responses to training in racing sled dogs fed diets containing medium, low, or zero carbohydrate. *Am J Clin Nutr* (1977) 30:419–30.
5. Downey RL, Kronfeld DS, Banta CA. Diet of Beagles affects stamina. *J Am Anim Hosp Assoc* (1980) 16:273–7.
6. Kronfeld DS, Adkins TO, Downey RL. Nutrition, anaerobic and aerobic exercise and stress. In: Burger IH, Rivers JPW, editors. *Nutrition of Dog and Cat: Waltham Symposium*. Cambridge: Cambridge University Press (1989). p. 133–45.
7. Reynolds AJ, Reinhart GA, Carey DP, Simmerman DA, Frank DA, Kallfelz FA. Effect of protein intake during training on biochemical and performance variables in sled dogs. *Am J Vet Res* (1999) 60(7):789–95.
8. Hill RC, Bloomberg MS, Legrand-Defretin V, Burger IH, Hillock SM, Sundstrom DA, et al. Maintenance energy requirements and the effect of diet on performance of racing Greyhounds. *Am J Vet Res* (2000) 61(12):1566–73. doi:10.2460/ajvr.2000.61.1566
9. Davenport GM, Kelley RL, Altom EK, Lepine AJ. Effect of diet on hunting performance of English pointers. *Vet Ther* (2001) 2(1):10–23.
10. Altom EK, Davenport GM, Myers LJ, Cummins KA. Effect of dietary fat source and exercise on odorant-detecting ability of canine athletes. *Res Vet Sci* (2003) 75(2):149–55. doi:10.1016/S0034-5288(03)00071-7
11. Fedorova I, Salem N. Omega three fatty acids and rodent behavior. *Prostagland Leukot Essent Fatty Acids* (2006) 75:271–89. doi:10.1016/j.plefa.2006.07.006
12. Seebungkert B, Lynch JW. Effects of polyunsaturated fatty acids on voltage-gated potassium and sodium channels in fat olfactory receptor neurons. *Eur J Neurosci* (2002) 16:2085–94. doi:10.1046/j.1460-9568.2002.02288.x
13. Angle CT, Wakshlag JJ, Gillette RL, Steury T, Haney P, Barrett J, et al. The effects of exercise and diet on olfactory capability in detection dogs. *J Nutr Sci* (2014) 3:e44. doi:10.1017/jns.2014.35
14. Greenleaf JE, Kruk B, Nazar K, Falecka-Wieczorek I, Kaciuba-Uscilko H. Glucose infusion into exercising dogs after confinement: rectal and active muscle temperatures. *Aviat Space Environ Med* (1995) 66(12):1169–74.

15. Huntingford JL, Kirn BN, Cramer K, Mann S, Wakshlag JJ. Evaluation of a performance enhancing supplement in American foxhounds during eventing. *J Nutr Sci* (2014) 3:e24. doi:10.1017/jns.2014.38
16. Strasser A, Simunek M, Seiser M, Hofecker G. Age-dependent changes in cardiovascular and metabolic responses to exercise in beagle dogs. *Zentralbl Veterinarmed A* (1997) 44:449–60. doi:10.1111/j.1439-0442.1997.tb01130.x
17. Larsson C, Ahlstrøm O, Junghans P, Jensen RB, Blache D, Tauson AH. The oral [(13)C]bicarbonate technique for measurement of short-term energy expenditure of sled dogs and their physiological response to diets with different fat:carbohydrate ratios. *J Nutr Sci* (2015) 4:e32. doi:10.1017/jns.2015.23
18. Ermon V, Yazwinski M, Milizio JG, Wakshlag JJ. Serum chemistry and electrolyte alterations in sled dogs before and after a 1600 km race: dietary sodium and hyponatraemia. *J Nutr Sci* (2014) 3:e26. doi:10.1017/jns.2014.39
19. Meyer M, Hahn G, Buess C, Mesch U, Piper J. Pulmonary gas exchange in panting dogs. *J Appl Phys* (1989) 68(3):1258–63.
20. Spoo JW, Zoran DL, Downey RL, Bischoff K, Wakshlag JJ. Serum biochemical, blood gas and antioxidant status in search and rescue dogs before and after simulated fieldwork. *Vet J* (2015) 206:47–53. doi:10.1016/j.tvjl.2015.07.002
21. Huntingford JL, Levine CB, Mustacich DJ, Corrigan D, Downey RL, Wakshlag JJ. The effects of low intensity endurance activity on various physiological parameters and exercise induced oxidative stress in dogs. *Open J Vet Med* (2014) 4(7):134–44. doi:10.4236/ojvm.2014.47016
22. Binns A, Gray M, Di Brezzo R. Thermic effect of food, exercise, and total energy expenditure in active females. *J Sci Med Sport* (2015) 18(2):204–8. doi:10.1016/j.jsams.2014.01.008
23. Angle CT, Wakshlag JJ, Gillette RL, Stokol T, Geske S, Adkins TO, et al. Hematologic, serum biochemical, and cortisol changes associated with anticipation of exercise and short duration high-intensity exercise in sled dogs. *Vet Clin Pathol* (2009) 38(3):370–4. doi:10.1111/j.1939-165X.2009.00122.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

REVIEW
published: 28 October 2016
doi: 10.3389/fvets.2016.00096

Using Scent Detection Dogs in Conservation Settings: A Review of Scientific Literature Regarding Their Selection

*Sarah C. Beebe, Tiffani J. Howell and Pauleen C. Bennett**

Anthrozoology Research Group, School of Psychology and Public Health, La Trobe University, Bendigo, VIC, Australia

Dogs are widely used for scent detection work, assisting in searches for, among other things, missing persons, explosives, and even cancers. They are also increasingly used in conservation settings, being deployed for a range of diverse purposes. Although scent detecting dogs have been used in conservation roles for over 100 years, it is only recently that the scientific literature has begun to document their effectiveness and, importantly, how suitable dogs should initially be selected by organizations wanting to develop a detection program. In this paper, we review this literature, with the aim of extracting information that might be of value to conservation groups considering whether to invest in the use of dogs. We conclude that selection of appropriate dogs is no easy task. While olfactory ability is critical, so also are a range of other characteristics. These include biological, psychological, and social traits. At present, no validated selection tools have been published. Existing organizations have adapted selection instruments from other contexts for their use, but very little published information is available regarding the effectiveness of these instruments in a conservation setting. In the absence of clear guidelines, we urge those wanting to invest in one or more dogs for conservation purposes to proceed with extreme caution and, preferably, under the watchful eyes of an experienced professional.

Keywords: conservation, detection dog, olfaction, scent, biopsychosocial, scat, wildlife, selection

OPEN ACCESS

Edited by:
Cynthia M. Otto,
University of Pennsylvania, USA

Reviewed by:
Craig A. Schultz,
Federal Bureau of Investigation, USA
Aimee Hurt,
Working Dogs for Conservation, USA

****Correspondence:***
Pauleen C. Bennett
pauleen.bennett@latrobe.edu.au

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *29 January 2016*
Accepted: *12 October 2016*
Published: *28 October 2016*

Citation:
Beebe SC, Howell TJ and Bennett PC (2016) Using Scent Detection Dogs in Conservation Settings: A Review of Scientific Literature Regarding Their Selection. Front. Vet. Sci. 3:96. doi: 10.3389/fvets.2016.00096

INTRODUCTION

The use of dogs in conservation detection first emerged in the 1890s, when dogs were successfully used to locate the New Zealand kiwi (*Apteryx* spp.) and kakapo (*Strigops habroptilus*) (1, 2). Up until the early 1990s, conservation detection dogs (CDDs) focused predominantly on detection of live birds [(3); see Ref. (4) for additional references]. Recent years, however, have seen a rapid expansion of the field. Conservation detection now encompasses an array of activities, including detection of live wildlife (5–8), carcass detection for birds and bats around wind turbines (9–12), and detection of scats, pathogens, and other biological materials (13–16). Several reports indicate that, in many cases, CDDs are more efficient than several other survey methods in detecting the presence/absence, and relative abundance, of plants and wildlife (3, 10, 15, 17, 18). These animals therefore represent an exciting opportunity which could substantially benefit conservation groups worldwide. However, the costly nature of selecting, training, and housing CDDs (2), and uncertainty regarding why some individual dogs succeed in tasks where others perform poorly (19, 20), may act as barriers preventing more widespread use.

In order to improve selection efficiency in other dog-based scent detection contexts, relevant organizations have developed comprehensive assessment tools. Not many of these are publicly accessible, although a few have been described (7, 14). For example, the Brownell–Marsolais scale (21), used with search and rescue (SAR) and disaster dogs, reportedly allows one to measure pack, food, and play drives, as well as motivation and nerve strength, in scent detection dog candidates. To the best of our knowledge, no such tools exist for CDD selection. Organizations selecting and assessing potential CDDs have reported adapting assessment instruments from other fields, but it seems likely that conservation detection has unique requirements, which might mean that existing tools do not capture the full catalog of required traits. They are also difficult for inexperienced persons to acquire or implement effectively.

A dearth of publicly available knowledge regarding how to select relevant dogs is potentially a significant barrier for organizations wanting to use scent detection dogs in conservation settings. Conservation groups often involve volunteers, and they are typically small, local organizations with access to limited expertise and resources, unlike police and military detection units, which are comparatively well resourced. Even larger organizations employing dogs for conservation purposes tend to source their dogs from shelters or from among those owned by volunteers and may benefit from access to information about successful selection practices used elsewhere. Hurt and Smith (22) reported that, of potential CDDs sourced from shelters, as few as one in every 200–300 dogs may be selected. Of these, only 40% may successfully complete the training program.

Low training-completion rates undoubtedly reflect many factors that intervene between selection and eventual certification, including health and performance issues not related to initial suitability. Nonetheless, there is a clear need for development of standardized tools, which may be deployed reliably throughout the entire industry. Experienced handlers typically report preferring to work with dogs with high energy and strong motivational drives, which may make them unsuitable as family pets, yet conservation groups may require their dogs to live primarily as pets. The dogs may also perform their conservation role infrequently, in fragile ecosystems, some containing critically endangered plants or animals, and in challenging search environments where success may be greatly affected by environmental conditions, such as wind direction and strength. It is therefore imperative that these dogs are highly responsive to their human handlers and able to perform consistently across trials regardless of challenges provided by local conditions.

A key benefit of established selection tools in other contexts is that they assess several different characteristics believed to be relevant to scent detection dog success. Similarly, we propose that, in the case of CDDs, a multidimensional model may provide a useful conceptual framework for informing development of selection tools. For this reason, we advocate use of the biopsychosocial (BPS) model, first developed as a tool for psychiatric medicine by Engel (23, 24). Engel (23) argued that the prevailing biomedical model was insufficient to account for many medical problems and for patient outcomes following treatment. Instead, he asserted, these outcomes typically reflect a combination of biological characteristics (genetic predispositions and physiological mechanisms), psychological processes (perceptions, beliefs, attitudes, personality, and attachment), and social contexts (social structure, cultural influences, and other interpersonal relationships).

The BPS model has widespread appeal because it reminds us to consider whole systems, assuming from the outset that most behavioral outcomes will reflect a complex combination of factors, some of which may interact in unexpected ways (25–27). With regards to scent detection dogs, it seems clear that many dog characteristics are relevant. Moreover, each dog's characteristics are likely to interact with those of its trainer, handler, and environment to determine overall success. One of the main advantages of a BPS approach, however, is that the system can operate on many levels. Analysis at one level (e.g., dog) can therefore be used to improve understanding at a higher level (e.g., dog-handler team). With that in mind, the aim in this review is to examine the importance of biological, psychological, and social characteristics in selection of candidates for training as CDDs.

LITERATURE SEARCH

First, we conducted a literature search using combinations of key words from among the following list: BPS, working dog, scent detection, wildlife detection, scat detection, carcass detection, breed, temperament, personality, behavior, social intelligence, and assessment tools. Sources searched included the Google Scholar, Science Direct, and Web of Science databases. Second, we examined the reference lists of studies from our first search in order to find relevant studies we may have missed. Finally, we searched reports from individual CDD organizations to determine whether more information was available on selection and training in cases where sufficient information was not provided in the published scientific literature. We compiled a list of studies which provided sufficient detail regarding the dogs used, selection, training and search methods employed, and accuracy/efficiency of CDD teams. Of the more than sixty studies identified, we selected 30 studies as a sample of available information (**Table 1**). These 30 studies where chosen in order to represent the broad diversity of search roles and environments in which CDDs are often employed.

OVERVIEW OF LITERATURE SEARCH RESULTS

As is evident from **Table 1**, current literature in the field primarily details what CDDs can do (4, 22, 30). The list is impressive! Detection of biological materials, such as scats, hair, urine, and burrows, makes up the majority of studies (76.7%), with studies focused solely on scat detection being most common of all (56.7%). Detection of live wildlife is reported in 20% of the studies, while 6.7% focus on carcass detection.

Of the studies in this review, 43.3% reported that dogs were acquired from professional CDD training organizations, or were selected using criteria developed by these organizations.

TABLE 1 | Summary of a sample of wildlife, carcass, scat, and nest detection studies and the techniques used/characteristics selected for when assessing dogs as potential conservation detection dogs (CDDs).

Reference	Target	Dog-handler teams	Accuracy/efficiency	Selection and training based on
Arandjelovic et al. (28)	Scat detection: lowland gorilla	3 CDDs; >1 year experience	Dogs detected 43 fresh and 288 old scat samples in 44 days Fresh scats were more likely to be detected by dogs (72%) than humans (39%)	Selection: professional CDD organization Reward: no mention Training: authors refer to published CDD literature
Brook et al. (29)	Scat detection: Javan rhinoceros	2 CDDs; >1 year experience	Dogs detected 22 scats over 118 days, and ~429 km	Selection: professional CDD organization Reward: no mention Training: professional CDD organization
Browne et al. (30)	Scat and skin detection: 3 species of reptile	20 CDDs; <1 year experience; 8 Retrievers, 2 Shepherds, 1 Spaniel, 5 working dog mixes, 4 non-working breeds	Average success by dogs to detect Tuatara (i) scent was 85.0%, (ii) scats was 97.8%, and (iii) skin was 95.6% Average success by dogs to detect Gecko (i) scent was 77.8%, (ii) scats was 77.8%, and (iii) skin was 51.8% (Detection rates for gecko increased with repeated trials)	Selection: experience in competitive obedience scent discrimination exercises Reward: no mention Training: standard scent obedience exercises
Cablk and Heaton (31)	Burrow and live reptile detection: desert tortoise	2 CDDs; no information	Accuracy for nest detection by dogs was 90% Efficiency between dogs and humans was similar to find live tortoises	Selection: play drive, low–moderate hunt drive, direction and control with handler Reward: play Training: described in article
Chambers et al. (32)	Roost and scat detection: bat	2CDDs; experienced	Accuracy of dogs to detect bat scats at 6 m above ground was 20% and at 2 m above ground was 60%	Selection: professional CDD organization Reward: no mention Training: professional CDD organization
Cristescu et al. (33)	Scat detection: koala	1 CDD; >1 year experience; Collie	Dogs were 153% more accurate than human surveyors Dogs were 19 times faster than human surveyors	Selection: ball drive and motivation Reward: play Training: professional dog trainer
Dematteo et al. (18)	Scat and burrow detection: bush dog	1 CDD; experienced	Dogs detected 11 dens over 72 days and 218.4 km	Selection: professional CDD organization. Also mentioned were body type (robust) and disposition (no territorial behavior) Reward: play Training: professional CDD organization
Duggan et al. (8)	Live animal detection: Franklin's ground squirrel	2 CDDs; experienced	Accuracy of dogs and human surveyors was similar at 83 and 84%, respectively Efficiency of dogs was 10 times faster than humans	Selection: professional CDD organization. Also mentioned were object obsession and play drive Reward: play Training: professional CDD organization. Authors also refer to published CDD literature
Hagell (34)	Scat detection: spider monkey	1 CDD; experienced	Dogs and human surveyors scored similarly in comparison trials. However dogs were less accurate (59%) than humans (82%)	Selection: professional CDD organization Reward: no mention Training: authors refer to published CDD literature
Harrison (17)	Scat detection: bobcat	1 CDD; no information	Dogs were over 10 times more effective in detecting bobcats than scent traps, camera traps, and hair scares combined	Selection: professional CDD organization Reward: play Training: professional CDD organization
Kerley and Salkina (19)	Scat discrimination: Amur tiger	5 CDDs; experienced; 1 Shepherd, 1 Pointer, 3 mixed breeds	Accuracy of scat discrimination increased from 87 to 98% with repeated trials in 4 of the 5 dogs	Selection: play/food drive of pups and their parents Reward: play/food Training: authors refer to published CDD and scent discrimination literature

(Continued)

TABLE 1 | **Continued**

Reference	Target	Dog-handler teams	Accuracy/efficiency	Selection and training based on
Leigh and Dominick (16)	Scat detection: spotted tailed quoll	1 CDD; <1 year experience; Shepherd	Accuracy of dogs in grassland and heath was 83% and in woodland was 87%	Selection: professional CDD organization. Also mentioned were play drive, temperament, high energy and intelligence. Authors also refer to published CDD literature Reward: play Training: authors refer to published CDD and scent-detection literature
Long et al. (6)	Scat detection: 3 forest carnivores	5 CDDs; experienced	1596 scats found over 2 years (~3.6 scats/km). Of these, 83% were unlikely to be found without CDDs	Selection: professional CDD organization. Also mentioned were drive/object orientation and appropriate temperament Reward: play Training: professional CDD organization. Authors also refer to published CDD literature
Mathews et al. (12)	Carcass detection: bat	2 CDDs; <1 year experience; 2 Retrievers	Accuracy of dogs was 75%, which was significantly higher than human surveyors who detected 20% of bat carcasses Efficiency of dogs over 4 times faster than humans	Selection: ball drive (search and play) Reward: play Training: trained by experienced police dog handlers
Nussear et al. (7)	Live reptile detection: desert tortoise	6 CDDs; varying experience; 1 Collie, 2 Shepherds, 1 Kelpie, 2 Retrievers	Accuracy was similar between human and dog teams of ~70%. Dogs found more targets under vegetation than humans	Selection: drive, previous scent experience, and training Reward: no mention Training: authors refer to published CDD literature
O'Connor et al. (20)	Nest searches: bumble bee	1 CDD; <1 year experience; Spaniel	Accuracy of dog to detect known bumble bee nests was 62.5% In uncontrolled test dog performed similar to human surveyors (CDD 1.41 nests/ha; humans 1.44 nests/ha)	Selection: authors refer to published CDD literature Reward: no mention Training: authors refer to published CDD literature
Oliveira et al. (35)	Scat detection: *Mazama* deer	1 CDD; <1 year experience; mixed breed	Dog detected 8 scat samples across 39 km of trails searched, approximately 0.21 samples/km. Human surveyors detected no scat samples, but did record 24 deer tracks	Selection: professional organization Reward: play Training: military police and narcotics detection dogs
Paula et al. (11)	Carcass detection: bird	1 CDD; <1 year experience; 1 Shepherd	Accuracy of dog was 96%, which was significantly higher than human surveyors who detected 9% of bird carcasses	Selection: object orientation, high drive, and appropriate temperament Reward: play Training: authors refer to published CDD literature
Reed et al. (36)	Scat detection: 6 carnivores	2 CDDs; <1 year experience; 2 mixed breeds	Accuracy decreased in dogs with increasing detection distance. At 10 m from transect, dog detection rates were >75%	Selection: professional CDD organization. Also mentioned were object obsession and agility. Authors also refer to published CDD and scent detection literature Reward: play Training: professional CDD organization. Authors refer to published CDD literature
Reindl (37)	Scat detection: black-footed ferret	2 CDDs; no information	Accuracy of dogs was 86% for areas of known ferret populations	Selection: professional CDD organization. Also mentioned were play/food drive and focus, consistent concentration, and agility Reward: play/food Training: professional CDD organization (details provided in text)

(Continued)

TABLE 1 | **Continued**

Reference	Target	Dog-handler teams	Accuracy/efficiency	Selection and training based on
Robertson and Fraser (38)	Live bird detection: kiwi and kakapo	3 CDDs; experienced; 2 Retrievers, 1 Setter	Of radio-tagged birds, dogs detected 36% adult kiwis and 24% sub-adults	Selection: temperament test by professional organization at 6–12 months into training Reward: no mention Training: described in article
Rolland et al. (39)	Scat detection: right whale	2 CDDs; no information	Detection by dogs (n = 97 scats) was more successful than humans (n = 30 scats). Efficiency of dogs was 4 times higher than human surveyors	Selection: calm disposition, physical stability, and persistence Reward: play Training: authors refer to published CDD literature
Savidge et al. (40)	Live reptile detection: brown tree snake	2 CDDs; 1 experienced and 1 inexp; 2 Retrievers	Accuracy of experienced CDD (44%) was higher than the inexperienced CDD (26%)	Selection: professional CDD organization Reward: play Training: narcotics, forensic, and SAR techniques
Smith et al. (13)	Scat detection: San Joaquin kit fox	7 CDDs; 2 Shepherds, 4 Retrievers, 1 mixed breed	Dogs correctly identified 100% of scats in scent line-up. Dogs correctly ignored incorrect scats 67% of the time In an uncontrolled field search dogs detected 0.43–5.37 scats/km	Selection: food or Play obsession Reward: play/food Training: authors refer to published CDD and scent detection literature
Stevenson et al. (41)	Live reptile detection and shed skins: eastern indigo snake	1 CDD; experienced; mixed breed	Accuracy for live snakes was 81%, and for shed skins was 100%	Selection: professional CDD organization Reward: play Training: professional CDD organization
Vynne et al. (42)	Scat detection: 5 mammals	3 CDDs; <1 year experience	Dogs detected 2683 scats over 407 surveys. This was ~6.6 scats per dog team per search day. Detection rates varied between target species	Selection: play drive Reward: play Training: professional CDD organization. Authors refer to published CDD literature
Wasser et al. (14)	Scat detection: black and grizzly bear	9 CDDs; <1 year experience	Detection rates differed between years. Dogs detected 0.63–3.76 scats/ha in 1999, and detected 0.35–1.89 scats/ha in 2001	Selection: play drive/object orientation, temperament, trainability, and motivation Reward: play Training: narcotics, bomb, and arson detection, and search and rescue
Wasser et al. (43)	Scat detection: Spotted and Barred owl	2 CDDs; experienced; 2 mixed breeds	Accuracy of dogs was significantly higher than bird vocalization surveys. Ratio of dog surveys:vocalization surveys for northern spotted owl was 87:59%, and for Barred owl was 20.1:7.3%	Selection: high play drive. Authors also refer to published CDD literature Reward: play Training: authors refer to published CDD literature
Waters et al. (44)	Nest searches: bumble bee	1 CDD; <1 year experience; Spaniel	Accuracy of dog to detect known bumble bee nests was 100% In uncontrolled tests dog found 33 nests	Selection: drug detection dog organization Reward: no mention Training: drug detection dog organization. Authors also refer to published CDD and scent detection literature
Wultsch et al. (45)	Scat detection: 5 native felines	1 CDD; <1 year experience	Dogs detected 1053 scats. 49% of these were identified to the species level	Selection: professional CDD organization Reward: no information Training: professional CDD organization

A further 40% of studies reported adapting tools used for the selection and training of narcotics, explosives, SAR, cadaver, police, and forensics detection dogs. Of these, only one study reported on the specific assessment tools which were adapted (36). Noted in the review was that very few studies provided detailed information on the methods used to test or score specific characteristics, or how selection of traits may vary with respect to the external search environment or search target being selected for.

The lack of a standardized and publicly available assessment tool for CDDs, along with a widespread failure in available literature to report on specific selection processes, is a significant omission, particularly given that several studies show that CDD performance may be impacted by many factors. For example, environmental terrain/vegetation density (16), specific search target (42), and whether the target is terrestrial, arboreal, or marine (32, 39), all impact CDD effectiveness. Experience of the CDD team (40), handler characteristics (46), and the individuality of the dog selected (1, 20) are also important factors in CDD success.

When analyzing the specific characteristics commonly selected for in CDDs, it appeared that a strong play/food drive was the most common trait selected for. Appropriate temperament for the field of conservation detection was also cited often, and traits relating to problem solving, intelligence, and trainability were reportedly selected for in many studies. This suggests a considerable focus on psychological factors above biological or social factors and is important, because these traits are typically very poorly defined and difficult to measure. Biological characteristics of the dog, including agility, physical stability, and body type, were included as selection considerations in only a few studies in our review. This is not to say that biological traits are unimportant to selection considerations, however, as most studies which provided information on dog breed included at least one working or sporting dog, or a mixed breed. Thus, breed characteristics are likely to be important, even if this is not overtly stated. Only a few studies in the review considered the importance of sociability or dog-handler cooperation.

While existing literature rarely included sufficient information about selection, and about the reliability and validity of selection instruments, from our reading of the literature, we identified a number of biological, psychological, and social traits as being important. These are reviewed below. Biological traits included: morphology, including characteristics associated with the original working function of the dog's breed, and olfactory, visual, and auditory acuity. Psychological traits included: personality; suitable levels of nerve strength; and drives, such as food, play, pack, and prey/hunt drives. Finally, social characteristics included: the possible importance of correctly pairing dog-handler teams based on the experience and psychological traits of both the dog and the handler.

BIOLOGICAL CHARACTERISTICS LIKELY TO AFFECT CONSERVATION DETECTION DOG SUCCESS

Several biological characteristics, grouped loosely into morphological characteristics and sensory capabilities, appear likely to be required for dogs to become successful CDDs. The perceived importance of morphological capabilities is not surprising since these dogs often work in challenging environmental landscapes (14, 16). Dogs working in SAR, cadaver, and disaster contexts may face similar challenges, which may explain why selection tools used in these contexts are frequently adapted for use with CDDs (36, 39, 44). The Brownell–Marsolais scale, for example, assesses morphological features (e.g., breed, size, health) and motor capabilities (e.g., agility, athleticism, and stamina) of candidate dogs.

Morphology

Available studies show a strong trend toward using working and sporting dog breeds for conservation work, breeds with morphological characteristics which allow them to maintain performance in the face of challenging search environments, long working hours, and unfavorable weather conditions (16). Many of the dog breeds selected for CDD work are medium to large sized dogs (13, 20). These may be most suitable as CDDs, as dogs which are too large or small may struggle during extreme weather conditions (47). While Pugs, for example, appear capable of performing scent detection tasks to a similar standard to German Shepherd Dogs (48), they are not able to maintain body heat in extreme cold. It is also generally thought that smaller dogs with short, flat snouts have limited olfactory capabilities compared with their larger counterparts (49). At the other extreme, Great Danes may also be unsuited to this line of work, as their large size may make it difficult for them to cool down when working in strenuous environmental conditions or hot weather.

Several studies have suggested that the breed of dog (e.g., working or sporting dogs) (4), or even the genetic line of the dog, such as whether the dog comes from a working dog line or a show dog line, may be of particular importance (49). Dogs bred specifically for a working purpose have typically been selected for robust morphologies and, thus, may be more capable of withstanding the challenging field conditions associated with conservation detection work. Furthermore, medium sized dogs may be more capable of maneuvering in difficult environments, and may maintain stamina for longer search durations. This is not to say that small dogs do not have potentially important roles to play in conservation, particularly in environmental contexts with limited space. However, these should likely be selected from among working dog breeds, with careful consideration of their physical robustness. It may also be important to consider coat length and type. Dogs working in challenging terrains may require short coats or coats sufficient to provide thermal protection (50).

Olfactory System

Olfaction is clearly a key sensory system for all scent detection dogs, regardless of context. Gazit and Terkel (47) demonstrated the importance of olfaction in a controlled environment when they showed that explosives dogs did not vary their search methods or detection success regardless of whether or not light conditions made the target visually obvious.

Selective breeding of dogs for scent work has resulted in some breeds of dog possessing several morphological features which facilitate their already exceptional olfactory capabilities.

These include large ears and dewlaps to catch scent particles, and wide, elongated noses with a large nasal cavity to house the olfactory epithelium and increase the number of odor receptor cells (ORCs), all of which aid in scent detection capabilities. Dog breeds differ in the number of ORCs they possess. Bloodhounds possess the largest number of ORCs of any dog breed, with 300 million. However, Bloodhounds are not commonly used in conservation detection work (4). Working and sporting dogs, such as Shepherds, Retrievers, Collies, and Setters, are more common (4) despite having fewer ORCs (e.g., Shepherds have around 225 million ORCs).

An individual's olfactory sensitivity alone, then, is evidently not the only relevant characteristic for this type of work. While one might presume that a number of ORCs above some theoretical threshold level is required, many other traits also determine success. In this respect, it is instructive to consider the type of scent being detected. In scent detection work there are three search types that a dog can perform: air-scenting, in which the nose of the dog is held in the air "sniffing" to catch scent on the wind; tracking, where the nose is held close to the ground, following the scent and direction of the target; and, trailing, in which dogs use a combination of air-scenting and tracking techniques (51, 52).

Air-scenting is the most common search technique used in conservation detection, and may be what enables CDDs to cope with the often challenging nature and long working hours of the search environment. Early work by Steen et al. (53) suggested that game hunting dogs are capable of maintaining strong olfactory capabilities while working in challenging environments, under strenuous physiological conditions. This ability appears to be due to the Bernoulli effect, which occurs when the dog runs at a high speed with its nose held up, "sniffing" into the air. Craven et al. (54) later determined that canine olfactory acuity should be most effective during sniffing. During sniffing each nostril acquires spatially separate odor samples (known as "bilateral scent intake"). Fluid dynamics within the nose increase aerodynamic flow of the odor sample, creating an active aerodynamic sampling system which is specialized for odor detection and discrimination (54, 55). Due to this discrimination function, it is likely that dogs which perform air-scenting are able to detect airborne scents in large area searches where there is no scent trail to follow.

There is extensive literature on the topics of tracking and trailing dogs (56), and the capabilities of scent detection dogs in game hunting, SAR, and several other high intensity search environments (52). However, there is very little information on how dogs maintain strong scenting abilities in varying environmental conditions, and practically no literature detailing the possible impacts of choosing a natural air-scenting dog breed (e.g., Spaniels) for conservation work (57). Currently available assessment tools used for scent detection dogs do not appear to account for the dogs' natural preference toward one type of scenting work.

Visual and Auditory Systems

Hurt and Smith (22) suggest that the specific auditory and visual acuity required of scent detection dogs may vary between the differing scent detection fields, and even within fields, based on the nature of the search environment and specific target of interest. Polgár et al. (58) have since shown that, while pet dogs are capable of using olfactory cues alone to make correct decisions in a basic choice task, they will often prioritize the use of other search strategies. CDDs should demonstrate a baseline level of visual acuity to aid them in searching complex environments (6). Greater visual acuity may also aid in detection of live wildlife (2). This is particularly likely in situations where environmental conditions, such as wind strength, are unfavorable, allowing the dog to locate movement in the distance, before honing in on the target's exact location using olfactory cues. However, for detection of stationary targets, such as scats and plants, it has been suggested that dogs do not rely heavily on visual acuity to assist in detection. Goodwin et al. (15) found that, in detection of the Spotted knapweed plant (*Centeurea maculosa*), human surveyors were more successful in locating large targets than small targets, whereas dogs were similarly successful in locating small and large targets. Brook et al. (29) also showed that, while dogs were effective at locating the area in which Javan rhinoceros (*Rhinoceros sondaicus*) dung was located, human surveyors were much faster than dogs at visually locating dung. It was suggested that, even when rhinoceros dung was visible, dogs still relied predominantly on olfaction to locate the target.

We could find no information regarding the importance of auditory acuity in conservation detection. However, Brownell and Marsolais (21) suggest that, for dogs working in SAR/disaster detection, auditory acuity should be relatively strong. This likely reflects the search environment which, similar to conservation detection environments, requires strong dog-handler communication (22, 46) to facilitate efficient searching in ever-changing environmental conditions (13). Several tools developed for other fields of scent detection, such as the United States Department of Agriculture (USDA) National Detector Dog scale (59), provide scoring systems to measure a dog's physical health, including a section to measure sensory systems. However, we could find no studies examining whether scent dog performance in any context varied with auditory acuity. Perhaps, as with olfactory ability, it is presumed that any dog with visual and auditory acuity above some theoretical baseline measure will be able to perform satisfactorily. Individual differences in sensory traits are not evaluated, much less reported upon, in available literature.

Summary of Important Biological Characteristics

From this brief review, we conclude that there are several biological traits which should be considered when selecting dogs for conservation detection. These include morphological characteristics, often associated with the original working function of the dogs' breed and olfactory, visual, and auditory capabilities. Because these biological traits are equally important across several scent detection disciplines, established tools for the assessment of scent detection dogs in other contexts should provide sufficient information regarding the types of biological traits to be measured and how this is best accomplished. This is indeed the case for the USDA National Detector Dog scale (59), which measures physical soundness. More specialized tests which account for the specific nature of the search environment and target scent should also be considered. SAR detection contexts are perhaps the most similar fields in terms of environmental search habitat; however, the desired biological traits may differ between these fields and environmental contexts due to differences in scenting types required based on the types of targets being detected.

PSYCHOLOGICAL CHARACTERISTICS LIKELY TO AFFECT CONSERVATION DETECTION DOG SUCCESS

While physical traits reflecting biological characteristics are undoubtedly important in determining the ability of a dog to work as a CDD, taken alone they are insufficient to ensure success. Temperament, personality, and behavior also play a critical role in determining CDD success or failure (2, 4). According to Ley and Bennett (60), *temperament* is a generalized behavioral style, present from birth and typically reflecting a genetic predisposition, while *personality* is functionally indistinguishable from temperament, but is the result of both the dog's genetics and experiences over time, which together shape the dog's characteristic style of responding to situational events. *Behavior*, meanwhile, is how a dog responds at any given moment to a situation (60). While behavior normally reflects underlying personality traits, it is also partially determined by situational factors.

Individual psychological differences between dogs are thought to influence performance in working tasks, even in dogs with similar biological traits (4, 61). Several reports in the scientific literature suggest the importance of considering specific psychological traits in potential CDDs (1, 4). The strongest evidence for this comes from studies where individual differences are pronounced, even when physiological and environmental factors are controlled for. O'Connor et al. (20) showed marked variability between two dogs trained to detect bumble bee nests in controlled scent line ups (68%: 100%). Another study by Kerley and Salkina (19) showed individual differences between five experienced CDDs, asked to detect and differentiate between the scat of Amur Tigers. While four of these dogs progressively became more proficient with repeated trials (87–98% accuracy over time), it was unclear why the fifth dog did not show similar improvements. It is possible that psychological factors, such as personality characteristics, nerve strength, and motivational drives, may have had some impact on these outcomes.

Personality Characteristics

One of the main difficulties faced by behavioral researchers, and indeed one of the difficulties we faced in reviewing the scientific literature on conservation detection, is that many different words may be used to describe very similar behavioral traits. This makes it difficult to pinpoint exactly which traits are of key importance to CDD success. To address this problem, several researchers have proposed using discrete categories to encompass a broad spectrum of temperament, personality, and behavioral traits (61–63). For example, Jones and Gosling (63) proposed that most psychological dimensions fit within seven broad temperament categories: reactivity/excitability–stability; fearfulness–courage/confidence; aggression–agreeableness; sociability/friendliness–lack of interest in others; responsiveness to training; dominance–submission; activity level.

Prior to the detailed conceptualization of behavioral traits provided by Jones and Gosling (63); Svartberg (61) determined that a boldness–shyness index could act as an umbrella variable under which several psychological traits predictive of success in working dogs could fit. They included sociability toward strangers, playfulness, interest to chase, exploration, and fearlessness as evidence of boldness. The scientific literature suggests that boldness may be a particularly important trait in successful completion of working tasks. This is because individuals with low boldness scores are more fearful, anxious, and easily distracted (61). Thus, they take longer and often require different training methods to become successful in working tasks, or may never be successful despite intensive training.

Nerve Strength

Many tools which are commonly used for assessment of scent detection dogs assess something called "nerve strength." This is perhaps associated with boldness in that it describes the behavioral response of a dog to an unfamiliar stimulus (e.g., tactile, auditory, or visual stimulus) at a point in time. The Brownell–Marsolais (21) scale, for example, which is used for SAR and disaster detection dogs, measures an individuals' behavioral responses to unfamiliar stimuli. Behaviors exhibited by the dog, such as fearfulness, curiosity, or anxiety, provide an indication of the dog's nerve strength, indicating to researchers the suitability of the dog for particular fields of scent detection.

Of particular relevance to conservation detection is tactile nerve strength, which measures a dog's response to unfamiliar surfaces and spaces. Auditory nerve strength, meanwhile, measures the dog's reactivity to unexpected/unfamiliar noises. Behaviors exhibited during nerve strength assessments may be suggestive of a dog's ability to work efficiently in unfamiliar search areas (also referred to as environmental stability). Rolland et al. (39) demonstrated the importance of tactile nerve strength for dogs working on boats to detect floating feces of marine mammals. Hurt and Smith (22) also suggest the importance of good tactile nerve strength due to the complex, highly variable nature of the search environment typical of conservation detection. They note, however, that nerve strength may be less important to dogs working in a conservation context than it would be to military/police dogs, due to the nature of the work and search environment (22).

Tests which measure nerve strength in potential CDDs may be adapted from those used to assess SAR, disaster, and cadaver dogs due to similarities in the search environments. However, the environments CDDs may work in are particularly diverse and, as yet, no standardized assessment exists to measure visual, auditory, or tactile nerve strength in potential CDDs. Development of a standardized tool to assess these psychological characteristics may be particularly important to conservation detection, as it may aid in assessing behaviors appropriate to each specific field of conservation detection.

Motivational Drives

Several authors have also stressed the importance of object obsession and strong drives, such as food and play drives, as contributors to the potential success of CDDs (14, 36). This is evidenced by the fact that, of the studies in **Table 1** which provided information on CDD selection, 80% stated food or play drives as selection criteria. These psychological factors are likely to be of great importance, but it is exceedingly difficult to operationalize what drives are and how they can be elicited in dogs in a way that can be reliably measured. In this review, we use the definitions for drives which were established by Brownell and Marsolais (21), who divided drives into six categories: pack/social; play; food; prey; hunt; and defense.

Brownell and Marsolais (21) proposed that drive-testing should be a critical part of the assessment process when selecting a dog for disaster/SAR scent work, as drives are thought to be strongly associated with sustained search intensity and trainability. The similar working requirements of SAR dogs and CDDs, including the requirement for sustained working motivation over several hours in challenging environments, suggests that the Brownell–Marsolais drive test may be applicable to selection of dogs for conservation scent detection. However, the unique ecosystems in which CDDs are sometimes required to work may mean that the existing test needs to be modified for use in this context.

Clear benefits of selecting dogs which demonstrate excessive food/play drives for scent detection roles have consistently been reported in the scientific literature. Work on the selection and training of narcotics dogs by Maejima et al. (64), for example, and selection of cadaver detection dogs by Dorriety (65) has confirmed that object obsession is associated with decreased distractibility and increased trainability, which should, in turn, be associated with scent detection dog success (64, 65). One reason why strong object/food obsession may be so important is that scent detection dog training requires the trainer to associate the target scent with an object of intrinsic pleasure for the dog, such as a ball or a food reward (66, 67). The more the dog desires the ball or food, the more successful the training process is, since this desire increases the dog's focus, decreases distractibility, and increases motivation to work for sustained periods of time (68).

Several studies documenting success in field studies by CDDs have stated that dogs were initially chosen on the basis of strong play or chase drives (14, 36). To the best of our knowledge, however, no studies have provided quantitative, laboratory-based evidence demonstrating the importance of specific drives for CDD success (22). Future research which documents learning times, success rates, and efficacy of current training methods for dogs which vary in these drives would be beneficial. This is especially relevant in the case of community run CDD programs, where there may be fewer options to reject dogs based on low drive, due to a dependence on volunteers. More research is needed to examine the extent to which drives are both necessary and sufficient for success. If found to be necessary, then strategies for identifying and quantifying specific drives at an early age require development.

The importance of food/play drive is thought applicable across all fields of scent detection, as it facilitates scent detection training and sustained motivation during searching. Other drives, however, including prey, hunt, and search drives, have also been related to sustained search motivation during scent detection tasks in various contexts (2). The applicability of these drives in the specific context of conservation work remains less certain. Cablk and Heaton (31) mention that dogs with high prey drive may be unsuited to live wildlife detection, due to the potential for harm to be caused to the target species. Dogs working in natural ecosystems may cause harm to the wildlife they are employed to detect, and may be too easily distracted by non-target scents. Hurt and Smith (22) similarly suggest that strong prey/hunt drives may be detrimental to conservation detection, although they also asserted that strong search drives should aid in sustained attention to the working task. This could present a problem to end users wishing to utilize existing assessment tools to identify potential CDDs, as desired levels of prey, hunt, and search drives are not well defined or addressed in existing tools. It is also not known whether good training can overcome any potential for high prey drive to be detrimental.

Another psychological characteristic of interest in this context is sociability or pack drive, the ability of a dog to cooperate with a handler, and to work in the presence of unfamiliar dogs or humans without exhibiting anxiety, fearfulness, or aggression. The importance of a moderate to high sociability/pack drive in fields such as biosecurity or narcotics detection is well established and reflects the human-oriented nature of the search environment, which requires that dogs exhibit specific, predictable behaviors around unfamiliar people. In contrast, the specific search environment of conservation detection is less focused on the dog's response to unfamiliar people, but may necessitate a moderate to high level of sociability/pack drive to facilitate cooperation with a human handler. The Brownell–Marsolais scales assess directability in potential dogs, which is likely to be applicable to CDDs. This is difficult to conclude with any certainty, however, as the perceived importance of sociability appears to vary between organizations and with respect to the specific search environment and target.

Summary of Psychological Characteristics

In summary, our review of the scientific literature suggests that many psychological traits are likely to impact on CDD success. Strong motivation and obsessive food/play drives are considered important in a large proportion of the scientific literature, although there is little evidence for whether one drive might be better than another in specific contexts. This may well vary according to the specific nature of conservation detection work being performed, but there is no way of assessing this based on existing tools. Dahlgren et al. (4) have suggested the importance of considering a dog breed's original working function, as this may make specific psychological characteristics more pronounced. For example, a Setter may be more skilled at detecting a live bird than a Shepherd, but may also have a higher hunt/chase drive (4). Individual variability within dog breeds is pronounced, however, and we would caution against selecting dogs purely on the basis of their breed heritage.

SOCIAL CHARACTERISTICS LIKELY TO AFFECT CONSERVATION DETECTION DOG SUCCESS

Dogs and humans have shared a close social relationship for thousands of years (69). This has resulted in the ability of humans and dogs to successfully interact in modern societies, governed by shared rules (70). Most studies involving CDDs have focused on the ability of dogs to find specific targets, and there is little doubt that more attention should be paid to other factors affecting their success. These include the dog-handler team and, specifically, the contested importance of the relationship between dog and handler in contributing to CDD success (22, 46, 71). In this section, we briefly review features of dogs' social intelligence, and discuss the likely contribution of dog sociability to the potential success of CDDs.

Social Intelligence

Over recent years, dog cognition research has increased, with several studies focusing specifically on dog-handler relationships and inter-species cooperation (72). Miklósi et al. (73) demonstrated a strong social intelligence in dogs by showing that dogs were able to interpret human hand gestures and subtle changes in body positioning to find hidden food in a manner similar to young children. Gácsi et al. (74) suggested that the success of dogs in recognizing subtle human communicative gestures is the result of selection for attention to, and cooperation with, humans.

We could find no studies specifically investigating how variability in canine social intelligence may affect the success of scent detection dogs, although it is instructive that experienced CDDs appear capable of working successfully with unfamiliar handlers to a sufficient operational standard. Brook et al. (29) successfully located Javan rhinoceros (*R. sondaicus*) dung using two professional CDDs, after just 3 weeks of handler training and dog acclimation to the new handler and new working environment. Another study, by Dematteo et al. (18), demonstrated the efficacy of professionally trained CDDs to work with an unfamiliar handler. These dogs successfully located the presence of bush dogs (*Speothos venaticus*) after only 2 weeks of handler training.

Handler Characteristics

While a growing body of literature shows that some CDDs can work with multiple or unfamiliar handlers (18, 29), we could find no studies in which the same dog's performance was compared when the dog was handled by a familiar versus unfamiliar, but equally experienced, handler. Hence, it is unclear whether these dogs would be more successful in their respective fields when paired with a familiar handler with experience in reading both the dog and the environment. It is also unclear whether a certain level of dog handling expertise is required before undertaking the training necessary to specifically handle CDDs, although the Scientific Working Group on Dog and Orthogonal detector Guidelines (SWGDOG) provide detailed recommendations concerning initial selection and training of scent dog handlers (75).

It is possible that handler experience may be particularly important for CDDs, due to the complex nature of working in unpredictable and constantly changing search environments. Furtado et al. (71) described a study which demonstrated an inexperienced handler effect during Jaguar (*Panthera onca*) scat detection. In this study, two experienced dog-handler teams had an 81% accuracy rate when collecting Jaguar and Puma scats, while an inexperienced dog-handler team collected 50% non-target species. This can be problematic, as misidentification of scats in the field may mean that inexperienced handlers reward incorrect dog behavior. On this basis, further research is needed into the effects of handler experience or handler characteristics on CDD outcomes. Inexperienced handlers may be less capable of directing dog searches in accordance with environmental conditions, and may be more likely to reward dogs too early or on the wrong scent, inadvertently reducing detection accuracy and success (71). Furthermore, dogs which do not demonstrate a strong sociability/pack drive may require a more confident/experienced handler to ensure cooperation between the dog-handler team.

Summary of Social Characteristics

It appears that little is known regarding how social characteristics of dogs can impact on search success. Yet, this is a critical issue, since the nature of conservation work requires close cooperation between dog and handler. As a species, dogs are highly competent in understanding humans, but individual and breed-based differences are likely to impact performance. These have not yet been investigated or documented. Handler characteristics, particularly their level of expertise, are also likely to be of critical importance, as is the nature of the relationship between an individual dog and his or her handler. The framework established by the BPS model may act as a tool to help us better understand how interactions between a dog's social environment and its psychological and biological traits may act together to influence performance, but conclusions regarding this are not yet supported by available literature.

CONCLUSION AND RECOMMENDATIONS

Many of the characteristics required in potential CDD candidates are similar to those assessed by instruments developed for selection of dogs in other contexts, such as narcotics, explosives, cadaver, forensics, and SAR detection dogs. This is to be expected since conservation detection shares many commonalities with these other fields of scent detection. However, many of the traits are psychological and social rather than physical, and this makes it difficult for them to be accurately assessed, particularly by novices. While several articles we reviewed reported adapting selection tools from other fields of scent detection, most gave little or no information regarding which tools were used, how they were adapted, or the specific selection criteria used to assess CDD candidates.

A lack of available information regarding the selection process is likely to make it extremely difficult for conservation groups to identify suitable dogs, upon which scarce resources can then be expended for training and deployment purposes. This, we believe, represents a substantial barrier to the wider use of CDDs. While any group of volunteers can acquire and utilize other resources for tracking endangered animals or detecting invasive plants, only those with access to already trained dogs, or to somebody who already possesses the skills required to select and train dogs, are likely to be able to benefit from the impressive skills appropriately selected and trained dogs demonstrate. We therefore urge those working in this field to be generous in providing access to information regarding selection of dogs, and also rigorous in documenting and empirically justifying their selection processes. We also urge those considering acquiring their first "potential" CDD to consult with experts first about the biological, psychological, and social characteristics most likely to predict success. Adapting existing tools which assess characteristics thought to be applicable across all fields of conservation detection (e.g., traits such as robust morphology, play/food drive, directability, and social cooperation) is likely to be the first step in developing a standardized assessment for CDDs. Unique requirements of the role, however, mean that careful adaptation is likely to be required.

AUTHOR CONTRIBUTIONS

SB conducted the literature searches and wrote the first draft of the manuscript. TH worked with SB to structure and edit the content. PB played a leadership role in devising and setting the scope for the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank La Trobe University's Securing Food, Water, and the Environment Research Focus Area for providing the scholarship funding and work space necessary to carry out this research. The authors also thank the Frontiers reviewers for providing valuable feedback to improve the manuscript.

REFERENCES

1. Robert M, Laporte P. Field techniques for studying breeding Yellow Rails. *J Field Ornithol* (1994) 68(1):56–63.
2. Helton WS. Introduction to the new science of working dogs. In: Helton WS, editor. *Canine Ergonomics: The Science of Working Dogs*. Boca Raton, FL: CRC Press (2009). p. 1–15.
3. Jenkins D, Watson A, Miller GR. Population studies on red grouse, *Lagopus lagopus scoticus* (Lath.) in north-east Scotland. *J Anim Ecol* (1963) 32(3):317–76. doi:10.2307/2598
4. Dahlgren DK, Elmore RD, Smith DA, Hurt A, Arnett EB, Connelly JW. Use of dogs in wildlife research and management. 7th ed. In: Silvy NJ, editor. *The Wildlife Techniques Manual Volume 1*. Baltimore, ML: The John Hopkins University Press (2012). p. 140–53.
5. Gutzwiller K. Minimizing dog-induced biases in game bird research. *Wildl Soc Bull* (1990) 18(3):351–6.
6. Long RA, Donovan TM, Mackay P, Zielinski WJ, Buzas JS. Comparing scat detection dogs, cameras, and hair snares for surveying carnivores. *J Wildl Manage* (2007) 71(6):2018–25. doi:10.2193/2006-292
7. Nussear KE, Esque TC, Heaton JS, Cablk ME, Drake KK, Valentin C, et al. Are wildlife detector dogs or people better at finding desert tortoises (*Gopherus Agassizii*)? *J Herpetol Conserv Biol* (2008) 3(1):103–15.
8. Duggan JM, Heske EJ, Schooley RL, Hurt A, Whitelaw A. Comparing detection dog and livetrapping surveys for a cryptic rodent. *J Wildl Manag* (2011) 75(5):1209–17. doi:10.1002/jwmg.150
9. Homan HJ, Linz G, Peer BD. Dogs increase recovery of passerine carcasses in dense vegetation. *Wildl Soc Bull* (2001) 29(1):292–6. doi:10.2307/3784011
10. Arnett EB. A preliminary evaluation on the use of dogs to recover bat fatalities at wind energy facilities. *Wildl Soc Bull* (2006) 34(5):1440–5. doi:10.2193/0091-7648(2006)34[1440:APEOTU]2.0.CO;2
11. Paula J, Leal MC, Silva MJ, Mascarenhas R, Costa H, Mascarenhas M. Dogs as a tool to improve bird-strike mortality estimates at wind farms. *J Nat Conserv* (2011) 19(4):202–8. doi:10.1016/j.jnc.2011.01.002
12. Mathews F, Swindells M, Goodhead R, August TA, Hardman P, Linton DM, et al. Effectiveness of search dogs compared with human observers in locating bat carcasses at wind-turbine sites: a blinded randomized trial. *Wildl Soc Bull* (2013) 37(1):34–40. doi:10.1002/wsb.256
13. Smith DA, Ralls K, Hurt A, Adams B, Parker M, Davenport B, et al. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). *Anim Conserv* (2003) 6(4):339–46. doi:10.1017/S136794300300341X
14. Wasser SK, Davenport B, Ramage ER, Hunt KE, Parker M, Clarke C, et al. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. *Can J Zool* (2004) 82(3):475–92. doi:10.1139/Z04-020
15. Goodwin KM, Engel RE, Weaver DK. Trained dogs outperform human surveyors in the detection of rare spotted knapweed (*Centaurea stoebe*). *Invasive Plant Sci Manag* (2010) 3(2):113–21. doi:10.1614/IPSM-D-09-00025.1
16. Leigh KA, Dominick M. An assessment of the effects of habitat structure on the scat finding performance of a wildlife detection dog. *Methods Ecol Evol* (2015) 6(7):745–54. doi:10.1111/2041-210X.12374
17. Harrison RL. A comparison of survey methods for detecting bobcats. *Wildl Soc Bull* (2006) 34(2):548–52. doi:10.2193/0091-7648(2006)34[548:ACOSMF]2.0.CO;2
18. Dematteo KE, Rinas MA, Sede MM, Davenport B, Argüelles CF, Lovett K, et al. Detection dogs: an effective technique for bush dog surveys. *J Wildl Manag* (2009) 73(8):1436–40. doi:10.2193/2008-545
19. Kerley LL, Salkina GP. Using scent-matching dogs to identify individual Amur Tigers from scats. *J Wildl Manage* (2007) 71(4):1349–56. doi:10.2193/2006-361
20. O'Connor S, Park KJ, Goulson D. Humans versus dogs; a comparison of methods for the detection of bumble bee nests. *J Apic Res* (2012) 51(2):204–11. doi:10.3896/IBRA.1.51.2.09
21. Brownell DA, Marsolais M. The Brownell-Marsolais scale: a proposal for the qualitative evaluation of SAR/disaster K9 candidates. *Adv Rescue Technol* (2002):57–67. Available from: www.operationtakemehome.org/sar/Canine%20Downloads/Forms/AllItems.aspx
22. Hurt A, Smith DA. Conservation dogs. In: Helton WS, editor. *Canine Ergonomics: The Science of Working Dogs*. Boca Raton, FL: CRC Press (2009). p. 175–94.
23. Engel GL. The need for a new medical model: a challenge for biomedicine. *Science* (1977) 196(4286):129–36. doi:10.1126/science.847460
24. Ghaemi SN. The biopsychosocial model in psychiatry: a critique. *Existenz* (2011) 6(1):1–8.
25. Olson CM, Strawderman MS. Modifiable behavioral factors in a biopsychosocial model predict inadequate and excessive gestational weight gain. *J Am Diet Assoc* (2003) 103(1):48–54. doi:10.1053/jada.2003.50001
26. Griffiths MD. A 'components' model of addiction within a biopsychosocial framework. *J Subst Use* (2005) 10(4):191–7. doi:10.1080/14659890500114359
27. Schotte CKW, Van Den Bossche B, De Doncker D, Claes S, Cosyns P. A biopsychosocial model as a guide for psychoeducation and treatment of depression. *Depress Anxiety* (2006) 23:312–24. doi:10.1002/da.20177
28. Arandjelovic M, Bergl RA, Ikfuingei R, Jameson C, Parker M, Vigilant L. Detection dog efficacy for collecting faecal samples from the critically endangered Cross River gorilla (*Gorilla gorilla diehli*) for genetic censusing. *R Soc Open Sci* (2015) 2:140423. doi:10.1098/rsos.140423
29. Brook SM, van Coeverden de Groot P, Scott C, Boag P, Long B, Ley RE, et al. Integrated and novel survey methods for rhinoceros populations confirm the extinction of *Rhinoceros sondaicus annamiticus* from Vietnam. *Biol Conserv* (2012) 155:59–67. doi:10.1016/j.biocon.2012.06.008
30. Browne CM, Stafford KJ, Fordham RA. The detection and identification of tuatara and gecko scents by dogs. *J Vet Behav* (2015) 10(6):496–503. doi:10.1016/j.jveb.2015.08.002
31. Cablk ME, Heaton JS. Accuracy and reliability of dogs in surveying for desert tortoise (*Gopherus agassizii*). *Ecol Appl* (2006) 16(5):1926–35. doi:10.1890/1051-0761(2006)016[1926:AARODI]2.0.CO;2

32. Chambers CL, Vojta CD, Mering ED, Davenport B. Efficacy of scent-detection dogs for locating bat roosts in trees and snags. *Wildl Soc Bull* (2015) 39(4):780–7. doi:10.1002/wsb.598
33. Cristescu RH, Foley E, Markula A, Jackson G, Jones D, Frère C. Accuracy and efficiency of detection dogs: a powerful new tool for koala conservation and management. *Sci Rep* (2015) 5:8349. doi:10.1038/srep08349
34. Hagell SE. *Conserving Forest Connectivity for the Central American Spider Monkey (Ateles geoffroyi) in Southwestern Nicaragua [Dissertation]*. Flagstaff, AZ: Northern Arizona University (2010).
35. Oliveira ML, de Norris D, Ramírez JFM, Peres PH, de F, Galetti M, et al. Dogs can detect scat samples more efficiently than humans: an experiment in a continuous Atlantic Forest remnant. *Zoologia* (2012) 29(2):183–6. doi:10.1590/s1984-46702012000200012
36. Reed SE, Bidlack AL, Hurt A, Getz WM. Detection distance and environmental factors in conservation detection dog surveys. *J Wildl Manage* (2011) 75(1):243–51. doi:10.1002/jwmg.8
37. Reindl SA. *Efficacy of Scent Dogs in Detecting Black-Footed Ferrets (Mustela nigripes) at a Reintroduction Site in South Dakota [Dissertation]*. Dakota, USA: South Dakota State University (2004).
38. Robertson HA, Fraser JR. Use of trained dogs to determine the age structure and conservation status of kiwi *Apteryx* spp. populations. *Bird Conserv Int* (2009) 19:121–9. doi:10.1017/S0959270908007673
39. Rolland RM, Hamilton PK, Kraus SD, Davenport B, Gillett RM, Wasser SK. Faecal sampling using detection dogs to study reproduction and health in North Atlantic right whales (*Eubalaena glacialis*). *J Cetacean Res Manag* (2006) 8(2):121–5.
40. Savidge JA, Stanford JW, Reed RN, Haddock GR, Adams AAY. Canine detection of free-ranging brown treesnakes on Guam. *N Z J Ecol* (2010) 35(2):174–81.
41. Stevenson DJ, Ravenscroft KR, Zappalorti RT, Ravenscroft MD, Weigley SW, Jenkins CL. Using a wildlife detector dog for locating Eastern Indigo snakes (*Drymarchon couperi*). *Herpetol Rev* (2010) 41(4):437–42.
42. Vynne C, Skalski JR, Machado RB, Groom MJ, Jácomo ATA, Marinho-Filho J, et al. Effectiveness of scat-detection dogs in determining species presence in a tropical savanna landscape. *Conserv Biol* (2010) 25(1):154–62. doi:10.1111/j.1523-1739.2010.01581.x
43. Wasser SK, Hayward LS, Hartman J, Booth RK, Broms K, Berg J, et al. Using detection dogs to conduct simultaneous surveys of northern spotted (*Strix occidentalis caurina*) and barred owls (*Strix varia*). *PLoS One* (2012) 7(8):e42892. doi:10.1371/journal.pone.0042892
44. Waters J, O'Connor S, Park K, Goulson D. Testing a detection dog to locate bumblebee colonies and estimate nest density. *Apidologie* (2010) 42(2):200–5. doi:10.1051/apido/2010056
45. Wultsch C, Waits LP, Kelly MJ. Noninvasive individual and species identification of jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) in Belize, Central America using cross-species microsatellites and faecal DNA. *Mol Ecol Resour* (2014) 14(6):1171–82. doi:10.1111/1755-0998.12266
46. Bennett E. Observations from the use of dogs to undertake carcass searches at wind facilities in Australia. In: Hull C, Bennett E, Stark E, Smales I, Lau J, Venosta M, editors. *Wind and Wildlife: Proceedings from the Conference on Wind Energy and Wildlife Impacts, October 2012, Melbourne Australia*. Netherlands: Springer Science (2015). p. 113–23.
47. Gazit I, Terkel J. Domination of olfaction over vision in explosives detection by dogs. *Appl Anim Behav Sci* (2003) 82(1):65–73. doi:10.1016/S0168-1591(03)00051-0
48. Hall NJ, Glenn K, Smith DW, Wynne CDL. Performance of pugs, German shepherds, and greyhounds (*Canis lupus familiaris*) on an odor-discrimination task. *J Comp Psychol* (2015) 129(3):237–46. doi:10.1037/a0039271
49. Polgár Z, Kinnunen M, Ujváry D, Miklósi A, Gácsi M. A test of canine olfactory capacity: comparing various dog breeds and wolves in a natural detection task. *PLoS One* (2016) 11(5):e0154087. doi:10.1371/journal.pone.0154087
50. Bulanda S. *Ready! Training the Search and Rescue Dog*. 2nd ed. Freehold, NJ: Kennel Club Books (2010).
51. Syrotuck WG. *Scent and the Scenting Dog*. Mechanicsburg, PA: Barkleigh Productions (1972).
52. Jones KE, Dashfield K, Dowend AB, Otto CM. Search-and-rescue dogs: an overview for veterinarians. *J Am Vet Med Assoc* (2004) 225(6):854–60. doi:10.2460/javma.2004.225.854
53. Steen JB, Mohus I, Kvesetberg T, Walløe L. Olfaction in bird dogs during hunting. *Acta Physiol Scand* (1996) 157:115–9. doi:10.1046/j.1365-201X.1996.479227000.x
54. Craven BA, Paterson EG, Settles GS. The fluid dynamics of canine olfaction: unique nasal airflow patterns as an explanation of macrosmia. *J R Soc Interface* (2009) 7(47):933–43. doi:10.1098/rsif.2009.0490
55. Settles GS, Kester DA, Dodson-Dreibelbis LJ. The external aerodynamics of canine olfaction. In: Bath FG, Humphrey JAC, Secomb TW, editors. *Sensors and Sensing in Biology and Engineering*. Vienna, Austria: Springer-Verlag (2003). p. 323–35.
56. Wells DL, Hepper PG. Directional tracking in the domestic dog, *Canis familiaris*. *Appl Anim Behav Sci* (2003) 84(4):297–305. doi:10.1016/j.applanim.2003.08.009
57. Bradshaw JWS, Jackson SL. Changes in rates of sniffing in air-trailing dogs. *J Vet Behav* (2009) 4(6):253–4. doi:10.1016/j.jveb.2009.06.005
58. Polgár Z, Miklósi A, Gácsi M. Strategies used by pet dogs for solving olfaction-based problems at various distances. *PLoS One* (2015) 10(7):e0131610. doi:10.1371/journal.pone.0131610
59. USDA Animal and Plant Health Inspection Service. *USDA National Detector Dog Manual*. (2012). Available from: www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/detector_dog.pdf
60. Ley JM, Bennett PC. Understanding personality by understanding companion dogs. *Anthrozoös* (2007) 20(2):113–24. doi:10.2752/175303707X207909
61. Svartberg K. Shyness–boldness predicts performance in working dogs. *Appl Anim Behav Sci* (2002) 79(2):157–74. doi:10.1016/S0168-1591(02)00120-X
62. Rooney NJ, Bradshaw JW. Breed and sex differences in the behavioural attributes of specialist search dogs – a questionnaire survey of trainers and handlers. *Appl Anim Behav Sci* (2004) 86(1):123–35. doi:10.1016/j.applanim.2003.12.007
63. Jones AC, Gosling SD. Temperament and personality in dogs (*Canis familiaris*): a review and evaluation of past research. *Appl Anim Behav Sci* (2005) 95(1–2):1–53. doi:10.1016/j.applanim.2005.04.008
64. Maejima M, Inoue-Murayama M, Tonosaki K, Matsuura N, Kato S, Saito Y, et al. Traits and genotypes may predict the successful training of drug detection dogs. *Appl Anim Behav Sci* (2007) 107(3–4):287–98. doi:10.1016/j.applanim.2006.10.005
65. Dorriety JK. Cadaver dogs as a forensic tool: an analysis of prior studies. *J Forensic Ident* (2007) 57(5):717–25.
66. Panksepp J, Biven L. *The Archaeology of Mind: Neuroevolutionary Origins of Human Emotions*. New York: WW Norton & Company (2012).
67. Berridge KC, Kringelbach ML. Neuroscience of affect: brain mechanisms of pleasure and displeasure. *Curr Opin Neurobiol* (2013) 23(3):294–303. doi:10.1016/j.conb.2013.01.017
68. Christiansen FO, Bakken M, Braastad B. Behavioural differences between three breed groups of hunting dogs confronted with domestic sheep. *Appl Anim Behav Sci* (2001) 72(2):115–29. doi:10.1016/S0168-1591(00)00202-1
69. Morey DF. Burying key evidence: the social bond between dogs and people. *J Archaeol Sci* (2006) 33(2):158–75. doi:10.1016/j.jas.2005.07.009
70. Hare B, Tomasello M. Human-like social skills in dogs? *Trends Cogn Sci* (2005) 9(9):439–44. doi:10.1016/j.tics.2005.07.003
71. Furtado MM, Carrillo-Percastegui SE, Jácomo ATA, Powell G, Silveira L, Vynne C, et al. Studying Jaguars in the wild: past experiences and future perspectives. *CAT News. Special Issue 4 – The Jaguar in Brazil*. (2008). p. 41–7. Available from: http://www.catsg.org/index.php?id=196
72. Topál J, Gácsi M, Miklósi A, Virányi Z, Kubinyi E, Csányi V. Attachment to humans: a comparative study on hand-reared wolves and differently socialized dog puppies. *Anim Behav* (2005) 70:1367–75. doi:10.1016/j.anbehav.2005.03.025
73. Miklósi A, Kubinyi E, Topál J, Gácsi M, Virányi Z, Csányi V. A simple reason for a big difference: wolves do not look back at humans, but dogs do. *Curr Biol* (2001) 13(9):763–6. doi:10.1016/S0960-9822(03)00263-X
74. Gácsi M, Gyoöri B, Virányi Z, Kubinyi E, Range F, Belényi B, et al. Explaining dog wolf differences in utilizing human pointing gestures: selection for synergistic shifts in the development of some social skills. *PLoS One* (2009) 4(8):e6584. doi:10.1371/journal.pone.0006584

75. SWGDOG. *Scientific Working Group on Dog and Orthogonal Detector Guidelines SC5 – Selection of Handlers (2006). Posted Under Approved Guidelines*. (2006). Available from: http://swgdog.fiu.edu/

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 16 December 2016
doi: 10.3389/fvets.2016.00114

An Investigation of the Complexities of Successful and Unsuccessful Guide Dog Matching and Partnerships

Janice Lloyd[1], Claire Budge[2], Steve La Grow[2] and Kevin Stafford[3]*

[1] Discipline of Veterinary Sciences, James Cook University, Townsville, QLD, Australia, [2] College of Health, Massey University, Palmerston North, New Zealand, [3] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

OPEN ACCESS

Edited by:
Mia Cobb, Working Dog Alliance Australia, Australia

Reviewed by:
Nathaniel James Hall, Arizona State University, USA
Mitsuaki Ohta, Tokyo University of Agriculture, Japan
Rachel Moxon, National Breeding Centre, UK

***Correspondence:**
Janice Lloyd
janice.lloyd@jcu.edu.au

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *04 April 2016*
Accepted: *29 November 2016*
Published: *16 December 2016*

Citation:
Lloyd J, Budge C, La Grow S and Stafford K (2016) An Investigation of the Complexities of Successful and Unsuccessful Guide Dog Matching and Partnerships. Front. Vet. Sci. 3:114. doi: 10.3389/fvets.2016.00114

Matching a person who is blind or visually impaired with a guide dog is a process of finding the most suitable guide dog available for that individual. Not all guide dog partnerships are successful, and the consequences of an unsuccessful partnership may result in reduced mobility and quality of life for the handler (owner), and are costly in time and resources for guide dog training establishments. This study examined 50 peoples' partnerships with one or more dogs (118 pairings) to ascertain the outcome of the relationship. Forty-three of the 118 dogs were returned to the guide dog training establishment before reaching retirement age, with the majority ($n = 40$) being categorized as having dog-related issues. Most ($n = 26$) of these dogs' issues were classified as being behavioral in character, including work-related and non-work-related behavior, and 14 were due to physical causes (mainly poor health). Three dogs were returned due to matters relating to the handlers' behavior. More second dogs were returned than the handlers' first or third dogs, and dogs that had been previously used as a guide could be rematched successfully. Defining matching success is not clear-cut. Not all dogs that were returned were considered by their handlers to have been mismatched, and not all dogs retained until retirement were thought to have been good matches, suggesting that some handlers were retaining what they considered to be a poorly matched dog. Almost all the handlers who regarded a dog as being mismatched conceded that some aspects of the match were good. For example, a dog deemed mismatched for poor working behavior may have shown good home and/or other social behaviors. The same principle was true for successful matches, where few handlers claimed to have had a perfect dog. It is hoped that these results may help the guide dog industry identify important aspects of the matching process, and/or be used to identify areas where a matching problem exists.

Keywords: guide dogs, matching success, human–animal relationships, vision impairment, blind mobility

INTRODUCTION

The guide dog, like the long cane, is a primary mobility aid intended to enhance the lifestyle of people with a visual disability (blind or visually impaired) by facilitating independent travel (1–5). Additional benefits imparted to a guide dog handler (the person who uses a guide dog) include friendship, companionship, increased social function, and improved self-esteem and confidence (3, 4, 6–14).

The process of producing guide dogs involves the selection and breeding of suitable dogs, raising and socialization of the pups, and their subsequent training as mobility aids (15–19). The making of a handler-guide dog pairing involves the matching of a trained dog to its handler, the training of the handler and dog as a team, and ongoing follow-up. Matching a person who has a visual disability with a guide dog is a process of finding the most suitable guide dog available for that individual, and a successful match is one of ongoing satisfaction with the partnership (20). However, not all guide dog partnerships are successful, and the consequences of an unsuccessful partnership may be severe in terms of the reduction in mobility and quality of life for the handler, and time and resources for guide dog training establishments (3, 4, 21, 22).

Guide dog schools worldwide pay a great deal of attention to the process of matching a dog to its new handler, but little evaluation has been carried out regarding the matching process or its subsequent outcome. Although a guide dog is principally an aid to mobility, the success of a team is not solely dependent on the dog's ability to lead an individual safely and efficiently through the environment (23, 24). Factors other than orientation and mobility (O&M) such as those relating to social situations and home environment are considered when making matches (25). Lane et al. (26) suggested that the dog's effects on enhancing the handler's social interactions may be at least as important as increased mobility and independence. This suggestion was supported in a study of first time guide dog handlers (24) who found that the dog's effects on the handler's social interactions appeared to be a significant predictor of matching success. Matching is an art as much as a science, and there may be no such thing as a perfect match. Hence, the purpose of this research was to explore handler and guide dog relationships, from the handlers' perspective, to identify characteristics of handler and dog that influence the success or failure of the match.

MATERIALS AND METHODS

This study examined 50 peoples' (26 females and 24 males) partnerships with one or more dogs (118 pairings). All the dogs in the study were trained by guide dog schools that are members of the International Guide Dog Federation (IGDF), and as such are accredited to the highest international standards. The method of participant recruitment is described in Lloyd et al. (3) (p. 21). Descriptive and inferential statistical techniques were used to analyze the data.

The study was carried out in accordance with the recommendations of Massey University Human Ethics Committee with written informed consent from all subjects.

Sample Description

To differentiate between the human and canine elements in the study, the term "handler" or "dog" will be used when referring to the 118 handler-dog teams (pairings), and the term "participant" will be used when referring in general to the 50 individuals involved in the study.

At the time the study was conducted, 39 of the 50 participants were currently using a dog; 14 were currently using their first dogs, 13 their second, 7 their third, 2 their fourth, 1 person a sixth, and 2 people were using their eighth dog. At this time, the age of the participants ranged from 21 to 86 years, with a mean of 50.3 years (SD = 15.61). Participants were on average 37.6 years old (SD = 15.46) when they received their first guide dog, with an age range from 17 to 75 years. More than a fifth of participants (n = 11) were not currently using a dog. Eight participants had decided not to use a dog in the future due to: having a limited workload (n = 3); family pressure/unsuitable living environment (n = 3); and two people whose relationship with the guide dog school had foundered. The remaining three participants were on the waiting list for a replacement dog. Information on how the end of the relationship affects people's desire to apply for a replacement can be found in Lloyd et al. (21).

Of the 118 dogs in the sample, 66.9% (n = 79) had been retired or withdrawn before the study commenced and 33.1% (n = 39) were currently in work (**Table 1**). There were nearly twice as many bitches as male dogs in the sample, and both sexes were neutered except for one male.[1] The Labrador Retriever was the most commonly used breed (57.6%), 11% were German Shepherd dogs, 11% were Labrador/Golden Retrievers (first crosses), and 4.3% were Golden Retrievers. Other breeds, including mix-breeds and "exotics" like Standard Poodles, Boxers, Giant Schnauzers, and Flat and Curly Coated Retrievers comprised 16.1% of the sample. Coat color was predominately yellow (39%) or black (36.5%) (**Table 1**).

Independence of Errors

Most of the participants (n = 32) had used more than one dog and were serially represented in the database. Hence, an "intraclass correlation coefficient" (ICC) was calculated to test for any "non-independent" observations (caused by potential clustering) on the outcome of matching success[2] using the values shown in **Table 2** and the following formula provided by Snedechor and Cochran (27) (pp. 242–244):

$$\text{ICC} = \rho = \frac{\text{MSB} - \text{MSW}}{\text{MSB} + \text{MSW}(\overline{n} - 1)} = -0.086$$

where n-bar = average group size = 118/50 = 2.36, MSB = mean square between subjects = 0.169, and MSW = mean square within subjects = 0.214.

[1] A sexually intact working guide dog is rare; permission was granted from the participant who used this dog to be identified in this manner.

[2] A discriminant functional analysis (Defining Matching Success) supported that "matching success," as defined by the handler, was a suitable outcome (dependent) variable for the ICC analysis.

TABLE 1 | Canine (*N* = 118) demographic data.

Canine demographic data	Dog 1 (*n* = 50)	Dog 2 (*n* = 32)	Dog 3 (*n* = 15)	Dog 4 (*n* = 8)	Dog 5 (*n* = 5)	Dog 6 (*n* = 4)	Dog 7 (*n* = 2)	Dog 8 (*n* = 2)	O/all (*N* = 118)
All dogs (*n*)	50	32	15	8	5	4	2	2	118
Months worked—range	1–138	1–144	4–132	2–156	3–96	2–24	9–24	42–72	1–156
Months worked (M)	70.22	46.56	48.33	39.63	37.80	12.00	16.50	57.00	54.47
Months worked (SD)	41.30	41.53	44.73	57.56	40.49	11.66	10.61	21.21	43.76
Current dogs (*n*)	14	13	7	2	0	1	0	2	39
Months worked—range	14–132	9–120	4–106	2–26	N/a	N/a	N/a	42–72	2–132
Months worked (M)	71.50	50.23	28.43	14.00	N/a	20	N/a	57.00	51.67
Months worked (SD)	42.98	32.90	35.76	16.97	N/a	N/a	N/a	21.21	39.28
Previous dogs (*n*)	36	19	8	6	5	3	2	0	79
Months worked—range	1–138	1–144	6–132	3–156	3–96	2–24	9–24	N/a	1–156
Months worked (M)	69.72	44.05	65.75	48.17	37.80	9.33	16.50	N/a	55.85
Months worked (SD)	41.24	47.25	46.51	65.05	40.49	12.70	10.61	N/a	45.98
All dogs—breed (%)									
Labrador Retriever	62.0	59.4	40.0	62.5	80.0	50.0	50.0	0	57.6
Golden Retriever	4.0	6.3	6.7	0	0	0	0	0	4.3
Lab Ret. × Golden Ret.	14.0	6.3	20.0	12.5	0	0	0	0	11.0
German Shepherd dog	12.0	12.5	13.3	0	0	0	0	50.0	11.0
Exotic/others	8.0	15.6	20.0	25.0	20.0	50.0	50.0	50.0	16.1
All dogs—sex (%)									
Male castrate[a]	34.0	43.7[a]	33.3	37.5	60.0	25.0	0	50.0	37.3[a]
Female spayed	66.0	56.3	66.7	62.5	40.0	75.0	100	50.0	62.7
All dogs—color (%)									
Yellow	46.0	46.9	26.7	12.5	20.0	50.0	0	0	39.0
Black	38.0	28.1	46.7	50.0	80.0	0	0	0	36.5
Chocolate	2.0	6.3	6.7	12.5	0	25.0	50.0	0	5.9
Black and Tan	14.0	12.5	6.7	12.5	0	0	50.0	50.0	12.7
Others	0	6.2	13.3	12.5	0	25.0	0	50.0	5.9

[a]*One dog not neutered (0.9%).*
N/a, not applicable.

TABLE 2 | Tests of between-subjects-effects generated to calculate the intra-class correlation coefficient for the outcome (dependent) variable of matching success.

Source	Type 111 sum of squares	df	Mean square	*F*	Sig. *p*
Corrected model	8.281[a]	49	0.169	0.788	0.808
Intercept	4.828	1	4.828	22.525	0.000
All cases	8.281	49	0.169	0.788	0.808
Error	14.575	68	0.214	–	–
Total	31.000	118	–	–	–
Corrected total	22.856	117	–	–	–

[a]*R^2 = 0.362; adjusted R^2 = 0.097.*

The resulting value (ICC = −0.086) was very small and negative, which according to Snedechor and Cochran (27) argues strongly against there being any meaningful positive correlation between measurements within the same handler. This value, along with the average number of dogs used in the sample being only 2.4 (118 dogs/50 people), supported the decision not to make any adjustments and to treat each handler-dog pairing as an independent observation.

Data Collection

Data was collected *via* a structured self-report questionnaire (Data Sheet S1 in Supplementary Material) that was delivered *via* telephone to 39 participants and face-to-face for 11. The method of data collection was either chosen by the participant or was by way of necessity due to the logistics of travel. There did not appear to be any discernible difference in the quality of the data obtained by the two methods of data collection.

Demographic data (e.g., age, gender) was collected on each participant. Participants were asked to comment on the "good" and "bad" behavioral and physical characteristics of each dog they had used and to rate the importance of these traits. Participants were also asked about the outcome of the handler-dog partnership regarding whether the dog was currently working, retired,[3] accidentally deceased, or had been returned[4] to the guide dog school. Further questions concerned: why dogs ceased working; mismatched dogs versus returned dogs; and reasons for successful and unsuccessful matches. As over a third (*n* = 17) of participants had used or were using dogs that had been matched with at least one other handler, participants were also asked to comment on the use of "rematched" dogs.

[3]Dogs that stopped working at age eight and older were classified as "retired," including dogs that died after this age.
[4]The term "returned" pertains to a dog that is less than 8 years old, which did not succeed as a guide for a particular handler. Many dogs that are returned are re-matched to other people with varying degrees of success. Dogs that were returned but not re-matched are classified as "withdrawn."

RESULTS

Characteristics of "Good" and "Bad" Dogs

The handlers' comments on what was good and bad, behaviorally and physically, about their dogs (118 pairings) are shown in decreasing order of frequency in **Table 3**. Handlers also rated the three most important traits within each of the four categories, denoted in **Table 3** with one, two, or three asterisks in decreasing order of importance.

The most commonly mentioned good behavior was related to "social behavior" (83.3%) including the dog being personable and well behaved at home and in other social settings, followed by "work rate" (capacity/ability to work) (75.7%) and "specific guiding tasks" (70.6%). These three characteristics were also considered to be the most important. The bad behavior most commonly reported concerned "specific guiding tasks" (29.8%) closely followed by "distractions (mainly to other dogs) while working" (28.1%) and "work rate" (27.2%). The three traits most often cited as being of first, second, and third equal importance regarding undesirable behavior are "distractions (mainly to other dogs) while working," "scavenging," and poor "coping skills" or "running away," respectively (**Table 3**).

Concerning physical characteristics, the "size" of the dog matters. Over half of the handlers (55.3% of 118 pairings) mentioned that they liked the size of their dogs, with most handlers preferring a compact dog as opposed to a large one—mainly for ease of fitting into confined spaces such as under a desk at work or in a transport vehicle. Tall handlers said that they required a dog to be big enough for them to use the harness handle effectively, but not to be taller or longer than necessary. Smaller dog were deemed easier to lift, bathe, and be less strong and hence not be able to pull as hard as a more

TABLE 3 | Good and bad canine (*N* = 118) behavioral and physical characteristics.

Good traits	%	Bad traits	%
Behavioral		**Behavioral**	
Social inc., home; personality[a]	83.3	Specific guiding tasks	29.8
Work rate/capacity to work[b]	75.7	Distractions when working (mostly to dogs)[a]	28.1
Specific guiding tasks[c]	70.6	Work rate/capacity to work	27.2
Speed—control/tension/sustainability	28.9	Social inc., home; personality	27.2
Coping	23.0	Scavenging/food oriented salivation[b]	24.7
Not overly sensitive	17.9	Escapism/poor recall[c]	23.8
Office behavior	13.6	Aggression/s (mostly to dogs)	23.0
Toileting habits	11.1	Speed—control/tension/sustainability	22.1
No scavenging/food-oriented salivation	11.1	No bad	17.9
Good with children	10.2	Coping[c]	16.2
No escapism/good recall	9.4	Toileting habits	14.5
Good with other pets	6.0	Overly sensitive	14.5
Acceptable distractions	5.1	Office behavior	6.8
Barking only when appropriate	4.3	Coprophagous	5.1
No good	3.4	Chased cats	5.1
No aggressive tendencies	2.6	Suspicious—people/objects	4.3
Discouraged unwanted cats at home	1.7	Aggressive to other pets	3.4
Retrieved objects	0.9	Barking	3.4
Not coprophagous	0.9	Will not retrieve objects	0.9
		Fussy/expensive eating habits	0.9
		Anxious re-car travel	0.9
Physical		**Physical**	
Size (mostly compact)[a]	55.3	No bad	36.6
Breed[b]	48.5	Health[a]	27.2
Good-looking[c]	40.8	Coat—shedding/high maintenance[b]	22.1
Sex (mostly female)	32.3	Size (mostly too big)[c]	16.2
Easy-care coat (mostly short hair)	25.5	Strength—pulling	14.5
Color—compliment/unlike previous dog	23.0	Breed	12.8
Tactility—soft coat/ears	12.8	Gait—hard to follow/unstable/veering	4.3
Gait—easy to follow/provide stability	7.7	Not good-looking	4.3
Strength—pulling uphill only	6.8	Sex (mostly male)	2.6
Nothing remarkable/acceptable	6.0	Age—too puppy-like/too mature	2.6
Weight	4.3	Tail not docked—nuisance factor	2.6
Docked tail—no nuisance factor	1.7	Overweight	2.6
		Color—hair noticeable on clothing/carpets	1.7
		Not tactile—coarse texture of coat/ears	1.7
		Malodorous	1.7

[a]Item most often cited as greatest importance for that category.
[b]Second most often cited.
[c]Third most often cited.
D, dog; H, handler; B, behavioral; P, physical; W, work; NW, non-work; N/a, not applicable.

powerfully built dog. "Breed" was the next frequently mentioned desirable physical trait (48.5%) followed by "attractiveness" (40.8%). One handler, who returned her dog said "I thought that everything would be alright if only my next dog was a Poodle," but then described the Standard Poodle as being "dizzy" and "unfocussed" when it was received. Reasons given for wanting a dog to be attractive included "I feel like I live my life in a fish bowl, with everyone watching—so why shouldn't my dog look nice?" and "I miss my old dog's soft, soft ears. I don't like [the new dog's] ears. I don't suppose [the instructor] would find that important as it's not a mobility thing." "Size," "breed," and "attractiveness" were also consecutively considered to be the three most important characteristics concerning physical traits (**Table 3**).

Over a third of handlers (36.6% of 118 pairings) stated that their dogs had no bad physical characteristics, 27.2% experienced dogs with health problems (including skin issues), and 22.1% expressed troublesome issues regarding the dogs' coat such as shedding and amount of care required (grooming/bathing). "Health" was rated as being of most importance in this category, followed by "coat," and the dog being too large in "size."

Rating Canine Qualities

To gain a more specific understanding of what qualities were found attractive and unattractive in guide dogs, the 32 participants who had worked with more than one dog were asked to state the *main* characteristic that they liked best about their favorite dog (n = 32), and the *main* characteristic they liked least about their least favorite dog (n = 32). The 18 participants who used only one dog were asked to state the best and worst qualities of that particular dog (n = 18). Participants were asked to categorize these responses into either work (W) or non-work (NW) scenarios, and state if this was behavioral (B), physical (P), or emotional[5] (E) in nature.

The results of these classifications (**Figure 1**) show that most of the favorite and least favorite traits were behavioral in nature. Specifically, half of the "favorite" responses were classified as non-working behavior (NW/B), followed by working behavior (W/B) (36.0%) and non-working emotional (NW/E) (14.0%). To illustrate with examples from this sample, the NW/B category is demonstrated by a dog that "was wonderful company at home"; W/B is shown by a dog that "was excellent at finding destinations"; and NW/E *via* a dog that "was a soulmate for almost 12 years." Likewise, the majority of the "least favorite" responses were categorized as NW/B (42.0%). This was followed by W/B (30.0%), non-working physical (NW/P) (16.0%), NW/E (6.0%), working physical (W/P) (4.0%), and working emotional (W/E) (2.0%). Illustrations of NW/B included a dog that "solicited too much attention at social functions" and another that "growled at visitors at home." A dog that "failed to stop consistently at the end of the pavement" (down kerb) exemplifies W/B, and a dog that "shed hair excessively" represents NW/P. The statement "I don't know why I did not like that dog… it was quite a good worker and well behaved at home, but we just did not gel" was coded as NW/E. A dog that was categorized W/P was considered "too sick to work," and a dog classified as W/E pertained to the

[5]"Emotional" is defined as a psychological response that could not be classified as either behavioural or physical.

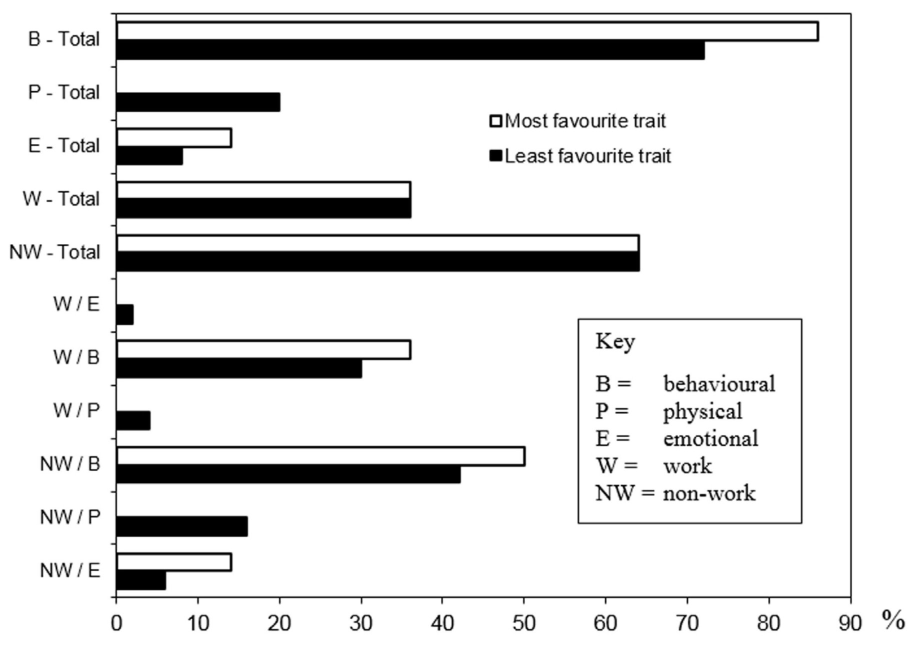


FIGURE 1 | **The participants' (N = 50) most favourite and least favourite characteristics of their dogs (n = 82) concerning behavior, physical, and emotional categories, and whether this relates to work or non-work**.

inexperience of the handler who professed that he "did not know how to work with my first dog."

Thirty of the 32 participants who had handled multiple dogs rated their dogs ($n = 93$) in order of favoritism. Sixteen of the 30 dogs considered to be the favorite had been the participants' first guide dog, seven were subsequent dogs previously employed as a guide, and seven were dogs in current use.

The Outcome of the Partnership

The outcome of all the handler-dog partnerships (118 pairings) in terms of whether a dog was currently working, retired, accidentally deceased, or returned to the guide dog school is illustrated in **Figure 2**. One-third (33.1%) of dogs in the sample were currently working. Of the two-thirds (66.9%) that were not, 36.4% were returned to the guide dog school before the dog reached retirement age, 27.1% were retired due to disorders related to old age (poor health, failing eyesight, slowing down, etc.) and 3.4% died from accidental causes prior to retiring. An itemization of the dogs that were currently working and the main general and specific explanation for why dogs either were returned or were under consideration for return are shown in **Table 4**.

Reasons Dogs Cease Working

Although the majority of dogs in the sample (66.9%) had ceased working, most (63.6%) had not been returned. The primary reasons for dogs not currently working or for being considered for return have been categorized as either dog (D) related or handler[6] (H) related, grouped into work (W) versus NW scenarios, and considered behavioral (B) or physical (P) in nature. Results fell into four of the eight possible combinations, which are displayed in **Figure 2**.

[6]The category "handler" incorporates the effects of Dahlgren and Whiteheads' (28) socioeconomic model where the handler's decision regarding the return of a dog may have been influenced by social or environmental factors not of the handler's choosing, such as a dog not being wanted by family or work-mates.

Forty of the 43 dogs that were returned were returned for dog-related problems. Of these 40 dogs, most ($n = 26$) were returned for dog behavioral problems, with almost two-thirds ($n = 17$) related to working behavior and nine dogs for behaviors unrelated to work (i.e., poor social and/or home behaviors) (**Table 4**). Problems relating to working behavior included dogs being distracted by and/or aggressive to other dogs, coping skills, and specific-guiding tasks. The few handlers who returned their dogs for incompatible walking speed said it was "frightening and uncomfortable to be dragged around" by a dog going too fast, "frustrating to be held back" by a slow dog and "confusing" if speed was inconsistent as the handler may not know why the dog slowed. Fourteen dogs were returned due to physical causes, that is, health issues that were unrelated to work including musculoskeletal disorders and cancer. The remaining three dogs were returned for NW-related handlers' behavior. One of these dogs had been matched on a temporary basis while the handler awaited a more permanent dog, one handler said he/she was pressurized not to have a dog in the workplace, and one handler's partner did not want a dog living in the house.

Of the 39 dogs (33.1%) working at the time, the study was conducted, five were being considered for return because of various dog-related health and behavior problems. In general, more second dogs were returned than first or third dogs, respectively, and around twice the number of second and third dogs that were currently working were being considered for return compared with first.

Most of the handlers who regarded their dogs as mismatched conceded that some aspects of the match were good. For example, a dog deemed mismatched for poor work may have shown good home and/or other social behaviors. The same principle held true for successful matches, where few handlers claimed to have had a perfect dog. However, it is noteworthy that the majority of dogs ($n = 24$ of 26) returned primarily for D/B reasons exhibited more than one undesirable behavior. Eight of the nine dogs that were returned for D/NW/B (primarily for poor home and social behavior) also had behavioral problems related to work. These included low coping skills, poor work rate, easily distracted, overly

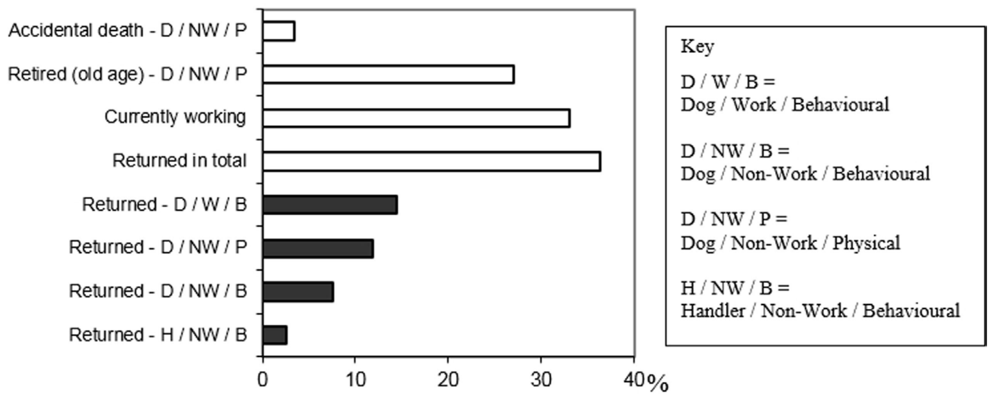


FIGURE 2 | The outcome of the dogs' ($N = 118$) working lives. The white bars show the broad outcomes and the black bars categorize the main reason why dogs were returned to the guide dog school.

TABLE 4 | The outcome of the dogs' (*N* = 118) working lives, and the general and specific categories for why dogs were returned or were being considered for return.

Outcome status of dogs' working lives	Dog 1 (*n* = 50)	Dog 2 (*n* = 32)	Dog 3 (*n* = 15)	Dog 4 (*n* = 8)	Dog 5 (*n* = 5)	Dog 6 (*n* = 4)	Dog 7 (*n* = 2)	Dog 8 (*n* = 2)	O/all (*N* = 118)
Currently working (%)	28.0	40.6	46.7	25.0	0	25.0	0	100	33.1
Retired—old age (≥8 years) (%)	40.0	18.8	26.7	25.0	0	0	0	0	27.1
Accidental death (≤8 years) (%)	4.0	0	6.7	0	0	0	50.0	0	3.4
Returned in total (≤8 years) (%)	28.0	40.6	20	50.0	100	75.0	50.0	0	36.4
Returned—general (%)									
Dog physical (D/P)	12.0	15.6	0	0	40.0	0	50.0	0	11.9
Dog behavior (D/B)	16.0	24.8	6.7	50.0	60.0	50.0	0	0	22.0
Handler behavior (H/B)	0	0	13.4	0	0	25.0	0	0	2.6
Returned—D/P specific—health (%)									
Musculoskeletal [non-work (NW)]	2.0	6.3	N/a	N/a	20.0	N/a	50.0	N/a	4.3
Cancer (malignant) (NW)	6.0	3.1	N/a	N/a	0	N/a	0	N/a	3.4
Endocrine (NW)	0	6.3	N/a	N/a	0	N/a	0	N/a	1.7
Gastrointestinal (NW)	2.0	0	N/a	N/a	0	N/a	0	N/a	0.9
Renal (NW)	2.0	0	N/a	N/a	0	N/a	0	N/a	0.9
Skin (NW)	0	0	N/a	N/a	20.0	N/a	0	N/a	0.9
Returned—D/B specific (%)									
Specific-guiding tasks (W)	2.0	0	0	0	20.0	0	0	0	1.7
Distracted/aggressive to dogs (W)	6.0	0	0	37.5	0	0	0	0	5.1
Social (inc., home) (NW)	4.0	12.5	6.7	0	20.0	25.0	0	0	7.6
Capacity to work (work rate) (W)	2.0	3.1	0	12.5	0	0	0	0	2.5
Coping (anxiety; adaptability) (W)	2.0	3.1	0	0	0	25.0	0	0	2.5
Working speed (W)	0	6.3	0	0	20.0	0	0	0	2.5
Returned—H/B specific (%)									
Temporary match (NW)	0	0	6.7	0	0	0	0	0	0.9
Family (NW)	0	0	6.7	0	0	0	0	0	0.9
Environment (NW)	0	0	0	0	0	25.0	0	0	0.9
Currently working—general (%)									
Going well	14.0	31.3	40.0	25.0	0	0	0	100	22.9
Good, but nearing retirement	12.0	3.1	0	0	0	0	0	0	5.9
Potential return—D/B and D/P	2.0	6.3	6.7	0	0	25.0	0	0	4.2
Currently working, but being considered for return—specific (%)									
Distract/aggress. To dogs (D/W/B)	2.0	0	0	0	0	0	0	0	0.9
Coping (D/W/B)	0	3.1	0	0	0	0	0	0	0.9
Scavenging (D/NW/B)	0	0	6.7	0	0	0	0	0	0.9
Aggressive to other pets (D/NW/B)	0	3.1	0	0	0	0	0	0	0.9
Ill-health—skin (D/NW/P)	0	0	0	0	0	25.0	0	0	0.9

sensitive, working speed too fast, and toileting on walks. Sixteen of the 17 dogs returned mainly for their W/B also displayed a range of NW-related problems including poor social behavior ($n = 8$) and three dogs were criticized for physical issues such as excessive hair shedding and for being a specific breed.

Classification of Outcome

The majority (73.7%, $n = 87$) of dogs in the sample were considered to have been successfully matched and 63.6% ($n = 75$) of all dogs were retained (**Table 5**). However, not all dogs that were returned before reaching retirement age were considered by their handlers to have been mismatched, and not all dogs retained until retirement were thought to have been good matches. Thus, the dogs were classified as:

Combination 1: successfully matched and retained (56.8%, $n = 67$)
Combination 2: mismatched, but retained (6.8%, $n = 8$)
Combination 3: successfully matched, but returned (17.0%, $n = 20$)
Combination 4: mismatched and returned (19.5%, $n = 23$).

Combination 1: Successfully Matched and Retained

Most of the dogs (56.8%, $n = 67$) in the sample were considered to be well matched and were kept by their handlers. Three dogs whose handlers believed them to be poor mobility aids were included in this group. This was due to the handlers feeling that they had enough useful residual vision to compensate for the dogs' lack of abilities and/or because the dogs were considered good companions.

Combination 2: Mismatched but Retained

The 6.8% ($n = 8$) of dogs that were considered mismatched, but not returned, were retained for several reasons. These included three participants who had used more than one dog becoming emotionally attached to their first dogs and being highly

TABLE 5 | Whether handlers deemed their dogs (*N* = 118) to be successfully matched or not and how this relates to the dogs being returned or retained.

Dogs' matching status and months worked	Dog 1 (*n* = 50)	Dog 2 (*n* = 32)	Dog 3 (*n* = 15)	Dog 4 (*n* = 8)	Dog 5 (*n* = 5)	Dog 6 (*n* = 4)	Dog 7 (*n* = 2)	Dog 8 (*n* = 2)	O/all (*N* = 118)
Successfully matched in total (%)	80.0	68.7	86.7	50.0	40.0	50.0	100	100	73.7
Months worked—range	1–132	1–144	4–132	2–156	62–96	2–20	9–24	42–72	1–156
Months worked—(M)	75.05	61.77	53.46	71.5	79	11	16.5	57	65.16
Months worked—(SD)	38.67	41.75	46.03	70.64	24.04	12.73	10.61	21.21	42.29
Mismatched in total (%)	20.0	31.3	13.3	50.0	60.0	50.0	0	0	26.3
Months worked—range	3–138	1–30	12–18	3–14	3–25	2–24	N/a	N/a	1–138
Months worked—(M)	50.9	13.1	15	7.75	10.33	13	N/a	N/a	24.45
Months worked—(SD)	47.86	8.01	4.24	5.62	12.7	15.56	N/a	N/a	32.81
Retained in total (%)	72.0	59.4	80.0	50.0	0	25.0	50.0	100	63.6
Months worked—range	12–138	9–144	4–132	2–156	N/a	N/a	N/a	42–72	2–156
Months worked—(M)	85.03	65.9	55.92	71.5	N/a	20	24	57	72.37
Months worked—(SD)	36.55	41.37	46.77	70.64	N/a	0	0	21.21	42.3
Returned in total (%)	28.0	40.6	20.0	50.0	100	75.0	50.0	0	36.4
Months worked—range	1–72	1–72	6–36	3–14	3–96	2–24	N/a	N/a	1–96
Months worked—(M)	32.14	18.31	18	7.75	37.8	9.33	9	N/a	23.23
Months worked—(SD)	25.76	20.76	15.88	5.62	40.49	12.7	0	N/a	24.68
Combination 1									
Successfully matched—retained (%)	62.0	53.1	73.3	50.0	0	25.0	50.0	100	56.8
Months worked—range	12–132	9–144	4–132	2–156	N/a	N/a	N/a	42–72	2–156
Months worked—(M)	85.71	70.94	59.36	71.5	N/a	20	24	57	74.03
Months worked—(SD)	35.78	40.78	47.42	70.64	N/a	0	0	21.21	41.87
Combination 2									
Mismatched—retained (%)	10.0	6.3	6.7	0	0	0	0	0	6.8
Months worked—range	14–138	16–30	N/a	N/a	N/a	N/a	N/a	N/a	14–138
Months worked—(M)	80.8	23	18	N/a	N/a	N/a	N/a	N/a	58.5
Months worked—(SD)	45.47	9.9	0	N/a	N/a	N/a	N/a	N/a	46.31
Combination 3									
Successfully matched—returned (%)	18.0	15.6	13.3	0	40.0	25.0	50.0	0	17.0
Months worked—range	1–72	1–72	6-36	N/a	62–96	N/a	N/a	N/a	1–96
Months worked—(M)	38.33	30.6	21	N/a	79	2	9	N/a	35.45
Months worked—(SD)	23.07	30.5	21.21	N/a	24.04	0	0	N/a	28.3
Combination 4									
Mismatched—returned (%)	10.0	25.0	6.7	50.0	60.0	50.0	0	0	19.5
Months worked—range	3–72	1–18	N/a	3–14	3–25	2-24	N/a	N/a	1–72
Months worked—(M)	21	10.63	12	7.75	10.33	13	N/a	N/a	12.61
Months worked—(SD)	29.16	5.78	0	5.62	12.7	15.56	N/a	N/a	14.76

motivated to make the partnership work, and another who claimed not to have known any better as it was his first dog and he had nothing to compare it to. The other four dogs that were retained despite being unsuccessfully matched were four of the five dogs in current use that were being considered for return (**Table 4**) for reasons of: being distracted by and aggressive to other dogs when working; being aggressive to other pets; being overly sensitive and not coping with the demands of guiding; and scavenging on and off the job.

Combination 3: Successfully Matched but Returned

Twenty dogs in the sample (17.0%) were returned before the dogs reached retirement age despite being successfully matched. Of these dogs, 12 were returned due to the dogs' unexpected ill-health, one dog was returned for slowing down through the normal aging process as it neared retirement age, one dog was withdrawn by the guide dog school because of protective aggressive tendencies, one dog was returned as it had been matched on a temporary basis until the handler's preference for a younger dog could be fulfilled, one dog was swapped with a close associate of the handler with the approval of the guide dog school, and one dog was withdrawn by the guide dog school for reasons unknown. The remaining three dogs that were returned, although successfully matched, were ultimately returned by handlers who had made informed choices to accept these dogs when the guide dog school discussed potential problems at the time of matching, and these problems were the reason for return. Two of these dogs were returned for dog distraction/aggression and one for an ongoing health problem.

Overall, handlers claimed to be very emotionally attached to 18 of the 20 dogs in this group. Regarding the other two dogs, one handler was moderately attached (temporary match), and the other, who returned her dog because of its poor health, felt guilty for not bonding more with a dog she had not realized at the time was too sick to work effectively.

Combination 4: Mismatched and Returned

Twenty-three dogs (19.5%) were returned for being considered to be poorly matched by the handler. Reasons provided for this included the dog having poor social/home behaviors, limited capacity to work (work rate), problems with specific-guiding tasks (including speed), and having poor coping skills. However, as shown above, more dogs were thought to be mismatched (26.3%, $n = 31$) than were actually returned for this reason. None of the three handlers who returned their dogs because of, or related to, their own behavior considered these dogs to be mismatched.

Relationships between the Combinations

Frequencies for the four combinations to which the handlers assigned their dogs are illustrated in **Figure 3**. A chi-square test for independence was conducted to examine these relationships. A significant result was obtained using Yates' Correction for Continuity [χ^2 (1, $N = 118$) = 23.71, $p < 0.0005$] suggesting that the proportion of dogs that was returned for being mismatched (74.2% of all mismatched dogs) was significantly different from the proportion of dogs that was returned although successfully matched (23% of all successfully matched dogs). A calculation of the odds ratio indicated that dogs were 9.6 times more likely to be returned if deemed mismatched.

Duration of the Partnership

Seventy-nine (66.9%) of the 118 dogs in the sample had reached the end of the working partnership (retired or returned) with a particular handler (**Table 1**). The duration of the partnership is calculated only on these 79 dogs, as the sample as a whole does not reflect the full working life of all partnerships. The 79 dogs previously employed as a guide worked from as little as 1 month to as long as 156 months[7] (13 years) with an average working life of 4.7 years (M = 55.9 months, SD = 46.0). Handler defined successful partnerships ($n = 53$), lasted from one month to 13 years, with an average service duration of 6 years (M = 72.3 months, SD = 42.8), and the largest number of dogs (mode) were retired at about 10 years of age (norm). Unsuccessful partnerships ($n = 26$) (inclusive of three dogs that were not returned) lasted from 1 month to 11.5 years (138 months), but dogs were returned on average at less than 2 years (M = 22.2 months, SD = 32.2), with the largest number (mode) being returned after 3 months. Dogs that were returned for being unsuccessfully matched ($n = 23$) worked from 1 month to 6 years (72 months) and were returned on average at just over 1 year (M = 12.6 months, SD = 14.7). All dogs that were returned for being unsuccessfully matched were returned within 2 years, with the exception of one dog that worked for 6 years. Excluding this dog, dogs that were returned for being unsuccessfully matched worked on average for just under 10 months, and the largest number were returned after 3 months.

Trends between Dogs

Concerning the relationships handlers had with their dogs, a recurring trend of "first dog best—second dog worst," with little apparent difference between the first and third dogs, was found. As this pattern may be of interest to the guide dog industry, this "second dog syndrome" was further investigated. The focus is on the trends between the first three dogs only, as these dogs comprise the majority of dogs (82.2%) in the sample.

When accounting for the proportion of dogs at the time the study was conducted, the number that were (a) returned, (b) mismatched, and (c) mismatched and returned were all highest for second dogs and lowest for third (**Figure 4**). Odds ratios indicated that the likelihood of a dog being (a) returned and (b) mismatched was nearly twice (1.8) as high for second dogs as first dogs, and second dogs were three times more likely to be (c) mismatched and returned than first dogs. Third dogs were around three times less likely than second dogs to be (a) returned (2.7) or (b) mismatched (3.0), and nearly five times less likely to be (c) returned for being mismatched. Only half of the mismatched first and third dogs were returned, but mismatched second dogs were returned four times as often. None of these trends reached statistical significance on chi-square analyses. However, second dogs were significantly (four times) more likely to be returned

[7] Only one dog worked for 13 years, and this was an unusual case that had a very limited workload for the last few years of its working life.

Returned \ Mismatched	No	Yes
No	Combination 1 **Successfully matched and Retained** 56.8% ($n = 67$)	Combination 2 **Mismatched but Retained** 6.8% ($n = 8$)
Yes	Combination 3 **Successfully matched but Returned** 17.0% ($n = 20$)	Combination 4 **Mismatched and Returned** 19.5% ($n = 23$)

Yates' Correction for Continuity χ^2 (1, $N = 118$) = 23.71, $p < .0005$

FIGURE 3 | Association between matching success and dogs that are returned or retained.

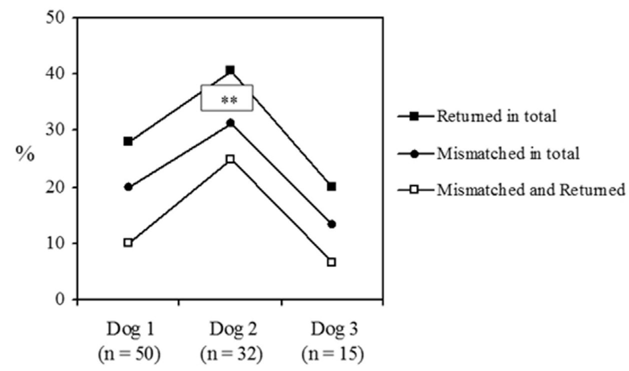


FIGURE 4 | Comparisons of the relationships between the first, second, and third dogs concerning the percent of dogs returned, mismatched, and those returned for being mismatched. **Denotes the significant relationship between the second dogs that are returned for being mismatched and those that are retained ($p < 0.004$).

if mismatched than retained (Fisher's Exact Probability test $p < 0.004$).

In light of this, it is not unexpected that the working life of dogs that had been retired or withdrawn before the study commenced ($n = 79$) is shortest in second dogs (**Table 1**). Independent samples *t*-tests revealed that second dogs (M = 44.1, SD = 47.3) were used for significantly less time than first dogs [M = 69.7, SD = 41.2, $t(53) = 2.09$, $p = 0.042$, $\eta^2 = 0.08$]. No significant difference was seen between second and third dogs (M = 65.8, SD = 46.5, $\eta^2 = 0.05$). However, this was likely due to the small number of third dogs that were not in current use in the sample ($n = 8$).

Defining Matching Success

Based on the study findings, defining matching success is not clear-cut. The results indicate that a substantial number of handlers return dogs (17.0%, $n = 20$) for reasons that do not pertain to being mismatched, mainly for the dogs' poor health. The results also suggest that more dogs were considered mismatched (26.3%, $n = 31$) than were returned for problems arising from these mismatches (19.5%, $n = 23$). As a goal of this research was to identify what factors were important in creating a successful match, it seemed sensible to focus on whether a dog was considered mismatched *per se* as opposed to whether it was returned for being mismatched.

A discriminant function analysis was conducted using the data relating to the four matching success/outcome categories to check that the above classification decision was viable. Although a significant Box's *M* value indicted that assumptions of equality of covariance matrices were not met, the results (**Figure 5**) suggest that there were three significantly distinct groups ($\chi^2 = 170.57$, $df = 36$, $p < 0.0005$): Combination 2, Combination 4, and Combinations 1 and 3 combined. In effect, the dogs deemed to be mismatched (Combinations 2 and 4) were considerably different from those that were not (Combinations 1 and 3). There were no meaningful differences between dogs that were considered successfully matched that were retained (Combination 1) and those that were returned (Combination 3). Dogs that were considered mismatched but retained (Combination 2) appeared to differ somewhat from those that were considered mismatched and returned (Combination 4). However, the number of dogs in Combination 2 ($n = 8$) was very small, and the decision to combine these dogs with the other mismatched dogs in Combination 4 was justified based on the qualitative data described in "Classification of Outcome." Hence, it was decided that a reasonable definition of matching success was whether dogs were considered to be mismatched or not by their handlers.

Rematched Dogs

Just over a third ($n = 17$) of the participants had used or were using dogs that had been matched with at least one other previous person. This scenario is common as dogs are returned to guide dog schools for a variety of reasons that do not preclude them from being rematched to others. These reasons include ill-health (of the person), emigration, or changes in individual mobility needs and/or family dynamics.

The majority (84%) of participants said that they were or would be content to be matched with a dog that had been previously used as a guide by another person, although caveats included "it is OK, as long as you know the dog's history" and "people need to know that it may take longer to bond [with the dog]." Some participants preferred dogs that had been used previously as a guide, as these dogs tended to be more mature and, therefore, less rambunctious than dogs fresh out of training, or "had been round the block and knew a thing or two about guiding." Eight participants (16%) stated they would not be happy if offered a dog that had been previously used as a guide because they were concerned that it may be more likely to have behavioral problems or take longer to adjust to a new home/working environment.

Twenty (17.2%) of the 118 dogs in the sample had been returned to the guide dog school by their previous handlers, and ultimately, 15 of these 20 rematches were successful. However, according to the participants in this study, four of these 15 dogs did not have a successful partnership with the first person they were rematched to, but did on a subsequent match, and three of these 15 dogs had the opposite experience. Of the five dogs that were not rematched successfully, three were eventually withdrawn; one for extreme excitability, one for marked aggression to other dogs, and one for a musculoskeletal problem. The other two dogs that were not rematched successfully, although currently working, were being considered for return, as one exhibited the same problem that its previous handler returned it for (scavenging) and the other developed an unrelated health issue (skin problem).

DISCUSSION

The purpose of this study was to investigate the relationship between handlers and their guide dogs to understand, from the handlers' perspective, why some partnerships worked while others did not, to improve the outcome of the matching process. Defining matching success is not clear-cut. Not all dogs that were returned were considered by their handlers to have been mismatched, and not all dogs retained until retirement were

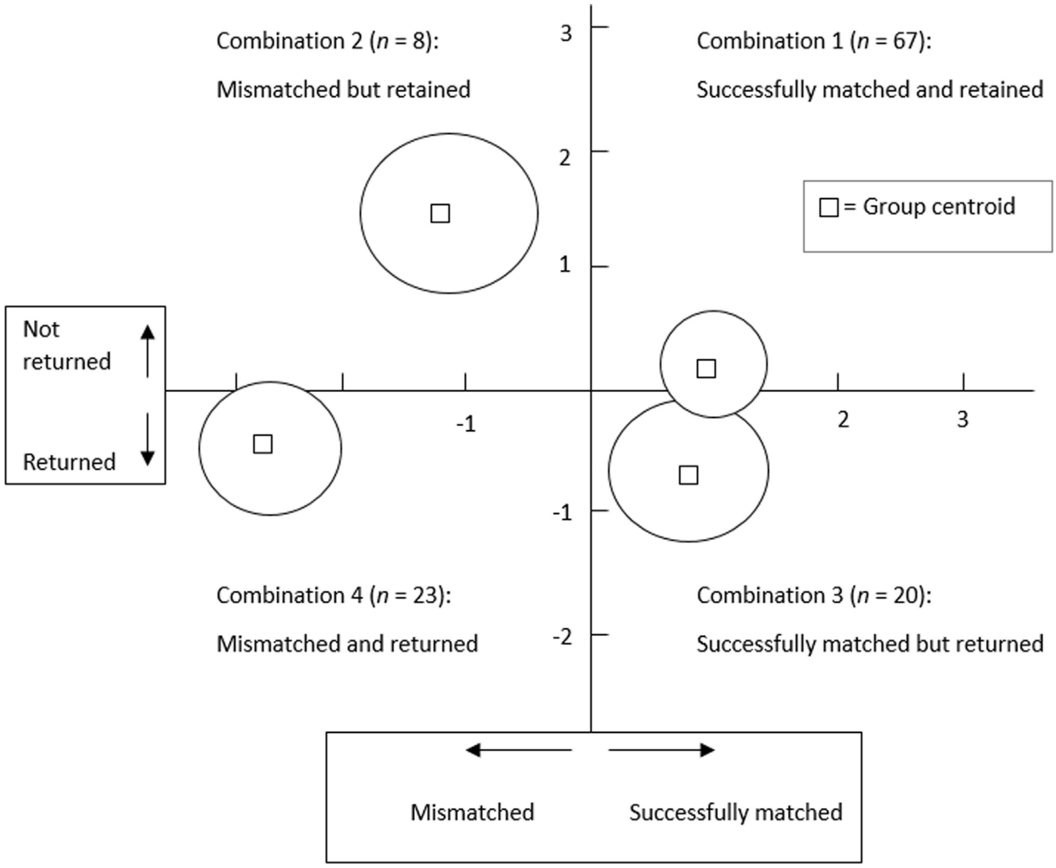


FIGURE 5 | A discriminant function analysis plot showing estimation of the group centroids and the corresponding confidence circles for matching success for Combinations 1–4. Note: the confidence circles were calculated as per Maxwell (29).

thought to have been good matches. The latter finding suggests that some handlers were retaining what they considered to be a poorly matched dog, which could have detrimental effects on both the handlers' and the dogs' quality of life.

This study measured the success of the match based on whether handlers thought the dog was mismatched or not. Ratings of good and bad behaviors and physical characteristics described what qualities handlers found attractive and unattractive in guide dogs in general, and how they related to work (mobility-related) versus NW (home/social) situations, and were primary used to describe the data in a meaningful way. While there are theoretical grounds to believe that a handler is more similar to themselves than another handler in how they perceive a dog, each human–animal relationship is unique and the decision to treat each handler-dog pairing as independent observations was supported by statistics (i.e., insignificant ICC value and average number of dogs used close to only two). However, although not detectable in the present study, it is possible that some handlers might be more likely to return dogs than others. For example, a person who may have had a specific problem with one dog might pay more attention to the same issue in a subsequent dog, and this should be taken into account during the matching process.

Although several studies have described peoples' attitudes toward guide dogs (1, 23, 30, 31), there appears to be little data available on the reasons why some matches fail. This may be because some guide dog schools compete with others for clients. However, it would benefit those involved with guide dogs if this information was more freely available.

The Outcome of the Partnership

Most dogs in this study were successfully matched. Partnerships ended for one of the three reasons: (1) the dog retired, (2) it was returned (whether mismatched or not), or (3) it died. Over a third of dogs were returned in total, primarily for problems concerning the dogs' behavior, followed by canine health problems. In addition, three handlers returned their dogs for personal or social reasons. More dogs were returned for behavior problems relating to work than for NW. However, the largest single behavior problem that dogs were returned for was poor home/social behavior. It would be advantageous for guide dog schools to pay equal attention to working and non-working behaviors when training dogs and making matches, as in addition to this finding, Lloyd et al. (24) and Lloyd (32) found that factors relating to both the working and the non-working relationship appeared to be significant predictors of matching success.

Just over a quarter of dogs were considered to be mismatched, but only a one-fifth of dogs were returned for this reason. Reasons for dogs being returned or considered to be mismatched related more to the dog than the handler, and problems were behavioral more than physical. More dogs were considered mismatched for reasons that pertained to work than for NW. This discrepancy was due to a number of dogs being returned, despite being considered successfully matched, for health reasons. The probability of a mismatched dog being returned was several fold higher than for successfully matched dogs, and as may be expected, the reasons that dogs are returned (whether mismatched or not) correspond with what handlers said they liked the least about their dogs. It should be appreciated that matching is not absolute; some unsuccessful matches had good points, and few successfully matched handlers claimed to have a perfect dog. However, some handlers kept dogs that they thought were mismatched because they were emotionally attached to the dog, had enough vision to compensate or were inexperienced. This was more likely to happen for first-time dogs than second ones, which is discussed below.

Successful partnerships lasted for an average of 6 years, with the largest number (mode) of dogs being returned after 10 years of service. This is lower than the average of 7 years reported by Nicholson et al. (22). However, the present study included dogs that had previously worked with other handlers and had been rematched, and it is possible that Nicholson et al. (22) were referring to successfully matched dogs that had worked with only one handler. A recent paper by Caron-Lormier et al. (33) who investigated aging in guide dogs also recorded the length of the dogs' working life. The researchers found that 84% of working guide dogs were able to function as guide dogs until they had worked for 8.5 years, when they retired. However, this sample excluded dogs that were withdrawn for behavioral reasons and so methodological issues make it difficult to compare this study with the present study.

In the present study, excluding one dog that worked for 6 years before being returned for behavioral problems, mismatched dogs worked for 10 months on average, but the largest number (mode) were returned after just 3 months—presumably before an emotional bond had fully developed. Therefore, a handler who is frustrated with a new partnership should be informed that the working and the non-working relationship might take longer than this to improve, possibly from 6 months up to a year (34). These findings are similar to those of Fuller (35) who reported that most unsuccessfully matched dogs were returned within the first year, and that several dogs were returned for behavioral problems after 5 years of use. Although not stated by Fuller (35), it is possible that these late returns were due to replacement dogs becoming more readily available at that time. Fuller (35) indicated that returns were due to handler-related reasons one-third of the time, and the remainder for dog-related reasons, with physical incapacity or death being the major factor in both categories. Both Fuller (35) and the present study suggests that the number of returns because the handler has personal or social problems is small, but results differ in that Fuller (35) reported more dogs stopped working for health (59.4%) than for behavioral problems (7.4%). Adjusting for the inclusion of the human's physical incapacity or demise as reasons for partnerships to end, the number of dogs returned for behavioral problems in the present study (22.0%) was double that of Fuller (35) figures (10.2%). This finding supports Ireson (15) theory of dogs being returned less often by previous generations of guide dog handlers. However, it could not be ascertained what the specific behavioral problems were in the Fuller (35) study nor whether they related to work and/or NW.

The number of dogs in the present study that were unsuccessfully matched (26.3%), and returned for this reason (19.5%) is comparable to the 25.4% that were withdrawn in Nicholson et al. (22) UK study. These researchers did not define the reasons dogs were withdrawn. However, conversations with guide dog professionals at symposiums attended during the course of this research, suggest that the current findings reflect the outcome of guide dog programs around the world in terms of numbers, reasons, and trends. These numbers also relate to the 16.0% of pet dogs adopted from animal shelters in New Zealand returned for unacceptable behaviors (36). Breakdowns in the owner–pet relationship may occur because the owner has unrealistic expectations of the role of a pet and/or is ignorant of breed-specific behaviors, and the time and money required for maintenance (37). Although guide dogs are provided free of charge in many countries, and guide dog schools protect the dogs' welfare in cases of hardship, the use of guide dogs can be expensive. Intriguingly, the return rate of dogs is analogous to the 29.3% (38) abandonment rate of assistance technology devices (excluding dogs). Scherer (38) found that the most influential factor was a change in needs/priorities of the user, but that the user's input into the selection of the device was important for a good outcome.

Only the main reasons dogs were returned were identified in the present study. However, it is likely that more than one problem contributed to their return. Future research could establish if handlers return dogs due to an accumulation of problems and if these problems interrelate. For example, if a dog's level of anxiety increases during its working life, the pace at which it walks may increase resulting in an incompatible match. In addition, an anxious dog may be overly sensitive and have poor social/home behavior, that is, multiple problems stemming from the same underlying concern. Physical attributes such as breed or size may also be linked to behavioral problems. For example, large dogs may be too strong if inclined to pull through the harness. A breed-specific behavioral problem, which confirms the importance of educating handlers about their choices and expectations, is epitomized by the handler who thought that everything would be alright if only her next dog was a Standard Poodle. However, when this eventuated, the Poodle was described as "dizzy" and "unfocussed," and was subsequently returned for this and other behaviors common within the breed.

Only one dog in the present study was withdrawn primarily for a skin problem, and one was being considered for return as the problem was severe, but "skin" was frequently mention as problematic. Furthermore, fieldwork associated with this research suggested that more dogs appeared to have health issues in recent times than in the past, especially skin problems, This is important, as Caron-Lormier et al. (33) found that of 14 groups of health problems in 8 of the most common breed (plus "other") of guide dogs, skin problems had the greatest impact on reducing working life, by an average of 5 years. There was no discernible rise in the small number of dogs being returned for health problems over the years dogs were used in the present study. However, only the main reasons for return were considered and it is possible that health issues were also a major, albeit secondary, concern. Musculoskeletal disorders were the most common reason for dogs being withdrawn prior to retirement in both the Caron-Lormier et al. (33) and the present study. In-depth examinations of guide dog schools' records to identify health problems and establish if these are a growing concern would be invaluable for making matching decisions, and for the breeding program if these conditions were heritable.

Not being able to walk at their preferred walking speed was also of concern to handlers in the present study as being forced to walk too fast can be frightening, and too slow frustrating. However, few dogs were returned primarily because of a speed mismatch. This suggests that instructors are adept at matching for speed because (a) they are aware of its importance and (b) because speed is more quantifiable (for human and canine) than many other matching criteria.

Of interest, is that many handlers appeared to feel the need to defend their preferences for physical traits in their dogs, including why they wanted a dog to be good-looking, and how the dog felt to touch (tactility). This concept is supported in a focus group discussion on guide dog usage (34) concerning the general public sometimes being insensitive to people with a visual disability preferring a dog of a certain color.

Another interesting finding was that three of the handlers who returned their problem dogs did not feel that they had been mismatched because the instructors discussed the potential for these particular problems at the time of matching, thus empowering the handlers to make informed choices. A similar concept exists when handlers do not consider dogs with health problems to be mismatched, if these problems were unforeseen. Conversely, handlers were upset and angry if they subsequently discovered they had been matched with a dog that had been returned by a previous handler for a problem that the new handler was unaware of. Candidness is arguably the best policy for client satisfaction and the opportunity for person and dog to work through problems together may actually strengthen the bond. An important aspect of the relationship is cooperation between dog and handler (39) with the handler being in control for some tasks but permitting the dog to also use its initiative in making suitable guiding decisions. Allowing the dog some freedom of choice might help a guide dog reach its potential in its working role; it is feasible that dogs that are not afforded some measure of choice have less chance to develop self-control (16).

Rematched Dogs

Problems between people and their guide dogs are common, and, as with pet ownership, relationships often break down. However, a problem for one person may not be a problem for another, and dogs are returned for reasons that do not preclude them from working with a subsequent handler, for example, ill-health of the previous handler. Twenty dogs in this sample had been rematched, some twice, with a success rate of 75% (i.e., comparable to that of all the dogs in the sample). The remainder were withdrawn from the program and rehomed as pets.

No patterns emerged regarding what might constitute success, as both the successfully and the unsuccessfully rematched groups had been rematched for a variety of issues. However, it is apparent that the rematching of dogs is an appropriate use of resources. Most participants were happy with the notion of being matched with a previously employed dog, provided they were aware of the dog's history. Fuller (35) commented that some returned dogs did well when rematched, although he provided no other details. Ledger and Baxter (40) concluded that successive owners of the same pet dog, which was repeatedly adopted from a UK animal shelter, reported different behavioral problems due to different attitudes and perceptions. Pet owner attitudes are believed to directly affect behavioral problems in dogs, particularly (what was previously known as), dominance aggression and displacement/excitement behaviors (41, 42). For these reasons, it would be interesting to compare the experiences of successive handlers of rematched dogs.

Second Dog Syndrome

An unexpected finding was that handlers described inferior relationships with their second dogs compared to their first and third dogs. First-time dogs were favored the most, but there was little apparent difference between the first and third dogs. The term "second dog syndrome" was coined for this discussion (32).

It is feasible that fewer first dogs were rejected for the same reasons that handlers did not return mismatched dogs, as discussed above. These include expectations being lower due to people not knowing what to expect (having had no other dog to compare), having enough vision to compensate for dogs that did not excel as mobility aids and being more emotionally attached to these dogs. As the use of more than one guide dog is common, it would be interesting for future research to compare people who had only used one dog with those who had used multiple dogs to ascertain if experience has an effect on matching success.

It is understandable why there should be a "first-dog" effect in the handlers' affections, as this dog was the one to initialize and/or improve independent mobility, thus being the catalyst for life changing events. Another possible explanation provided by Lloyd et al. (21) is "distortion of memory" where handlers may have forgotten the boisterous behavior of the first dog when it was young, and are comparing a youthful, exuberant second dog to that of the first dog at the end of the partnership when it had matured. It is also possible that guide dog schools might match second-time dog users with less than optimal dogs in the belief that the handler will cope with a dog that is more of a "challenge." However, anecdotal evidence from this study suggests that some dogs that were returned were successfully matched to first time as well as experienced users, and third dogs were less likely to be returned or deemed to be mismatched than second dogs. Regardless, knowing that a second dog is likely to be perceived as second best is useful knowledge for guide dog instructors to help prepare clients who are about to receive a second dog. Following on from Lloyd et al. (21) work, Ward and Peirce (43) created a client- driven information resource for second time guide dog applicants to aid in the transition of dogs.

CONCLUSION

This research serves to increase awareness of what is happening in guide dog partnerships post-qualification. This research is intrinsically important for the guide dog industry in several ways. It examined, in a real-life setting, the outcome of the matching process; a process that is widely practised, but little assessed, and highlights the need to consider not only working behaviors but also social/home behaviors when making matching decisions. Understanding what makes a successful partnership is becoming increasingly important as there has been a steady increase in the number of handler-guide dog teams graduating around the world, as well as in the number of other service (or assistance) animals. Guide dogs are expensive to produce, as well as being expensive in personal terms for all concerned if a match is unsuccessful, and it may be assumed that the number of unsuccessful matches is likely to increase relative to the total number of matches made. Although feelings at the end of the partnership were not a focus of this study, nearly two-thirds of participants had used more than one dog. As the transition from one dog to the next is a recurring feature, handlers probably experience the end of more relationships than the average pet dog owner (22). Retiring a guide dog is not only difficult for the handler but also for the handler's family and friends (21) and no doubt also for the dog. Every participant in this study was matched successfully at least once, and most dogs that were rematched went on to have a successful relationship with a different handler. This shows that the success of the handler and guide dog partnership does not solely depend on either the person or the dog, but relies on the interplay between them, that is, the match.

ETHICS STATEMENT

Massey University Human Ethics Committee. The study was carried out in accordance with the recommendations of Massey University Human Ethics Committee with written informed consent from all subjects. The participants in the study were vision impaired.

AUTHOR CONTRIBUTIONS

All the authors (JL, CB, SL, and KS) on this publication have contributed to the conception and design of this work. The first author (JL) undertook the research and wrote the article with the approval of the other authors (CB, SL, and KS) who have critically revised the content. All the authors (JL, CB, SL, and KS) agreed to be accountable for the content.

ACKNOWLEDGMENTS

The authors wish to thank the people who participated in this study, and the staff of the Blind Foundation's Guide Dog Services for their support throughout the process. Thanks are also extended to Professor Nigel Perkins for his advice on statistical matters. The authors would like to note the contribution of the doctoral dissertation "Exploring the match between people and their guide dogs" (32). Support for this publication has been provided by Douglas Pharmaceuticals Ltd. The views in this publication do not necessarily reflect the views of Douglas Pharmaceuticals Ltd.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fvets.2016.00114/full#supplementary-material.

REFERENCES

1. Deshen S, Deshen H. On social aspects of the usage of guide-dogs and long canes. *Sociol Rev* (1989) 37:89–103. doi:10.1111/j.1467-954X.1989.tb00022.x
2. Goddard ME, Beilharz RG. A factor analysis of fearfulness in potential guide dogs. *Appl Anim Behav Sci* (1984) 12(3):253–65. doi:10.1016/0168-1591(84)90118-7
3. Lloyd JKF, La Grow S, Stafford KJ, Budge RC. The guide dog as a mobility aid part 1: perceived effectiveness on travel performance. *IJOM* (2008) 1(1):17–33.
4. Lloyd JKF, La Grow S, Stafford KJ, Budge RC. The guide dog as a mobility aid part 2: perceived changes to travel habits. *IJOM* (2008) 1(1):34–45.
5. Oxley J. Tail with a happy ending: Kevin Carey interviews Julian Oxley, the director of the Guide Dogs for the Blind Association. *Br J Visual Impairment* (1995) 13(2):77–9. doi:10.1177/026461969501300205
6. Delafield G. *The Effects of Guide Dog Training on Some Aspects of Adjustment in Blind People [Unpublished Doctoral Dissertation]*. England: University of Nottingham (1974).
7. Muldoon C. Does the presence of a guide dog enhance feelings of social acceptance in guide dog users? [CD-ROM]. *Proceedings of the 10th International Mobility Conference*. Coventry, England: Guide Dogs for the Blind Association (2000). p. 258–61.
8. Refson K, Jackson AJ, Dusoir AE, Archer DB. Ophthalmic and visual profile of guide dog owners in Scotland. *Br J Ophthalmol* (1998) 83(4):470–7. doi:10.1136/bjo.83.4.470
9. Refson K, Jackson AJ, Dusoir AE, Archer DB. The health and social status of guide dog owners and other visually impaired adults in Scotland. *Vis Impair Res* (1999) 1(2):95–109. doi:10.1076/vimr.1.2.95.4411
10. Sanders CR. *Understanding Dogs: Living and Working with Canine Companions*. Philadelphia, PA: Temple University Press (1999).
11. Sanders CR. The impact of guide dogs on the identity of people with visual impairments. *Anthrozoos* (2000) 13(3):131–9. doi:10.2752/089279300786999815
12. Steffans MC, Bergler R. Blind people and their dogs. In: Wilson CC, Turner DC, editors. *Companion Animals in Human Health*. Thousand Oaks, CA: SAGE (1998). p. 149–57.
13. Whitmarsh LE. The benefits of guide dog ownership. *Vis Impair Res* (2005) 7(1):27–42. doi:10.1080/13882350590956439
14. Zee A. Guide dogs and their owners: assistance and friendship. In: Katcher AH, Beck AM, editors. *New Perspectives on Our Lives with Companion Animals*. Philadelphia, PA: University of Pennsylvania Press (1983). p. 472–83.
15. Ireson P. *Another Pair of Eyes: The Story of Guide Dogs in Britain*. London: Pelham (1991).
16. Lloyd J, Roe E. Using TTouch to reduce stress and enhance learning when training guide dogs. *IJOM* (2014) 6(1):8–20.
17. Pfaffenberger CJ, Scott JP, Fuller JL, Ginsburg BE, Bielfelt SW. *Guide Dogs for the Blind: Their Selection, Development and Training*. Amsterdam: Elsevier (1976).
18. Purves P, Godwin F. *Tess: The Story of a Guide Dog*. London: Gollancz (1981).

19. Whitstock R, Franck L, Haneline R. Dog guides. 2nd ed. In: Blasch B, Wiener W, Welsh R, editors. *Foundations of Orientation and Mobility*. New York, NY: American Foundation for the Blind (1997). p. 260–83.
20. Lloyd J, Stafford K, La Grow S, Budge RC. Matching guide dogs to people: assessing the relationship between 50 people and their first guide dogs. *Book of Abstracts of the Inaugural Australian Working Dog Conference of the Working Dog Alliance Australia*. Sydney: University of Sydney (2013). p. 26–7.
21. Lloyd JKF, Budge RC, Stafford KJ, La Grow S. The end of the partnership with a guide dog: emotional response and relationships with subsequent dogs. *Proceedings of the 13th International Mobility Conference*. Marburg, Germany (2009).
22. Nicholson J, Kemp-Wheeler S, Griffiths D. Distress arising from the end of a guide dog partnership. *Anthrozoös* (1995) 8(2):100–10. doi:10.2752/089279395787156419
23. Koda N, Kubo M, Ishigami T, Furuhashi H. Assessment of dog guides by users in Japan and suggestions for improvement. *J Vis Impair Blind* (2011) 105(11):591–600.
24. Lloyd JKF, La Grow SJ, Budge RC, Stafford KJ. Matching guide dogs: mobility and compatibility outcomes. *Proceedings of the 11th International Mobility Conference*. Stellenbosch, South Africa (2003).
25. Farrugia C, Gillard M, Tomlinson K. Orientation of a guide dog team. *Proceedings of the 9th International Mobility Conference*. Atlanta, GA: American Foundation for the Blind (1998). 346 p.
26. Lane DR, McNicholas J, Collis GM. Dogs for the disabled: benefits to recipients and welfare of the dog. *Appl Anim Behav Sci* (1998) 59(1–3):49–60. doi:10.1016/S0168-1591(98)00120-8
27. Snedechor GW, Cochran WG. *Statistical Methods*. 8th ed. Ames: Iowa State University Press (1989).
28. Dahlgren G, Whitehead M. *Policies and Strategies to Promote Social Equity in Health*. Stockholm: Institute for Future Studies (1991).
29. Maxwell AE. *Multivariate Analysis in Behavioural Research: Monographs on Applied Probability and Statistics*. London: Chapman and Hall (1977).
30. Audrestch HM, Whelan CT, Grice D, Asher L, England GC, Freeman SL. Recognizing the value of assistance dogs in society. *Disabil Health J* (2015) 8:469–74. doi:10.1016/j.dhjo.2015.07.001
31. Finestone S, Lukoff IF, Whiteman M. *The Demand for Dog Guides: And the Travel Adjustment of Blind Persons*. New York, NY: Columbia University, Research Center, The New York School of Social Work (1960).
32. Lloyd JKF. *Exploring the Match between People and Their Guide Dogs [Doctoral Dissertation]*. Palmerston North, New Zealand: Massey University (2004).
33. Caron-Lormier G, England GCW, Green MJ, Asher L. Using the incidence and impact of health conditions in guide dogs to investigate healthy ageing in working dogs. *Vet J* (2016) 207:124–30. doi:10.1016/j.tvjl.2015.10.046
34. Lloyd JKF, Budge RC, Stafford KJ, La Grow S. A focus group discussion on using guide dogs. *IJOM* (2009) 2(1):52–64.
35. Fuller JL. The human + guide partnership. In: Pfaffenberger CJ, Scott JP, Fuller JL, Ginsburg BE, Bielfelt SW, editors. *Guide Dogs for the Blind: Their Selection, Development and Training*. Amsterdam: Elsevier (1976). p. 127–47.
36. Stafford K, Erceg V, Kyono M, Lloyd J, Phipps N. The dog/human dyad: a match made in heaven? *Proceedings of the World Small Animal Veterinary Association*. Bangkok, Thailand: World Small Animal Veterinary Association (2003). p. 225–7.
37. Kidd AH, Kidd RM, George CC. Successful and unsuccessful pet adoptions. *Psychol Rep* (1992) 70(2):547–61. doi:10.2466/pr0.1992.70.2.547
38. Scherer MJ. Outcomes of assistive technology use on quality of life. *Disabil Rehabil* (1996) 18(9):439–48. doi:10.3109/09638289609165907
39. Naderi Sz, MiklÓsi Á, DÓka A, Csányi V. Co-operative interactions between blind persons and their dogs. *Appl Animal Behav Sci* (2001) 74(1):59–80. doi:10.1016/S0168-1591(01)00152-6
40. Ledger RA, Baxter MR. Assessing owner attitudes to dog behaviour: a case for owner-dog matching. *Proceedings of the First International Conference on Veterinary Behavioural Medicine*. Birmingham: Universities Federation for Animal Welfare (1997). 226 p.
41. O'Farrell V. Owner attitudes and dog behaviour problems. *Appl Anim Behav Sci* (1997) 52(3):205–13. doi:10.1016/S0168-1591(96)01123-9
42. Podberscek AL, Serpell JA. Aggressive behaviour in English cocker spaniels and the personality of their owners. *Vet Rec* (1997) 141(3):73–6. doi:10.1136/vr.141.3.73
43. Ward K, Peirce K. A client driven information resource for second time guide dog applicants. *IJOM* (2011) 3(1):36–40.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 19 December 2016
doi: 10.3389/fvets.2016.00111

Eye and Ear Temperature Using Infrared Thermography Are Related to Rectal Temperature in Dogs at Rest or With Exercise

Brian M. Zanghi*

Nestlé Purina Research, St Louis, MO, USA

Rectal body temperature (BT) has been documented in exercising dogs to monitor thermoregulation, heat stress risk, and performance during physical activity. Eye (BT_{eye}) and ear (BT_{ear}) temperature measured with infrared thermography (IRT) were compared to rectal (BT_{rec}) temperature as the reference method and assess alternative sites to track hyperthermia, possibly to establish BT_{eye} IRT as a passive and non-contact method. BT measures were recorded at 09:00, 11:30, 12:30, and 02:30 from Labrador Retrievers ($N = 16$) and Beagles ($N = 16$) while sedentary and with 30-min play-exercise (pre- and 0, 15, 30-min post-exercise). Total exercise locomotor activity counts were recorded to compare relative intensity of play-exercise between breeds. BT_{rec}, BT_{eye}, and BT_{ear} were measured within 5 min of the target time. Each BT method was analyzed by analysis of variance for main effects of breed and time. Method differences were compared using Bland–Altman plots and linear regression. Sedentary BT differed by breed for BT_{rec} ($p < 0.0001$), BT_{ear} ($p < 0.0001$), and BT_{eye} ($p = 0.06$) with Labs having on average 0.3–0.8°C higher BT compared to Beagles. Readings also declined over time for BT_{eye} ($p < 0.0001$) and BT_{ear} ($p < 0.0001$), but not for BT_{rec} ($p = 0.63$) for both breeds. Total exercise (30-min) activity counts did not differ ($p = 0.53$) between breeds. Time and breed interaction was significant in response to exercise for both BT_{rec} and BT_{ear} ($p = 0.035$ and $p = 0.005$, respectively), with a marginal interaction ($p = 0.09$) for BT_{eye}. All the three methods detected hyperthermia with Labs having a higher increase compared to Beagles. Both BT_{ear} and BT_{eye} were significantly ($p < 0.0001$) related to BT_{rec} in all dogs with sedentary or exercise activity. The relationship between BT_{eye} and BT_{rec} improved when monitoring exercise hyperthermia ($r = 0.674$) versus measures at rest ($r = 0.381$), whereas BT_{ear} was significantly related to BT_{rec} regardless of activity ($r = 0.615$–0.735). Although BT readings were significantly related, method bias ($p < 0.02$) was observed for BT_{eye} to slightly underestimate BT_{rec}, whereas no bias was observed between BT_{ear} and BT_{rec}. This study demonstrates that IRT technology effectively measures both ear and eye temperature and enables effective monitoring of BT changes at rest, with exercise, and between breeds. However, ear, and not eye, temperature is a better reflection of rectal temperature.

Keywords: core body temperature, exercise hyperthermia, canine, brain temperature, infrared thermography

Edited by:
Lynette Arnason Hart, University of California Davis, USA

Reviewed by:
Mitsuaki Ohta, Tokyo University of Agriculture, Japan
Joseph Wakshlag, Cornell University, USA
Vera Baumans, Utrecht University, Netherlands

****Correspondence:***
Brian M. Zanghi
brian.zanghi@rd.nestle.com

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *19 September 2016*
Accepted: *21 November 2016*
Published: *19 December 2016*

Citation:
Zanghi BM (2016) Eye and Ear Temperature Using Infrared Thermography Are Related to Rectal Temperature in Dogs at Rest or With Exercise. Front. Vet. Sci. 3:111. doi: 10.3389/fvets.2016.00111

INTRODUCTION

Hyperthermia has been documented in dogs, in response to exercise at various durations and intensities, and is an important physiological measure of thermoregulation, heat stress risk, and a factor limiting performance during physical activity (1–4). These early characterizations of hyperthermia during and after exercise were largely examined through recording rectal temperature, but newer technologies like ingestible sensors (5–7) and non-contact infrared thermography (IRT) (8) are enabling sensitive and alternative methods of measuring body temperatures (BTs) to monitor hyperthermia. Although rectal BT measures are sensitive in detecting changes in thermoregulatory responses because of dehydration (1, 2) and body cooling (3, 9), the need to stop the dog to obtain a measure, or have a continuously placed thermocouple for recording during laboratory-based treadmill exercise, are minor disadvantages. More recent work in exercising dogs has demonstrated the advantages of using an indigestible telemetric sensor that is swallowed and records core BT during exercise on various sampling intervals as it passes through the gastrointestinal tract (5–7).

Alternatively, IRT is useful and routinely used by veterinary clinicians to measure auricular (ear) temperature (10, 11) but requires contact of the animal for probe placement, and some animals exhibit apprehensive behaviors during attempted measurement. IRT has also been employed as a passive and non-contact method of measuring surface temperature of a subject's infrared radiation (12). Non-contact IRT is a promising technology that recently has been reviewed for its use in veterinary applications (13). Among other applications, non-contact IRT has been examined to successfully detect inflammatory conditions or fever in cows, horses, or ponies (14–16), as well as stress-induced hyperthermia in dogs (8). The research with ponies and dogs has demonstrated that IRT of the eye is a valid index to measure core BT and is sensitive to detect physiological fluctuations when compared to rectal temperature (8, 16).

Although IRT of the eye and ear temperature have been used to assess BT in a variety of canine research studies and clinical settings, the use of these two anatomical locations to assess the natural increase in BT with exercise has not been reported. The study was designed to examine BT of eye and ear in dogs of two different breeds (Labrador Retrievers and Beagles) at rest or following play-exercise using IRT technology compared to rectal temperature as the traditional standard method of measuring BT. Two different breeds of dogs were examined because BT differs with breed size (17). Ultimately, if the IRT eye method is successful, then the potential exists to use remote and non-contact IRT to monitor hyperthermia in the field in working dogs (possibly in real time) without disturbing work or performance.

MATERIALS AND METHODS

Animal Care and Feeding

Thirty-two dogs (16 males and 16 females) of two different breeds; 16 Labrador Retrievers (Labs) and 16 Beagles were evaluated. The Beagles had an average age of 4.0 ± 2.4 years, average weight of 11.7 ± 1.8 kg SD, and body condition score of 5–6 on a scale of 1–9 (18). The Labrador retrievers had an average age of 5.2 ± 1.5 years, average weight of 29.3 ± 2.5 kg SD, and body condition score of 5–7 on a scale of 1–9 (18). All dogs used in the exercise trial were required to have overall good general health prior to beginning the trial and evaluated by a veterinarian on the morning of each exercise day.

All dogs were fed once daily to maintain body weight with *ad libitum* access to water. Dogs were maintained on existing food (Purina Pro Plan Sport Active 26/16 Formula Dry Dog Food, Nestlé Purina PetCare Company, St. Louis, MO, USA) through the duration of the trial. Dogs were given *ad libitum* access to water before the exercise challenge and immediately after the 30-min exercise. Dogs were housed indoors with natural lighting and exposure to natural light cycles in pens (1.5 m × 4.5 m; 2 dogs per pen). Dogs were grouped on the basis of age, compatibility, and sex. All dogs were housed at the same kennel location and could see other dogs in adjacent pens. All dogs had direct interaction and socialization with caretakers on a daily basis and had continuous access to multiple toys.

Experimental Design and Play-Exercise

Body temperature of sedentary activity was measured on a day when the dogs were resting prior to any outside play time with indoor ambient temperature between 68 and 70°F, whereas measurement of BT on play-exercise days occurred 3 days later with a warm indoor ambient temperature between 78 and 80°F with 78% humidity. Sedentary activity consisted of normal indoor-only kennel activity during the 6-h BT recording period. Outdoor kennel access was provided after the last BT measurement was recorded.

To conduct the bout of play-exercise for all dogs on a single day, dogs were allotted into groups of four based on compatibility, and balanced for gender and breed. Play and subsequent data collection for a given play-group occurred within approximately a 1.25-h block of time, with group 1 beginning at 08:00. Group 2 began after the completion of group 1, etc. Exercise consisted of group play and interaction among the four dogs, which included voluntary running and playing, as well as interaction with familiar pet-care staff to encourage voluntary ball and/or toy chasing and retrieving if individual dogs were not playing with other playmates. Exercise occurred in a designated indoor play area (10 ft wide by 70 ft long) with multiple familiar balls and toys of various sizes and textures to facilitate play and retrieving.

Body Temperature Measurements

Body temperature was determined using three measurement locations. Rectal body temperature (BT_{rec}) was measured by insertion of a digital thermometer (Accuflex Pro, Model 016-639, Physiologic, Montreal, QC, Canada) approximately 1 cm to acquire an automated reading upon pressing the measurement button. Ear temperature (BT_{ear}) of the tympanic membrane and ear canal was detected by infrared detection of both ears (Pet-Temp PT-300, Advanced Monitors Corp., San Diego, CA, USA). Dogs with hanging pinna had the pinna temporarily pulled back to allow access to the ear canal for immediate insertion of the device probe. Reading was obtained upon pressing the measurement button and recorded from the digital output screen. Eye temperature (BT_{eye}) using a portable thermal camera (IR966 professional radiometric video camera, Sierra Pacific Innovations Corp., Las Vegas, NV, USA). BT_{eye} was determined from thermographic infrared images of each dog's face (8) encompassing both the right and left eye to assess fluctuations in BT. Two images were captured once the autofocus feature established a clear image at a distance of approximately 1 m and same angle. All the images were analyzed using thermal imaging analysis software (SPI IrAnalyser version 5.0.21.95) where both the maximum and average temperature of each eye were calculated within a rectangular area traced around the eye to include the entire eyeball and approximately 1 cm around the outside of the eyelids.

For BT recorded when the dogs were at rest, all three BT measurement sites were recorded within 5 min at 09:00, 11:30, 12:30, and 02:30. For assessment of BT before and after exercise, all BT measurement sites were recorded within 5 min immediately pre-exercise, immediately (0-min), 15-, and 30-min post-exercise play-bout. Pre-exercise and 0-min post-exercise measures were determined while the dog was outside of its kennel and outside of the exercise area, whereas 15 and 30-min post-exercise measures were obtained when the dog was in its home kennel run.

Locomotor Activity Recording

Locomotor activity counts were recorded for 24 h using the MotionWatch 8 activity monitoring system (CamNtech Ltd., Cambridgeshire, UK). The MotionWatch 8 is tri-axial accelerometer placed inside a specially designed case and attached to a collar around the dog's neck the evening before the play-exercise day. Placement of the device on the collar permitted normal rest, exercise, and feeding activity levels to be measured (19). The devices recorded activity counts on a 30-s epoch setting. Following removal of the device from the dog's neck, data were downloaded to a PC-based MotionWare Software (version 1.0.3, CamNtech Ltd., Cambridgeshire, UK), and data were exported as a MotionWare-generated ".mtn" file. Each ".mtn" file was converted to a text file with Notepad (version 6.1, Microsoft Corporation, Redmond, WA, USA), and activity data transferred to Microsoft® Excel® 2013 (Microsoft Corporation, Redmond, WA, USA) for alignment with epoch recorded time. Total locomotor activity counts were calculated for the 30-min duration of play-exercise.

Statistical Analysis

All statistical analyses were conducted using PROC MIXED in SAS (SAS 9.3., Copyright© 2002–2010 by SAS Institute Inc., Cary, NC, USA). To characterize BT measurements obtained from the eye, ear, or rectum, a separate analysis of variance (ANOVA) was performed for each BT location to assess the main effects of breed and time, and interaction between main effects. *Post hoc* analyses were performed using a Protected ($p < 0.05$) Fisher's LSD to separate means of dependent variable that differed with time or breed. Regression coefficients and prediction equations were generated by use of linear regression analysis. Paired *t* tests were used to assess differences in mean values for BTs, as measured *via* the various methods (eye or ear) versus the reference method (rectum) for Bland–Altman plot analysis. Significance was determined at a value of $\alpha = 0.05$.

RESULTS

Daytime Body Temperature Measurements

To characterize resting daytime BT across multiple methods, measures were recorded at approximately the same time from the ear, eye, and rectum. BT_{rec} measures are used to establish "normal" daytime fluctuation at thermo-neutral indoor temperature between 20 and 21°C (68–70°F). BT in Celsius degrees is plotted over time for each breed with data obtained from rectum (**Figure 1A**), ear (**Figure 1B**), or eye (**Figure 1C**).

Temperature measures for each method were analyzed separately for ANOVA to evaluate the main effects of breed and time. BT_{rec} was measured as the reference value, and there was a significant effect of breed (ANOVA $p = 0.001$) with Labs having a mean temperature of 0.5°C higher (38.3 ± 0.1°C SE) compared to Beagles (37.8 ± 0.1°C SE). Labs were always numerically higher across all four daytime measurement occasions, this difference ranged from 0.3 to 0.8°C (**Figure 1A**). Neither the main effect of time (ANOVA $p = 0.63$) nor the interaction between breed and time (ANOVA $p = 0.40$) were significantly different.

BT_{ear} measures resulted in a significant effect of breed (ANOVA $p = 0.02$), as well as a main effect of time (ANOVA $p < 0.0001$). The interaction between breed x and time (ANOVA $p = 0.39$) was not significant. Mean BTear at 14:30 was approximately 0.7°C lower compared to 11:30, and Labs (38.2 ± 0.1°C SE) on average had a 0.3°C higher ear temperature compared to Beagles (37.9 ± 0.1°C SE).

For BT_{eye}, there was a significant effect of time (ANOVA $p < 0.0001$), with afternoon measurement times being lower by approximately 0.8°C compared to morning times. There was also a marginal effect of breed (ANOVA $p = 0.06$) with Labs having a mean temperature of 0.4°C higher (37.3 ± 0.1 C SE) compared to Beagles (36.9 ± 0.1°C SE). The interaction between breed and time (ANOVA $p = 0.24$) was not significant.

Comparison of Methods of Body Temperature Measurement between Different Anatomical Locations with Sedentary Dogs

The relationship between the various BT methods and locations were analyzed by linear regression, and correlation data are listed in **Table 1**. Based on all the resting daytime BT measurements, BT_{eye} and BT_{ear} were significantly ($p < 0.0001$), albeit only moderately, related to BT_{rec} ($r = 0.381$ and $r = 0.615$, respectively). Comparison of BT_{eye} to BT_{rec}, or BT_{ear} to BT_{rec}, indicated that BT_{ear} was similar to BT_{rec} at 09:00 and 11:30 with less than 0.26% (0.1°C) difference in recorded temperatures but underestimated BT_{rec} by 0.8% (0.3°C) at both 12:30 and 14:30, respectively (**Table 2**). BT_{eye} underestimated BT_{rec} by 1.9% up to 3.3% across all measurement times (**Table 2**). On average, BT_{eye} was approximately 1.5 or 1.7°F cooler compared to BT_{ear} and BT_{rec}, respectively, for all four daytime measurement occasions and breed data combined.

To determine method bias without measurement replication, the four daytime recordings were combined for each dog, and

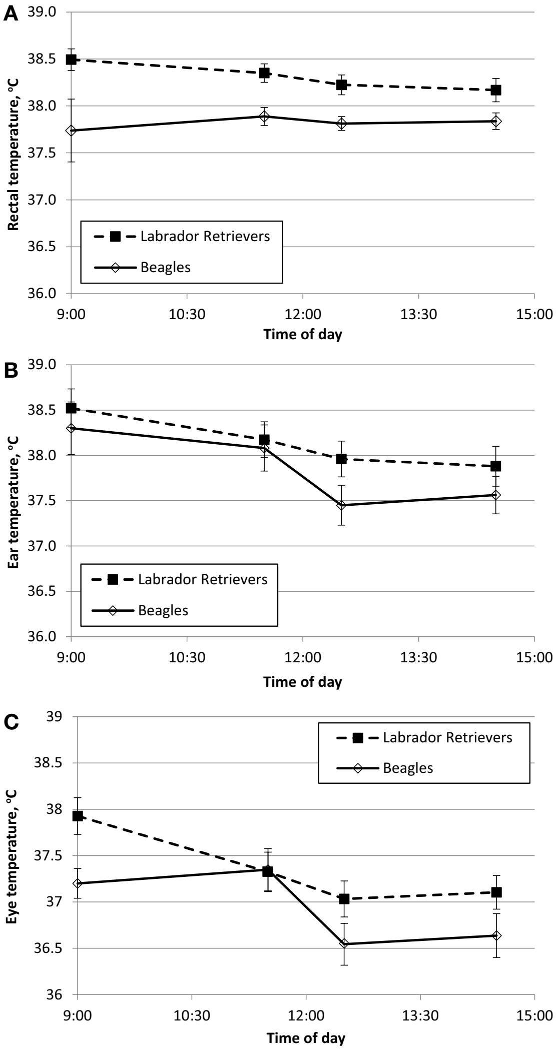


FIGURE 1 | Phase 1 mean (±SE) body temperature over time within a day in sedentary Labrador Retrievers or Beagles. **(A)** rectal body temperature, **(B)** ear temperature, and **(C)** eye temperature.

TABLE 1 | Correlations between measurement locations for data acquired while dogs were sedentary or with exercise.

Dependent variable	*r*	*p*-Value
Daytime and sedentary body temperature		
Eye versus rectal	0.381	<0.0001
Ear versus rectal	0.615	<0.0001
Eye versus ear	0.458	<0.0001
Body temperature before and after exercise		
Eye versus rectal	0.674	<0.0001
Ear versus rectal	0.735	<0.0001
Eye versus ear	0.640	<0.0001

the mean was used for analysis. Evaluation of a Bland–Altman plot revealed that a significant bias ($p < 0.001$) existed for the IRT method measuring BT_{eye} to underestimate BT_{rec}, the slope significantly ($p < 0.001$) differed from 0 with increasing underestimation at lower temperatures, and the 95% limit of agreement was ±0.925 (**Figure 2A**). By contrast, no bias was observed ($p = 1.0$) between the IRT method of BT_{ear} and BT_{rec}, the slope was not different ($p = 0.98$) from 0, and the 95% limit of agreement was ±0.453 (**Figure 2B**).

Exercise-Related Locomotor Activity and Body Temperature

Body temperature was measured before and at various times after a 30-min bout of play-exercise to characterize the effect of exercise on changes in BT between the breeds and at the same three anatomical sites as previously measured. Locomotor activity counts were recorded during the 30 min bout of play-exercise to assess exercise-related activity as a potential factor influencing BT. Activity counts did not differ ($p = 0.53$) between breeds (mean counts ±SE; Beagles = 42,700 ± 2,680; Labs = 40,500 ± 2,300). Ambient temperature of the indoor play area ranged from 25.6 to 26.7°C (78–80°F) for all groups of dogs.

Temperature measures for each method were analyzed separately for ANOVA to evaluate the main effects of breed and time. BT in Celsius degrees is plotted separately for each breed over time with data from rectum (**Figure 3A**), ear (**Figure 3B**), or eye (**Figure 3C**). For both BT_{rec} and BT_{ear}, a significant effect was observed for the interaction of time and breed in response to exercise (ANOVA $p = 0.035$ and $p = 0.005$, respectively). LS means for pre-exercise BT_{rec} and BT_{ear} were not different ($p = 0.38$ and $p = 0.27$, respectively) between breeds. On average, immediate post-exercise BT_{rec} and BT_{ear} significantly ($p < 0.001$) increased from pre-exercise temperature (**Figures 3A,B**) by 1.3 and 1.1°C, respectively, in Beagles or 2.0 and 2.3°C, respectively, in Labs. In addition, both BT_{rec} and BT_{ear} differed between breed ($p = 0.001$ and $p = 0.001$, respectively) with Labs measured to have higher BT_{rec} and BT_{ear}.

A significant ($p < 0.01$) post-exercise recovery of BT_{rec} and BT_{ear} was observed in both breeds by 15-min. Furthermore, BT_{rec} and BT_{ear} recordings in Beagles at 15-min post-exercise had recovered to initial pre-exercise temperatures ($p = 0.34$ and $p = 0.43$, respectively). By contrast in Labs, although BT_{rec} and BT_{ear} had significantly decreased from post-exercise peak, both BT_{rec} and BT_{ear} recordings were still significantly higher ($p < 0.01$) from initial pre-exercise recordings by at least 1.1°C. By 30 min of recovery from exercise BT_{rec} had recovered to pre-exercise values, but BT_{ear} was still significantly elevated 0.9°C.

A marginally significant effect for BT_{eye} was observed for the interaction of time and breed in response to exercise (ANOVA $p = 0.09$). LS means for pre-exercise BT_{eye} were not different ($p = 0.48$) between breeds with minimal numerical difference by approximately by 0.3°C. For general comparison purposes, it is important to note that pre-exercise BT recordings were obtained at different times of day for each exercise group between 08:00 and 14:00. By contrast, during the sedentary study, the first BT recording was obtained for all dogs at approximately the same time of day (09:00). Immediate post-exercise, BT_{eye} significantly ($p < 0.001$) increased by 2.1 or 2.8°C in Beagles or Labs, respectively, from pre-exercise temperature. Although the main effect of breed for BT_{eye} was not significant ($p = 0.18$) and both breeds had

TABLE 2 | Mean (±SD) temperature measured at various body locations of dogs for comparison between methods within a measurement time at different times of day while sedentary or relative to exercise.[a]

Measurement sites	**Time of day**			
Daytime measures at rest	**09:00**	**11:30**	**12:30**	**14:30**
Rectal	38.3 ± 0.5	38.1 ± 0.5	38.0 ± 0.4	38.0 ± 0.4
Ear	38.4 ± 0.6	38.1 ± 0.5	37.7 ± 0.5	37.7 ± 0.5
Eye	37.5 ± 0.8	37.3 ± 0.9	36.7 ± 0.8	36.9 ± 0.9
	Pre-exercise	**Post-exercise**		
Daytime measures before and after exercise	**–30 min**	**0 min**	**15 min**	**30 min**
Rectal	38.3 ± 0.5	39.7 ± 0.9	38.8 ± 0.7	38.3 ± 0.6
Ear	37.5 ± 0.8	39.2 ± 1.1	38.4 ± 0.6	37.9 ± 0.7
Eye	37.5 ± 1.1	39.9 ± 1.3	38.7 ± 0.9	38.4 ± 1.0

[a]*Data are representative of combining data from both Labrador Retriever and Beagles.*

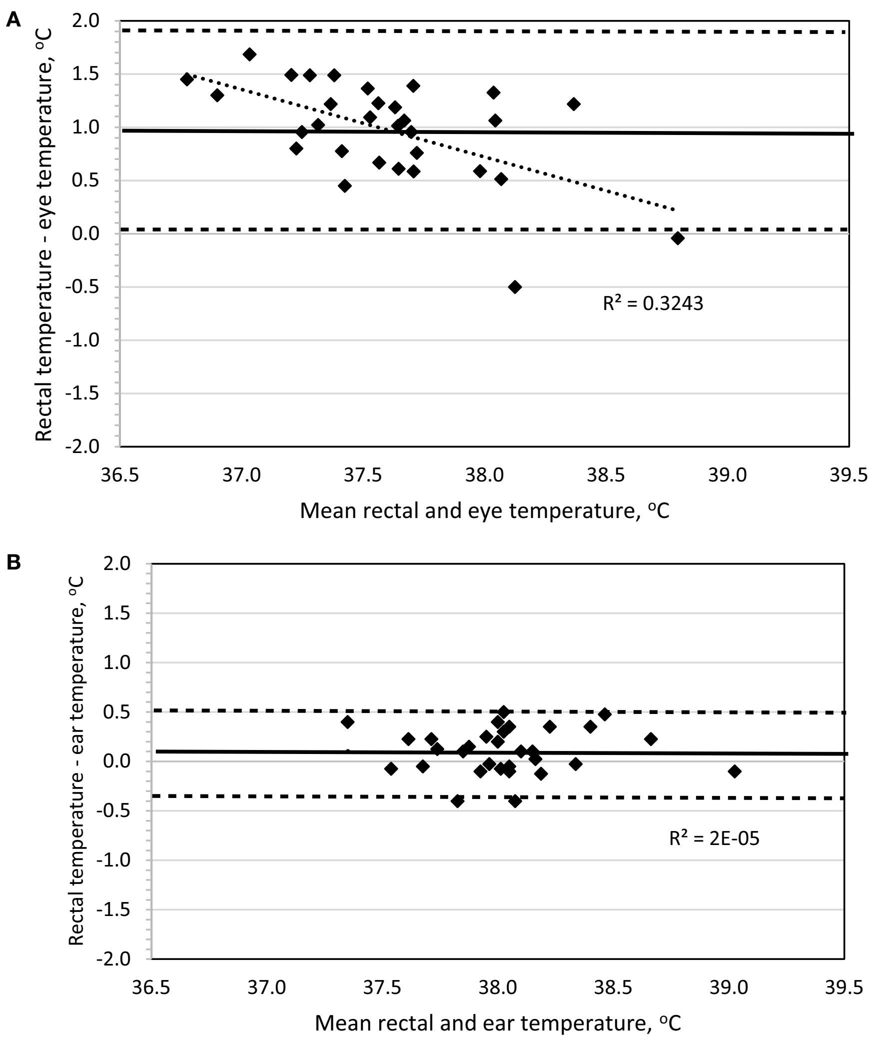


FIGURE 2 | **Bland–Altman plots of body temperature in sedentary Labrador Retrievers or Beagles comparing (A) rectal and eye or (B) rectal and ear**. In both panels, the thin dotted line is the line of best fit for the data, the thick solid horizontal line is the mean difference (bias), and the horizontal dashed lines are the 95% limits of agreement (mean difference ± 1.96 SD). Points located above 0 on the *y*-axis represent an underestimation of temperature by the method in comparison to rectal core body temperature as measured by digital thermometer.

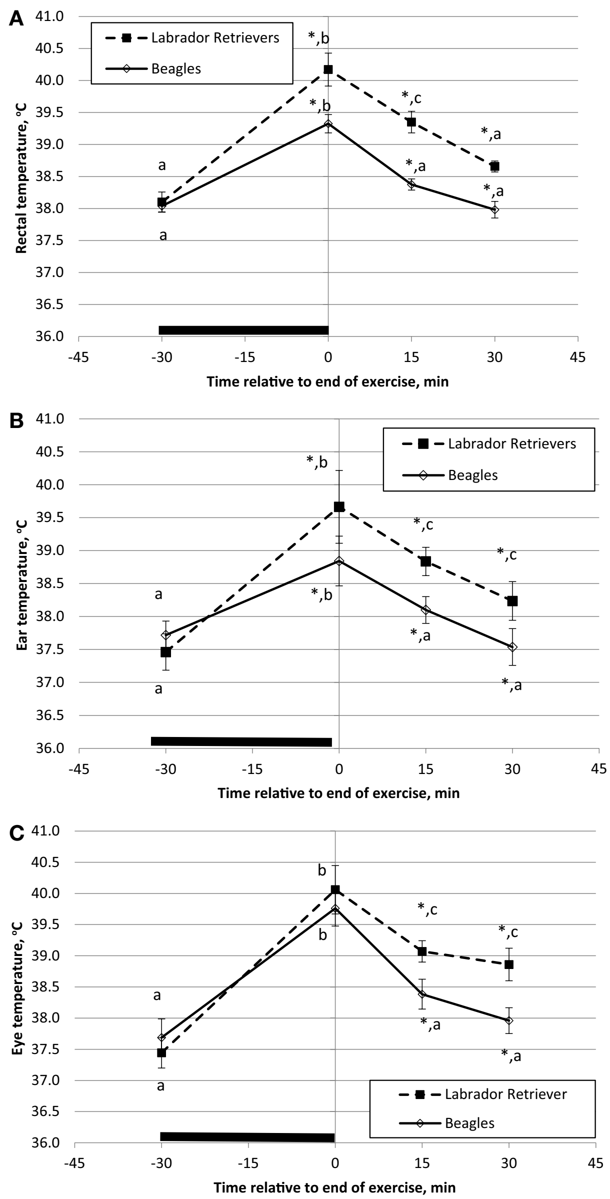


FIGURE 3 | **Phase 1 mean (±SE) body temperature before and after play-exercise in Labrador Retrievers or Beagles**. **(A)** rectal body temperature, **(B)** ear temperature, and **(C)** eye temperature. Differences between breed ($p < 0.05$) at a given time have a *. Differences ($p < 0.05$) within a breed and between measurement times are indicated by letters (a, b, and c).

similar locomotor activity counts over the 30-min exercise bout, on average Labs had a BT_{eye} of 40.3°C, which was approximately 0.5°C higher compared to Beagles. Linear regression analysis of the total exercise activity counts and the immediate post-exercise BT_{eye} revealed a significant, but only moderate, positive relationship ($r = 0.35$; $p = 0.05$).

Similar to BT_{rec} and BT_{ear}, by 15-min post-exercise BT_{eye} significantly ($p < 0.004$) recovered in both breeds, as Beagles significantly declined by 1.4°C and Labs that declined by 1.2°C. Again, average Beagle BT_{eye} at 15 and 30-min post-exercise were similar to the pre-exercise readings, whereas Labs were still significantly elevated ($p < 0.01$) by 1.3°C after 30-min of recovery.

Comparison of Methods of Body Temperature Measurement between Different Anatomical Locations with Exercising Dogs

The relationship between the various BT methods and locations were analyzed by linear regression, and correlation data are listed in **Table 1**. The relationship of BT measures between methods in response to play-exercise improved considerably compared to measurements recorded on rest days. Based on all the exercise BT measurements, BT_{eye} and BT_{ear} were significantly ($p < 0.0001$) related to BT_{rec} ($r = 0.674$ and $r = 0.735$, respectively). On average, BT_{eye} at all post-exercise times differed by less than 0.2°C compared to rectal temperatures, but BT_{eye} at pre-exercise underestimated BT_{rec} by 1.4% (0.5°C; **Table 2**). By contrast, BT_{ear} underestimated BT_{rec} by 1.0% (0.4°C) to 1.3% (0.6°C) across all measurement times (**Table 2**).

To determine method bias without measurement replication, the four recordings were combined for each dog, and the mean was used for analysis. Evaluation of a Bland–Altman plot revealed that a significant bias ($p = 0.02$) exists between the IRT method of BT_{eye} and BT_{rec}, and the slope is significantly different from 0 ($p = 0.02$) such that BT_{eye} will underestimate BT_{rec} at lower temperatures and overestimate BT_{rec} with increasing temperatures (**Figure 4A**). No bias ($p = 0.67$) exists between the IRT method of BT_{ear} and BT_{rec}, the 95% limit of agreement was ±0.76, and the slope is not different ($p = 0.62$) from 0 (**Figure 4B**).

DISCUSSION

The primary focus of this study was to evaluate hyperthermia in response to play-bout exercise with the use of IRT methods to measure eye and ear temperature of dogs. We report that both eye and ear temperature significantly relate to rectal BT and uniquely demonstrate that both are sensitive to accurately detect hyperthermia changes associated with exercise. In addition, this research also demonstrates that ear measures in the resting dog are sensitive and accurate, whereas eye temperature, while sensitive, is considerably less accurate when related to rectal temperature.

Prior to pursuing the primary objective, 16 Labrador Retrievers and 16 Beagles were initially used to characterize multiple daytime eye and ear temperature during sedentary activity relative to rectal temperature as the best estimate of core BT (20). To achieve the primary objective, the same dogs were exercised for 30 min and temperature of the eye, ear, and rectum measured before and after exercise. Accurately monitoring core BT during exercise in the field is critical and one of the ways to assess the risk of heat stress (4, 21) and exercise intolerance (22, 23). However, to obtain a rectal reading, exercise must be halted, measurement requires temporary restraint, and measurement recording may be challenging because of refusal by the pet. Thus, monitoring BT at alternative body sites or using different detection methods could address some of the challenges with rectal temperature measures.

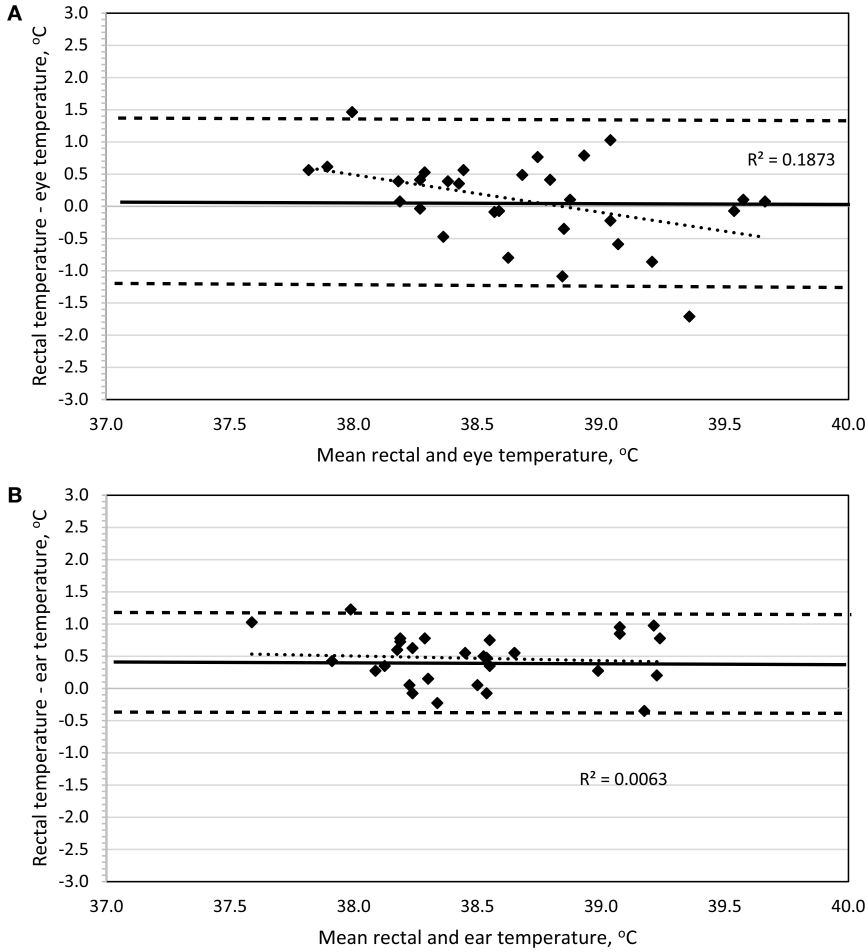


FIGURE 4 | Bland–Altman plots of body temperature in Labrador Retrievers or Beagles before and after 30-min of play-exercise comparing (A) rectal and eye or (B) rectal and ear. In both panels, the thin dotted line is the line of best fit for the data, the thick solid horizontal line is the mean difference (bias), and the horizontal dashed lines are the 95% limits of agreement (mean difference ± 1.96 SD). Points located above 0 on the *y*-axis represent an underestimation of temperature by the method in comparison to rectal core body temperature as measured by digital thermometer.

Past research has reported that tympanic membrane and hypothalamus share blood supply from the carotid arteries (24, 25), and a reading is reflective of heat from both tympanic membrane and ear canal (26). Areas of the eye, especially around the posterior border of the eyelid and the lacrimal caruncle, have rich capillary beds that respond to changes in blood flow resulting in localized temperature changes in people (27), cattle (28), and dogs (8, 29). Therefore, eye and ear temperature in sedentary and exercising Beagles and Labrador Retrievers are not only the surrogates of brain temperature but also reasonable alternatives to assess core BT fluctuations.

This study evaluating sedentary dogs indicated that ear temperature is better related ($r = 0.615$) than eye temperature ($r = 0.381$) when compared to rectal temperature as the reference method. When examined to monitor hyperthermia after exercise, the relationship for both ear ($r = 0.735$) and eye ($r = 0.674$) temperature compared to rectal temperature improved considerably (**Table 1**). The results generated from sedentary dogs confirm previous observations that ear and eye temperature significantly relate to and underestimate rectal BT (10, 11, 20).

Based on all daytime measurements, ear temperature underestimated rectal temperature on average by 0.1–0.3°C, which is similar to previous reports using infrared auricular thermometers versus rectal temperatures in dogs (10, 11). This underestimation increased to 0.4–0.6°C when measured to assess hyperthermia following exercise. Regardless of this minor loss of accuracy, Bland–Altman plot analysis indicates there is significant agreement, thus no bias, between the measurements of ear and rectal temperatures when measuring sedentary or hyperthermic BTs. In addition, sedentary data revealed that the 95% limit of agreement of ±0.45 is below the suggested, albeit arbitrary, limit of ±0.5 utilized in veterinary-based method evaluation (6, 10, 11).

A secondary goal was to explore the use of a remote-detection method of measuring eye temperature based on IRT to eliminate the need to insert a thermometer probe into the ear or rectum. This would potentially serve the dual benefit of improved pet compliance and ultimately reduce the need to halt the dog while working or exercising. As stated above, the relationship between eye ($r = 0.674$) temperature compared to rectal temperature improved considerably when measured for exercise-induced hyperthermia. Other canine studies evaluating eye temperature with IRT indicate a similar relationship between eye and rectal measures [$r = 0.661$; Ref. (8)]. However, evaluation of eye temperature in the sedentary dog revealed a greater underestimation of rectal temperature (**Table 2**) versus ear temperature and resulted in a broader 95% limit of agreement (±0.925), which worsened when used to measure BT after exercise. This indicates that eye temperature should not be used interchangeably with rectal temperature but still appears to be a feasible methodology and anatomical location to effectively monitor exercise-induced hyperthermia.

The daytime temperatures measured between 09:00 and 14:30 were obtained while the dogs were considered sedentary and did not exhibit the natural diurnal fluctuation of BT that normally increases 0.5–0.8°C from early morning (08:00–10:00) and peaks between 17:00 and 20:00 (17, 30, 31). This lack of diurnal fluctuation was observed across all three methods, and as shown in **Figure 1**, the temperatures were relatively consistent or slightly declined over the day. This observation is likely reflective of a natural increase in daytime BT because the dogs were in a kennel facility, slightly excited by entrance of familiar pet-care staff, and temporarily walked out of the kennel area for BT measures. Since the goal of the study was to examine BT methodology in sedentary and then in response to exercise, precautions such as temporary housing in metabolism cages to minimize external stimulation were excluded from the study design, thus preventing observation of the subtle diurnal fluctuation.

Finally, the third goal was to evaluate BT fluctuations between dogs of different body size to determine if breed differences were apparent between the three different temperature measurement methods. This study also reports observations of breed size differences in BT with all three methods resulting in Labrador Retrievers having a higher BT compared to Beagles, with no observed interaction between breed and time in the sedentary portion of the study. Other studies have reported an opposite observation of small breed dogs having a higher BT compared to larger breed dogs (17). It is unclear of the discrepancy between breed sizes between this study and others. However, other research by our lab has observed that Labrador Retrievers exhibit higher BT compared to similarly sized dogs (Shepherd breeds; Zanghi and Otto, unpublished data). Therefore, it appears to be important to consider the actual breed, and not just relative breed size, when considering "normal" BT ranges. Furthermore, breeds like Labrador Retrievers may be more prone to elevated BT, thus more at risk of heat stress, during exercise at higher ambient environmental temperatures.

Piccione et al. (17) did observe breed differences with diurnal change in daytime and nighttime BT, which, as stated above, is sensitive to masking because of physical activity or environmental stimuli. Thus in the current study, the movement of the dogs in the AM to obtain the measurements may have influenced the morning BTs that was sustained throughout the day with subsequent measurement collection. If so, Labs in this study appear to be more sensitive to external stimulation, and/or exercise that causes a rise in BT. The breed difference was consistent even when BT was measured in response to exercise. Therefore, it is difficult to attribute physical movement of the dogs during the sedentary study as the primary confounding factor, as exercise activity counts were similar between Labs and Beagles, but all three BT methods generated data that resulted in a significant or marginally significant breed × time interaction with Labs having a higher BT compared to Beagles. It is possible that the Labs respond differently with exercise that may be a result of lower thermal dissipation, which results in a higher BT.

In conclusion, both eye and ear temperature are effective for monitoring exercise hyperthermia, which is significantly related to rectal BT. However, eye temperature should not be used interchangeably with rectal temperature. This initial study provides evidence supporting the use of IRT measurement of eye temperature as an effective method of monitoring BT in a passive manner and remotely without having to physically contact the dog. Therefore, using a remote and passive eye temperature technology appears to address some of the challenges of pet restraint and pet compliance during probe insertion. It should be noted that although these data are representative of hyperthermia during exercise, exercise was only moderate rigorous, and average BT measures only reached approximately 40°C (104°F). Thus, additional research is warranted to examine the use of eye temperature in dogs experiencing higher BTs, in which remote detection of eye temperature of a dog approaching a higher risk of heat stress would be of significant value to the health of the pet. Finally, because this IRT device was deemed industrial-grade and used for research purposes, additional research to examine other similar devices, but possibly less sensitive and less expensive, would be valuable and likely more feasible for widespread use.

ETHICS STATEMENT

Study protocol was designed and followed in strict accordance with the guidelines established by the Nestlé Purina PetCare Animal Care and Use Advisory Committee at the pet-care facilities of Nestle Purina PetCare.

AUTHOR CONTRIBUTIONS

The author was the primary person responsible for designing the study, analyzing the data, and drafting the manuscript.

ACKNOWLEDGMENTS

The author would like to thank Xuemei Si for her statistical analysis assistance, Aaron Parker for managing the pets and coordinating the body temperature data collections and activity devices, and Hallie Barnett for operating the IRT unit and data generation for eye temperature measures.

FUNDING

All the funding was provided and generated by Nestle Purina PetCare Research for the purpose of conducting the study.

REFERENCES

1. Kozłowski S, Greenleaf JE, Turlejska E, Nazar K. Extracellular hyperosmolality and body temperature during physical exercise in dogs. *Am J Physiol* (1980) 239:R180–3.
2. Baker MA. Thermoregulatory responses to exercise in dehydrated dogs. *J Appl Physiol Respir Environ Exerc Physiol* (1984) 56:635–40.
3. Kozłowski S, Brzezińska Z, Kruk B, Kaciuba-Uściłko H, Greenleaf JE, Nazar K. Exercise hyperthermia as a factor limiting physical performance: temperature effect on muscle metabolism. *J Appl Physiol* (1985) 59:766–73.
4. Matwichuk CL, Taylor S, Shmon CL, Kass PH, Shelton GD. Changes in rectal temperature and hematologic, biochemical, blood gas, and acid-base values in healthy Labrador retrievers before and after strenuous exercise. *Am J Vet Res* (1999) 60:88–92.
5. Angle TC, Gillette RL. Telemetric measurement of body core temperature in exercising unconditioned Labrador retrievers. *Can J Vet Res* (2011) 75:157–9.
6. Osinchuk S, Taylor SM, Shmon CL, Pharr J, Campbell J. Comparison between core temperatures measured telemetrically using the CorTemp® ingestible temperature sensor and rectal temperature in healthy Labrador retrievers. *Can Vet J* (2014) 55:939–45.
7. Ober J, Gillette R, Angle T, Haney P, Fletcher D, Wakshlag J. The effects of varying concentrations of dietary protein and fat on blood gas, hematologic serum chemistry, and body temperature before and after exercise in Labrador retrievers. *Front Vet Sci* (2016) 3:59. doi:10.3389/fvets.2016.00059
8. Travain T, Colombo ES, Heinzl E, Bellucci D, Previde EP, Valsecchi P. Hot dogs: thermography in the assessment of stress in dogs (*Canis familiaris*)-a pilot study. *J Vet Behav* (2015) 10:17–23. doi:10.1016/j.jveb.2014.11.003
9. Kruk B, Kaciuba-Uściłko H, Nazar K, Greenleaf JE, Kozłowski S. Hypothalamic, rectal, and muscle temperatures in exercising dogs: effect of cooling. *J Appl Physiol* (1985) 58:1444–8.
10. Sousa MG, Carareto R, Pereira-Junior VA, Aquino MC. Comparison between auricular and standard rectal thermometers for the measurement of body temperature in dogs. *Can Vet J* (2011) 52:403–6.
11. Lamb V, McBrearty AR. Comparison of rectal, tympanic membrane and axillary temperature measurement methods in dogs. *Vet Rec* (2013) 173:524. doi:10.1136/vr.101806
12. Speakman JR, Ward S. Infrared thermography: principles and applications. *Zoology* (1998) 101:224–32.
13. Rekant SI, Lyons MA, Pacheco JM, Arzt J, Rodriguez LL. Veterinary applications of infrared thermography. *Am J Vet Res* (2016) 77:98–107. doi:10.2460/ajvr.77.1.98
14. Colak A, Polat B, Okumus Z, Kaya M, Yanmaz LE, Hayirli A. Short communication: early detection of mastitis using infrared thermography in dairy cows. *J Dairy Sci* (2008) 91:4244–8. doi:10.3168/jds.2008-1258
15. Fonseca BPA, Alves A, Nicoletti J, Thomassian A, Hussni CA, Mikail S. Thermography and ultrasonography in back pain diagnosis of equine athletes. *J Equine Vet Sci* (2006) 26:507–16. doi:10.1016/j.jevs.2006.09.007
16. Johnson SR, Rao S, Hussey S, Morley P, Traub-Dargatz J. Thermographic eye temperature as an index to body temperature in ponies. *J Equine Vet Sci* (2011) 31:63–6. doi:10.1016/j.jevs.2010.12.004
17. Piccione G, Giudice E, Fazio F, Refinetti R. Association between obesity and reduced body temperature in dogs. *Int J Obes (Lond)* (2011) 35:1011–8. doi:10.1038/ijo.2010.253
18. Laflamme D. Development and validation of a body condition score system for dogs. *Canine Pract* (1997) 22:10–5.
19. Zanghi BM, Kerr W, deRivera C, Araujo J, Milgram NW. Characterization of diurnal rest/activity rhythms in adult dogs of various cages fed once or twice daily. *J Vet Behav Clin Appl Res* (2012) 7:339–47. doi:10.1016/j.jveb.2012.01.004
20. Greer RJ, Cohn LA, Dodam JR, Wagner-Mann CC, Mann FA. Comparison of three methods of temperature measurement in hypothermic, euthermic, and hyperthermic dogs. *J Am Vet Med Assoc* (2007) 230:1841–8. doi:10.2460/javma.230.12.1841
21. Steiss JE, Wright JC. Respiratory alkalosis and primary hypocapnia in Labrador Retrievers participating in field trials in high-ambient-temperature conditions. *Am J Vet Res* (2008) 69(10):1262–7. doi:10.2460/ajvr.69.10.1262
22. Taylor SM, Shmon CL, Shelton GD, Patterson EN, Minor K, Mickelson JR. Exercise-induced collapse of Labrador retrievers: survey results and preliminary investigation of heritability. *J Am Anim Hosp Assoc* (2008) 44:295–301. doi:10.5326/0440295
23. Taylor SM, Shmon CL, Adams VJ, Mickelson JR, Patterson EN, Shelton GD. Evaluations of Labrador retrievers with exercise-induced collapse, including response to a standardized strenuous exercise protocol. *J Am Anim Hosp Assoc* (2009) 45:3–13. doi:10.5326/0450003
24. Benzinger M. Tympanic thermometry in anesthesia and surgery. *J Am Med Assoc* (1969) 209:1207–11. doi:10.1001/jama.209.8.1207
25. Giuliano K, Giuliano A, Scott S, MacLachlan E, Pysznik E, Elliot S, et al. Temperature measurement in critically ill adults: a comparison of tympanic and oral methods. *Am J Crit Care* (2000) 9:254–61.
26. Fraden J. The development of the thermoscan instant thermometer. *Clin Pediatr (Phila)* (1991) 30(4 Suppl):11–2.
27. Pavlidis I, Eberhardt NL, Levine JA. Seeing through the face of deception. *Nature* (2002) 415:35. Erratum in: *Nature* (2002) 415:602 doi:10.1038/415035a
28. Stewart M, Webster JR, Verkerk GA, Schaefer AL, Colyn JJ, Stafford KJ. Non-invasive measurement of stress in dairy cows using infrared thermography. *Physiol Behav* (2007) 92:520–5. doi:10.1016/j.physbeh.2007.04.034
29. Biondi F, Dornbusch PT, Sampaio M, Montiani-Ferreira F. Infrared ocular thermography in dogs with and without keratoconjunctivitis sicca. *Vet Ophthalmol* (2015) 18:28–34. doi:10.1111/vop.12086
30. Refinetti R, Piccione G. Daily rhythmicity of body temperature in the dog. *J Vet Med Sci* (2003) 65:935–7. doi:10.1292/jvms.65.935
31. Zanghi BM, Gardner C, Araujo J, Milgram NW. Diurnal changes in core body temperature, day/night locomotor activity patterns, and actigraphy-generated behavioral sleep in aged canines with varying levels of cognitive dysfunction. *Neurobiol Sleep Circ Rhythms* (2016) 1:8–18. doi:10.1016/j.nbscr.2016.07.001

Conflict of Interest Statement: The author would like to indicate that funding was solely provided by Nestle Purina Research and that the author is employed by Nestle Purina PetCare. No other conflict of interests exists, and all temperature and activity devices were purchased from the manufacturers. The data included in the manuscript have not been previously published, and animal protocols were conducted in accordance with approved Animal Care and Use Committee protocols.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 19 January 2017
doi: 10.3389/fvets.2017.00001

Assistance Dogs: Historic Patterns and Roles of Dogs Placed by ADI or IGDF Accredited Facilities and by Non-Accredited U.S. Facilities

*Sandra Walther[1], Mariko Yamamoto[2], Abigail Paige Thigpen[1], Anaissa Garcia[1], Neil H. Willits[3] and Lynette A. Hart[1]**

[1] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [2] Teikyo University of Science, Yamanashi, Japan, [3] Department of Statistics, University of California, Davis, Davis, CA, USA

OPEN ACCESS

Edited by:
Mia Cobb, Working Dog Alliance Australia, Australia

Reviewed by:
Geoffrey Wandesforde-Smith, University of California, Davis, USA
Hsin-YI Weng, Purdue University, USA
Mitsuaki Ohta, Tokyo University of Agriculture, Japan

***Correspondence:**
Lynette A. Hart
lahart@ucdavis.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *20 October 2016*
Accepted: *04 January 2017*
Published: *19 January 2017*

Citation:
Walther S, Yamamoto M, Thigpen AP, Garcia A, Willits NH and Hart LA (2017) Assistance Dogs: Historic Patterns and Roles of Dogs Placed by ADI or IGDF Accredited Facilities and by Non-Accredited U.S. Facilities. Front. Vet. Sci. 4:1. doi: 10.3389/fvets.2017.00001

Dogs' roles to support people with disabilities are increasing. Existing U.S. laws and regulations pertaining to the use of dogs for people with disabilities are only minimally enforced. Pushback legislation against some aspects of uses of assistance dogs currently is being passed or proposed in several states. Further, the U.S. Department of the Army and the Veterans' Administration support only dogs trained by an Assistance Dogs International (ADI) or International Guide Dog Federation (IGDF) accredited facility. Lacking a mandatory national process for screening the selection, training, and placement of assistance dogs with persons who have disabilities, the U.S. offers a creative but confusing opportunity for people to train their own dogs for any disability. While no U.S. surveillance system monitors assistance dogs, other countries generally have a legislated or regulatory process for approving assistance dogs or a cultural convention for obtaining dogs from accredited facilities. We conducted an online survey investigating current demographics of assistance dogs placed in 2013 and 2014 with persons who have disabilities, by facilities worldwide that are associated with ADI or IGDF and by some non-accredited U.S. facilities. Placement data from ADI and IGDF facilities revealed that in most countries aside from the U.S., guide dogs were by far the main type of assistance dog placed. In the U.S., there were about equal numbers of mobility and guide dogs placed, including many placed by large older facilities, along with smaller numbers of other types of assistance dogs. In non-accredited U.S. facilities, psychiatric dogs accounted for most placements. Dogs for families with an autistic child were increasing in all regions around the world. Of dog breeds placed, accredited facilities usually mentioned Labrador Retrievers and Golden Retrievers, and sometimes, German Shepherd Dogs. The facilities bred their dogs in-house, or acquired them from certain breeders. Non-accredited facilities more often used dogs from shelters or assisted people in training their own dogs. Facilities in Europe and the U.S. place dogs in all roles; other parts of the world primarily focus on guide dogs. Expansion of assistance dogs in many roles is continuing, with numbers of dogs placed accelerating internationally.

Keywords: Assistance Dogs International, autism dogs, diabetes dogs, hearing dogs, International Guide Dog Federation, mobility dogs, seizure dogs, service dogs

INTRODUCTION

The longstanding guiding role of a dog for a person with a visual impairment is obvious, and other roles of dogs have become evident. Guide dog owners report significantly increased social contacts and enhanced mental and physical well-being, as compared with visually impaired individuals without guide dogs. The close partnership is based on cooperative interactions between the person and dog in which they alternate the role of initiator for their joint actions (1). At the same time, guide dog owners walk faster and more efficiently than long-cane users (2). The very personal identity of the blind person changes to a person–dog team, with a softening of their former feelings of stigma (3). Reports describe the experience of owning a guide dog as life-changing, with both positive and negative consequences (4, 5). The loss of a guide dog at the end of a working partnership is especially difficult and distressing for the human partner (6).

Dogs to assist people with physical disabilities are referred to as mobility dogs. Canine Companions for Independence (CCI) (7) has placed almost 5,000 dogs since 1975, most for assistance with mobility. CCI mobility dog users report an increased sense of safety and peace of mind and greater independence. A systematic study examining the effects of service dogs for people with mobility (ambulatory) disabilities reviewed papers from the U.S., UK, and Japan and acknowledged the assistance with mobility while also highlighting the dogs' roles for social participation and some psychological issues (8).

The value of guide dogs and mobility dogs goes beyond their performance of specific working tasks. They also promote self-confidence, peace of mind, greater independence, a sense of safety, and enhanced social interaction. This broader range of values has opened the door to other creative uses of dogs for people with special needs. These uses range from calming or protecting individuals with autism spectrum disorder or with post-traumatic stress disorder, to alerting people with diabetes to blood hypoglycemia and alerting people to a forthcoming seizure (9). The detection roles presumably stem from the dogs' exceptional olfactory abilities and experimentally include detection of some types of cancer.

While taking a guide dog or mobility dog into a public place, airplane or train to help the disabled owner has generally been accepted because of the obvious need for the dog, there can be issues with regard to taking other types of assistance dogs onto planes, trains or into restaurants. To address these issues where the owner lacks a visibly apparent disability, most developed countries now have a centralized process by which persons with a disability can address specific requirements, often registering the disability and then legally registering the officially trained assistance dog that can be taken into public places and on airplanes, e.g., in Scotland (10). The international organizations, Assistance Dogs International (ADI) (11) and International Guide Dog Federation (IGDF) (12), have established basic guidelines for training, team training, and non-profit status to provide well-trained dogs to people with disabilities at low cost; each offers an accreditation process and posts the names of member facilities. Even with careful selection and training of dogs, predicting dogs that will be successful is challenging (13). As an alternative to legally requiring a registration process, countries may simply have a cultural convention for the person to partner only with a dog trained by an accredited facility.

The U.S. lacks a centralized process by which persons with a disability can legally register an assistance dog and take the dog into public places and on airplanes. Inconsistent nomenclature is used for the dogs' various special roles (14), and the U.S. regulations on public access are complicated (15). U.S. Department of Justice (U.S. DOJ) regulations allow a person with a disability who has an assistance dog the privilege to take their dog into restaurants, planes, etc., requiring only that the person has a disability (physical, mental, or medical) and that the specially trained dog performs a task related to the disability (16, 17). Lacking any centralized legal system of registration, and no convention of only partnering with dogs from accredited facilities, the U.S. is a dynamic site for experimenting with, and developing, new roles for assistance dogs. Not being addressed here is further complexity that, in the U.S., people with emotional disabilities are allowed public access by the U.S. Department of Housing and Urban Development (HUD) in housing (18, 19) and by the U.S. Department of Transportation (DOT) on airlines (20) for their emotional support animals that do not have a specific, trained task. These rather liberal U.S. regulations regarding emotional support animals allow housing and airplane access for birds, monkeys, and even miniature horses that need not perform any tasks (21). The U.S. situation is confusing for persons having disabilities who seek to acquire a well-trained dog that can offer effective assistance.

Assistance dog placements and roles are growing rapidly in the U.S., where the focus is on providing equal or reasonable accommodation for people with disabilities. Regulations of the various U.S. agencies assure privacy for persons with disabilities and allow them to have full public access with dogs that are presumably trained for assistance tasks for persons having any of a broadly defined range of disabilities. The open-ended U.S. regulatory process for assistance dogs has allowed for creative development of new roles for assistance by dogs.

Assistance Dogs International specifies three main categories of assistance dogs: guide dogs for the blind, hearing dogs for the hard of hearing, and service dogs. Service dogs include varied roles, such as wheelchair assistance for mobility, epilepsy monitoring of seizures, aid for families with autistic children, hypoglycemic detection for diabetes, and psychiatric support. Whereas the term assistance dogs in the international ADI world refers to all specially trained dogs assisting persons with disabilities, one comparable term adopted in the U.S. is service dogs (16, 17), a term including all the roles just mentioned. However, one complication is that HUD recently began using the term assistance animal to also include an animal that provides emotional support to a person with a disability (21).

Department of Transportation (20) and HUD (18, 19) have sometimes suggested requiring increased scrutiny for the use of psychiatric service dogs in public places or housing. The Department of Veterans Affairs and the Department of the Army only provide financial support for personnel when the dogs were trained by ADI or IGDF accredited facilities: psychiatric dogs are excluded.

The names of ADI and IGDF facilities are listed online, but little information is readily available to the public on the numbers of dogs being placed for various assisting roles, or their breeds and sources. Guide Dog Users, Inc. (GDUI) (22) posted surveys from 13 U.S. facilities, including detailed information to inform prospective applicants. Those facilities ranged in number of placements from 6 to 310 dogs per year, with a median of 47 dogs. With the exception of a facility specializing for children, placements require applicants to be 16–18 years of age.

California offers optional free registrations of assistance dogs supplanting the need for licensing dogs, and some data are available for 1999–2012 registrations. By 2005, dogs of small body size were registered at a similar frequency as those of large body sizes (23). Chihuahuas became the most frequently registered small breed, and Pit Bulls (although not a recognized breed) became the fourth most frequently registered large "breed." Demographics in psychiatric service dogs and emotional support service dogs changed rapidly; in early years of this study (2000–2002), psychiatric service dogs accounted for 17.1% of registrations and emotional support dogs were 0.0%, but in more recent years (2010–2012), psychiatric service dogs accounted for 31.9% of registrations and emotional support dogs were 19.0%.

In Europe, ADI lists 56 facilities in 19 countries; the Netherlands and Belgium each list 9 facilities, and the UK lists 8, whereas 8 countries list just one facility (11). The 51 facilities listed by IGDF are located in 20 countries; France has 12 facilities, and 10 countries have only one facility (12).

In Asia, ADI lists one facility in Japan and one in Taiwan that is also accredited with IGDF (11, 12). An additional facility for IGDF is in Korea, and one more in Taiwan, plus nine facilities in Japan. Thus, there is relatively little activity and research in Asia regarding assistance dogs apart from Japan. The legal qualification system under Japan's Act on Assistance Dogs for Physically Disabled Persons specifies what is required for assistance dogs (24). When someone with a disability pairs with an assistance dog, the team needs to be assessed and qualified by one of a few designated organizations. To be qualified, the assisting behavior and health of the dog are assessed as well as the adequacy of the person with a disability in having the ability to accompany and handle the dog in public. Thus, not all people who are interested in living with an assistance dog can acquire one. While these governmental initiatives help assure order in public when assistance dogs are used in Japan, they also likely reduce the number of assistance dogs in Japan (25).

Additionally, Japanese historical, cultural, and environmental factors may also slow the development of assistance dogs in Japan (25). For example, training dogs for human use, including hunting and herding, was not historically common in Japan, and Japanese had relatively negative attitudes toward dogs having utilitarian roles compared to the UK (26, 27). In addition, the Japanese cultural characteristic regarding cleanliness requires that guide dog owners give a higher consideration for dogs' excretions and shedding hair in public (28). The dense population and limited interpersonal space in homes in Japan amplify this concern. Little interest in acquiring a guide dog was also reported in Israel, where generally only veterans and members of upper strata used guide dogs (29). The authors attributed this to the great challenges in acquiring a dog and perhaps a cultural view by some of dogs as unclean.

Assistance Dogs International offers its certification process for non-profit facilities that place assistance dogs. Facilities initially enroll for candidate status and then work toward full accreditation. During this study's data collection in 2015, the U.S./Canada region listed 98 accredited and candidate facilities and Europe listed 56. Guide dog facilities that are accredited with IGDF and that place dogs in additional roles beyond guiding also are eligible to seek ADI accreditation.

In this paper, we describe the breeds, sources, and numbers of dogs with each role placed in 2013 and 2014 worldwide by responding facilities of ADI or IGDF, and the year of each facility's establishment. Additionally, we similarly surveyed responding non-accredited facilities in the U.S. that placed dogs.

MATERIALS AND METHODS

We first developed a brief survey with questions for each facility placing assistance dogs and uploaded it on SurveyMonkey®. Questions asked for contact and location information for the facility, the year of establishment, the total numbers of dogs placed in 2013 and 2014, and then the numbers of dogs placed each year for each category—guide dogs, hearing dogs, and service dogs for mobility, seizure alert, autism, psychiatric disorders, diabetic alert, and others. We asked whether handler-dog team training was provided, how long it lasts, and where it occurs. We asked the sources of the dogs and the breeds used. Finally, we asked the accreditation status of the facility, and for any comments. We contacted all accredited and candidate facilities worldwide that are associated with ADI and IGDF, sending them an email letter containing the survey link. Some facilities have dual accreditation; IGDF facilities placing guide dogs may apply for ADI accreditation if they place dogs in additional roles. This dual accreditation currently is held by eight North American facilities and five other international facilities. For non-responding ADI facilities in the U.S. and Europe, we accessed the facility's year of establishment and identified a primary role of dogs that the facility placed, based on the information available on the facility's website.

We also e-mailed a survey to all U.S. facilities that are not accredited that we could find listed online. To develop this list of 170 facilities, we searched by assistance dogs, service dogs, seizure dogs, diabetes alert dogs, autism dogs, PTSD dogs, and psychiatric dogs; we also gathered lists of facilities that were posted online. All of these facilities needed to be deleted if they also appeared on ADI or IGDF lists, or if they were duplicated.

We sent two reminder emails to all non-respondents and responded to numerous queries with follow-up replies and reminders. In addition, to gain maximal participation from ADI facilities in the U.S., we completed up to three phone calls to answer questions and remind non-responding U.S. facilities listed with ADI about the survey. Phone calls were not made to international facilities due to time zone and language differences.

We summarized the numerical information in several tables. ADI and IGDF present their members' information by continent, and we followed that pattern for the introductory **Table 1** and the final summarizing **Figure 5**, presenting North America, other international, and U.S. non-accredited facilities, to show the numbers of dogs by type placed around the world, and the extent to which current placements for each type are by older or newer facilities. **Table 2** and the timelines also show the results by continent, but separate Canada and the U.S. This allows easy comparison between the accredited and non-accredited U.S. facilities and between the U.S. and Europe. Canada and the U.S. differ in their regulations. Further, focusing particularly on contrasts between Europe and the U.S. seems useful because other parts of the world have very few facilities, whereas the numbers of facilities are somewhat comparable between Europe and the U.S. Simple descriptive statistics describe uses, sources, and types of these assistance dogs being placed, and their patterns as related to geographic category, the facility's age, and its accreditation status. To depict the numbers of dogs being placed in each role as related to the years the facilities were established, we created separate timelines for responding facilities in the various geographic areas (U.S., Canada, Europe, and other international countries) and non-accredited U.S. facilities. We plotted each facility on its geographic timeline by its year of establishment and indicated the roles and approximate total numbers of dogs placed, with the total numbers from 2013 and 2014 combined. To create separate timelines for non-responding accredited facilities in the U.S. and Europe, the regions having the greatest number of facilities, we used the date of establishment for each facility as indicated on their website and selected the most prominent role of dogs for the facility profiled online to show on the timeline.

No personal information was obtained from any individual; only facilities were contacted and asked to provide information about the facility. Thus, IRB approval was not sought.

To assess the historical opening of facilities and the specific roles they primarily addressed in 2013 and 2014, the year of 1915 was taken as the recent, starting point year for the formal training of assisting dogs. Each facility was categorized by primary role of dogs it places and the years lapsed since 1915 until the facility was established; for Europe and the U.S., responding and non-responding ADI/IGDF facilities were combined, as they did not significantly differ. Kruskal–Wallis tests were employed to test for differences between Europe, the U.S./Canada, and other international facilities in establishment dates of facilities for each of the primary roles of dogs for which there were sufficient numbers (e.g., guide, mobility, and autism). None of these tests showed significant differences, so the specifics are not included in the results.

To assess the historical development of each of these dogs' roles in various parts of the world (e.g., Europe, other international, and U.S./Canada), for each region, we listed all facilities placing a specific type of dog in 2013 in order of the facilities' year of establishment. Then for each role, we determined the number of dogs placed by each facility during 2013 and computed the weighted median year of facility establishment, weighted by the median numbers of dogs placed. This measure was determined in each region—Europe, other international, and U.S./Canada—for the seven roles of dogs. It was calculated for responding ADI and IGDF facilities and then also for non-accredited U.S. facilities. Finally, a canonical correspondence analysis (CCA) was used to

TABLE 1 | Total numbers of dogs placed in 2013 and 2014, categorized by types of dogs, for accredited international or North American facilities, or non-accredited U.S. facilities.

	Type of dog	# Dogs 2013/2014	% of Total dogs	# Facilities 2013/2014	Mdn dogs/year/facil (range of total dogs placed by all facilities/year)
International Assistance Dogs International and International Guide Dog Federation facilities (*n* = 34)	Guide	249/261	45	18/17	8.3 (1–77.5)
	Mobility	99/106	18	16/19	5.5 (1–14)
	Autism	53/67	10	9/11	4.3 (1.5–18)
	Hearing	118/141	23	3/5	9 (1–110)
	Psychiatric	3/20	2	2/6	1.5 (1–6.5)
	Diabetes	0/10	1	0/5	1 (0.5–2)
	Seizure	6/10	1	2/3	3.8 (1–5.5)
	Total	528/615 = 1,143	100	34	Mdn = 10 (1–110)
North American ADI and IGDF facilities (*n* = 55)	Guide	442/476	39	9/11	20 (2–199)
	Mobility	471/472	40	41/40	3.5 (1–177)
	Autism	95/110	9	18/19	3 (1–26.5)
	Hearing	59/50	5	8/7	4 (1–30)
	Psychiatric	52/67	5	14/16	2.3 (1–11)
	Diabetes	37/32	3	7/8	3 (1–10.5)
	Seizure	7/4	0	6/3	1 (1–2)
	Total	1,163/1,211 = 2,374	100	55	Mdn = 10 (1–233.5)
U.S. non-accredited facilities (*n* = 22)	Guide	2/1	0	1/1	1.5 (1.5–1.5)
	Mobility	59/52	14	15/12	2.5 (1–13)
	Autism	38/34	9	8/8	1.4 (1–14.5)
	Hearing	10/7	2	5/4	1.8 (1–3)
	Psychiatric	232/294	66	11/11	5 (1–136.5)
	Diabetes	17/23	5	6/4	3.5 (1–9.5)
	Seizure	15/13	4	6/5	2 (1–5)
	Total	373/424 = 797	100	22	Mdn = 8 (1–136.5)

TABLE 2 | Total Assistance Dogs International/International Guide Dog Federation dogs placed, by regions.

Regions	Total dogs 2013/2014	Total dogs	# Facilities 2013/2014	Median dogs/facility in 2013/2014	% Increase 2013–2014
Europe	397/485	882	22/24	10/11	23
Australia/NZ	46/50	96	5/5	8/9	9
Asia	87/91	178	5/5	10/12	5
Canada	43/50	93	5/5	7/12	16
U.S.	1,120/1,153	2,273	49/48	5/6.5	3
Western	350/370	720	14/13	6/8	6
Central	315/310	625	11/11	5/6	–2
Southern	196/218	414	16/16	5/7	12
Eastern	259/255	514	8/8	22/17.5	–2

depict the relationships among data, especially the characteristics of accredited, candidate, and non-accredited facilities with respect to types of dogs they placed and sources of the dogs.

RESULTS

Among the 229 invitations sent to ADI or IGDF facilities, only one was returned due to an inactive email address with no forwarding suggestion. In contrast, among the 170 invitations sent to non-accredited facilities, 37 (22%) bounced back, suggesting a high rate of turnover. Response rates from ADI facilities were 35% internationally and 57% in North America; response rates from facilities only in IGDF were 16% and 25%, respectively. Considering only the invited non-accredited U.S. facilities whose invitations were not bounced back, the response rate was 17%.

Characteristics of Facilities Internationally, in the U.S./Canada, and in Specific Geographic Regions

Accredited facilities in North America had a similar median number of dogs placed overall to those internationally, 10 per year (**Table 1**), whereas U.S. non-accredited facilities produced somewhat fewer dogs, 8 per year (**Table 1**). The ranges extended to 110 and 136.5 dogs placed per year for international and U.S. non-accredited facilities, respectively, whereas U.S. facilities extended to 233.5 dogs placed per year, reflecting some very large U.S. accredited facilities.

Accredited or Candidates: International (Excluding North America)

When considering only facilities outside North America, ADI and IGDF each list similar numbers of facilities, 68 (belonging to ADI and some also to IGDF) and 62 (belonging to IGDF only). Among the 34 responding facilities, close to 45% of the dogs placed in 2013 and 2014 were guide dogs (**Table 1**). Hearing dogs accounted for 23%. Mobility and autism dogs accounted for 18 and 10%, respectively; psychiatric, seizure, and diabetic alert each accounted for a small number of placements. The numbers of facilities involved in placing these types of dogs were similar for guide and mobility dogs, 18 and 19, respectively; the number of facilities placing autism dogs was next, and then hearing, psychiatric, diabetic and seizure dogs were each placed by only a few facilities. Generally, facilities placing hearing dogs placed the largest number of dogs in a specific role (**Table 1**: median = 9), guide dogs per facility per year were second, and facilities placing mobility dogs had the third largest number of placements. Autism and seizure placements per facility were next, and psychiatric and diabetic had a median of only 1–2 per facility. Overall, the international placements of dogs increased 16% in just the 1 year from 2013 to 2014.

Accredited or Candidates: U.S. and Canada

As shown in **Table 1**, and unlike the international facilities, the 55 responding North American facilities placed approximately equal numbers of guide and mobility dogs in 2013 and 2014, 39 and 40% of the total 2,374 dogs, respectively. Autism, hearing, psychiatric, diabetic alert, and seizure dogs accounted for the remaining 22% of the total, in that order. Mobility dogs were placed by the most facilities, by far, with autism and psychiatric dogs next. The guide dog facilities each placed a median of 20 guide dogs, several fold more than the facilities placed for other roles. The overall increase in number of North American placements from 2013 to 2014 was a modest 4%.

Non-Accredited: U.S.

As shown in **Table 1**, the 22 responding non-accredited U.S. facilities had a strong majority of placements of psychiatric dogs (66%). Remaining placements were for mobility, autism, diabetic alert, seizure, hearing, and guide dogs, in that order. Mobility dogs accounted for the greatest number of facilities, and psychiatric dogs were next, with autism, seizure, diabetic, hearing, and guide dogs following. The median number of dogs placed per facility for any particular role was only a few, with psychiatric being the highest (median = 5). The total number of dog placements increased 14% at these facilities from 2013 to 2014.

Geographic Regions

As shown in **Table 2**, among geographic regions, facilities in Europe reported the highest rate of growth from 2013 to 2014, with an overall 23% increase in total numbers of dogs placed. The five respondents each from Australia and Asia also increased their numbers of dogs placed, by 9 and 5%, respectively. Five respondents from Canada reported a 16% increase.

The number of responding ADI or IGDF facilities in the U.S. was more than double the number from Europe. Yet, overall, the U.S. had only a 3% increase in total number of dogs placed in 2014 as compared with 2013. In fact, the Central and Eastern states each reported a decline of 2% in their numbers of dogs placed. Facilities in the Eastern states differed from other regions in placing a large number of dogs per facility, reflecting their large guide dog facilities (medians 22 and 17.5 for 2013 and 2014). Southern states had the greatest increase in total number of dogs placed in 2014 compared with 2013, 12%, and the Western states were intermediate, 6%.

Characteristics of Facilities by Their Year Established: Numbers and Roles of Dogs

Examining changes over time, we considered facilities that were established: prior to 1980, when primarily only guide dogs and hearing dogs were placed; 1981–2000, a period when the new service roles were developed; and 2001–2014, when the new service roles continued growing.

Facilities Established Prior to 1980

Except for the U.S., the pioneering facilities that were established early generally continue to focus primarily on placing traditional guide dogs (**Figure 1**: Europe; **Figure 2** left: international—Australia and Asia); but a few respondents in Europe now also place dogs for mobility assistance and for families with autistic children. These dogs often were bred in-house.

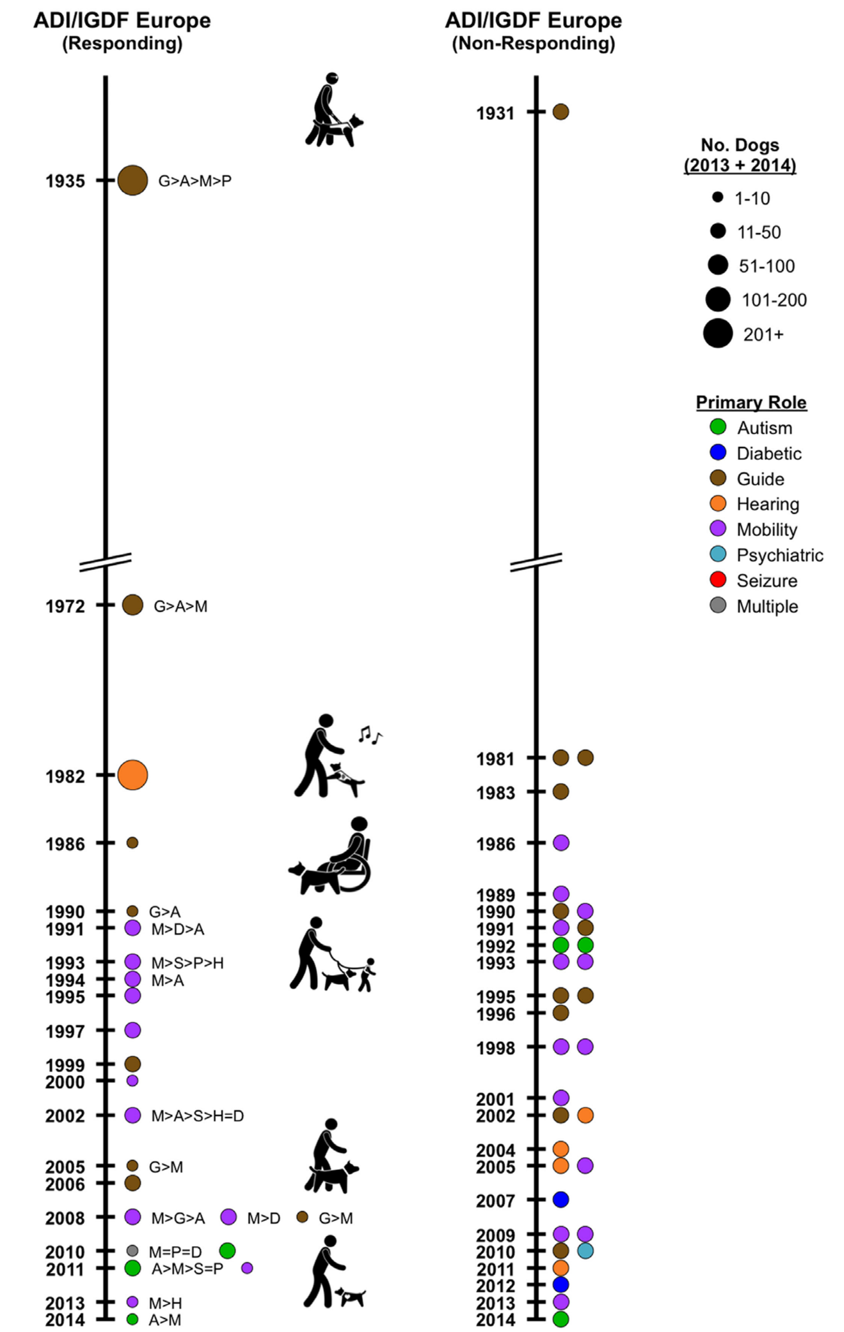


FIGURE 1 | Timeline depicting Assistance Dogs International or International Guide Dog Federation facilities in Europe, by year of establishment. *Left*: responding facilities. Colored circles represent the primary type of assistance dogs placed; the circle size indicates the numerical range for the total dogs placed by the facility in 2013 and 2014. If more than one type of dog is placed, letters indicate the types placed, from most to least. *Right*: non-responding facilities. Year of establishment and a major type of dogs placed were acquired recently from facilities' websites; no information is available on numbers of dogs placed.

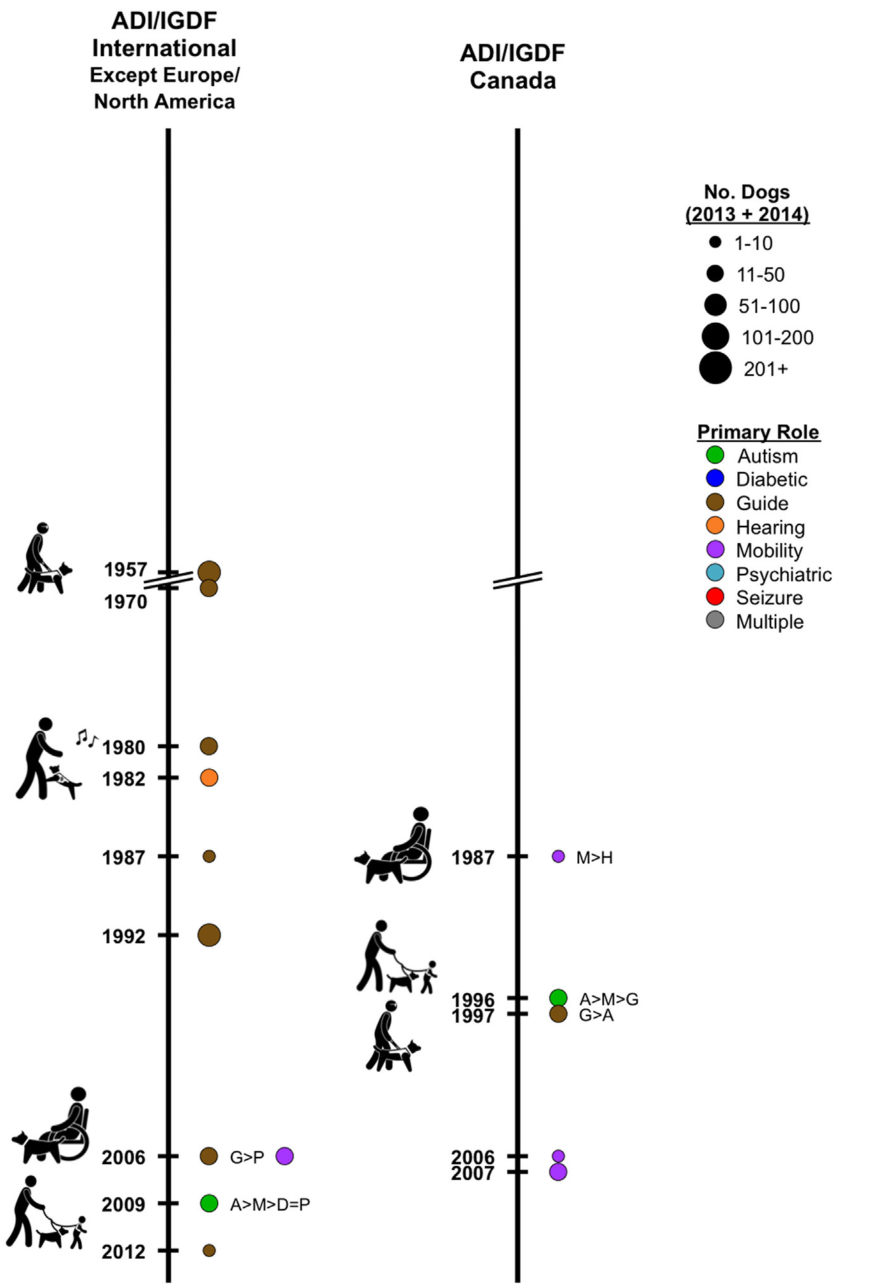


FIGURE 2 | ***Left*: timeline depicting responding International Assistance Dogs International or International Guide Dog Federation facilities, except in Europe or North America, by year of establishment**. Colored circles represent the primary type of assistance dogs placed; the circle size indicates the numerical range for the total dogs placed in 2013 and 2014. If more than one type of dog is placed, letters indicate the types placed, from most to least. *Right*: similar timeline for Canada.

No reporting Canadian facilities were yet established in this early period.

In the U.S., three responding accredited facilities, already established by 1948, continued placing primarily guide dogs, though one also diversified and placed mobility, psychiatric and hearing dogs (**Figure 3** left). Three responding facilities established in the 1970s now place primarily mobility dogs, but all also placed hearing dogs, two placed autism dogs, and one placed psychiatric dogs. Similarly, three non-responding, accreditation facilities established by 1956 place guide dogs (**Figure 3** center). Of three non-responding accreditation facilities established in the 1970s, one in 2016 profiles placing mobility dogs, and two profile hearing dogs. None of the responding, non-accredited U.S. facilities was established prior to 1980.

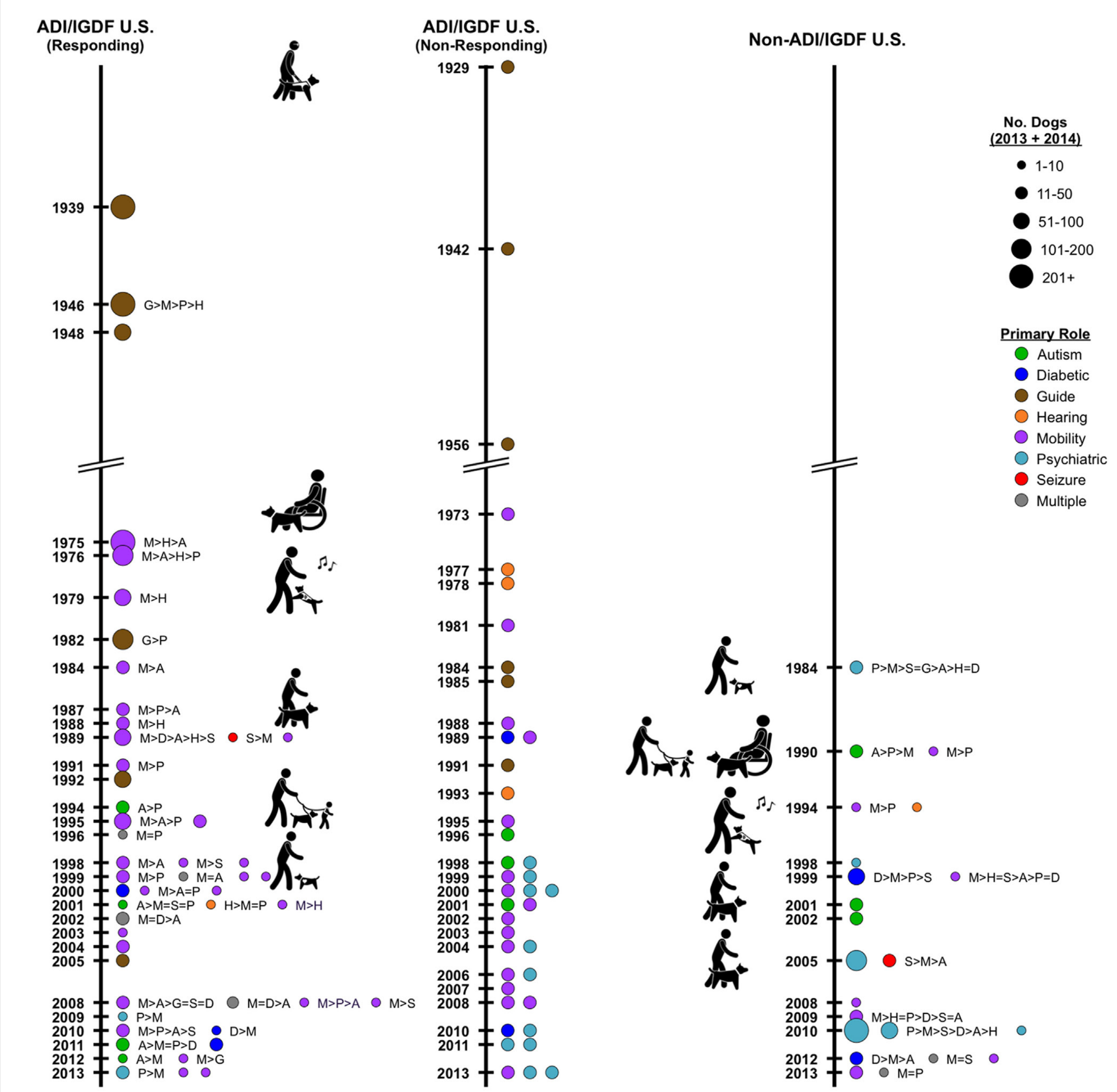


FIGURE 3 | Timeline depicting Assistance Dogs International or International Guide Dog Federation facilities in the U.S. by year of establishment. *Left*: responding facilities. Colored circles represent the primary type of assistance dogs placed; the circle size indicates the numerical range for the total dogs placed in 2013 and 2014. If more than one type of dog is placed, letters indicate the types placed, from most to least. *Center*: non-responding facilities. Year of establishment and a major type of dogs placed recently were acquired from facilities' websites; no information is available on numbers of dogs placed. *Right*: timeline depicting responding non-accredited facilities in the U.S. by year of establishment.

Facilities Established 1981–2000

Of the three international responding facilities in this category and outside Canada and Europe, two currently placed only guide dogs and one placed only hearing dogs (**Figure 2** left). The 10 responding facilities in Europe placed hearing (1), primarily guide (3), and mobility (6) dogs (**Figure 1** left). Autism (3) and psychiatric dogs (1) were also mentioned. The 18 non-responding facilities in Europe primarily profile in their websites, guide (8), mobility (8), psychiatric (1), and autism (1) dogs (**Figure 1** right). Of the three responding accreditation facilities from Canada, mobility, autism, and guide dogs were each primary from one facility (**Figure 2** right: Canada).

Twenty-three U.S. ADI facilities responded, including three guide dog facilities, two of which place a large number of dogs

(**Figure 3** left). Eighteen of these facilities placed mobility dogs, usually as a primary role for the facility. Other roles are autism (8), psychiatric (8), seizure (3), hearing (2), and diabetes (1). In the U.S., 17 non-responding accreditation facilities profile mobility (6), psychiatric (4), guide (3), autism (2), diabetes (1), and hearing (1) dogs (**Figure 3** center). These facilities typically obtained their dogs from outside breeders, or in the case of dogs for guide work, dogs were bred in-house. Placements of hearing dogs were not increasing but were being continued, primarily by a small number of long-established facilities that seemed unlikely to add new roles for the types of dogs they placed.

Eight responding non-accredited U.S. facilities were established in this time frame (**Figure 3** right). One facility placed solely hearing dogs. The others placed some psychiatric (7) dogs; facilities also placed mobility (6) and autism (3) dogs, as well as some diabetes, guide, and seizure dogs.

Facilities Established 2001–2014

Of the four responding facilities in this category and outside the U.S./Canada and Europe, two primarily placed guide dogs (**Figure 2** left). Mobility and autism dogs were each primary for one facility. The 12 responding facilities in Europe primarily placed mobility (6), guide (3), and autism (3) dogs (**Figure 1** left). The 15 non-responding facilities in Europe profile mobility (5), hearing (4), guide (2), diabetic (2), psychiatric (1), and autism (1) dogs (**Figure 1** right). Two responding Canadian facilities placed mobility dogs (**Figure 2** right).

Of 21 responding U.S. ADI facilities, 18 placed some mobility dogs; other roles addressed by facilities were autism (8), psychiatric (7), diabetes (6), seizure (4), guide (3), and hearing (2) (**Figure 3** left). Eighteen non-responding U.S. ADI facilities profile on their websites mobility (9), psychiatric (7), diabetic (1), and autism (1) dogs (**Figure 3** center).

From 14 responding non-accredited U.S. facilities, mobility (9), autism (6), and psychiatric (5) dogs, and seizure, hearing, and diabetes dogs also were represented (**Figure 3** right).

Characteristics of Facilities by Accreditation Status

Breeds that were almost invariably mentioned by accredited facilities include Golden Retrievers and Labrador Retrievers, sometimes with crosses, and often German Shepherd Dogs. Although a few facilities favored another specific breed CCAs addressing breeds by role or geography were unremarkable for other breeds.

Accredited

As revealed in CCAs, fully accredited facilities very often bred their own dogs (**Figure 4**). Typically, they did not use dogs from shelters or assist persons in training their own dogs. They often placed guide, mobility, and hearing dogs.

Candidate

These facilities often placed diabetes, seizure, and autism dogs (**Figure 4**).

Non-Accredited

Facilities that are non-accredited often acquired dogs from shelters or worked with persons who trained their own companion dogs (**Figure 4**), as indicated by the distance of these variables from the origin, in a similar direction. Psychiatric dogs tended to be placed by non-accredited facilities, indicated by their colocation distant from the origin.

Characteristics of Facilities throughout the World by the Development of Specific Roles of Dogs

The years of establishment for all facilities currently placing dogs in the various roles do not significantly differ for Europe and the U.S. However, the picture changes when considering the numbers of all dogs placed in the various roles and the years the relevant facilities were established. **Figure 5** depicts the weighted median years of facility establishments (weighted by the median number of dogs placed in each of the seven roles at each facility during 2013), shown for accredited North American and International facilities, as well as non-accredited U.S. facilities. A few of North America's facilities that were established early continue to have high outputs of assistance dogs, especially for guide dogs and mobility dogs, as compared with other international facilities, except for seizure dogs. On this measure, non-accredited U.S. facilities preceded the international facilities' placements of numbers of guide, autistic, and psychiatric assistance dogs (**Figure 5**).

Guide Dogs

In the U.S., guide dogs often are trained and placed by large facilities (median per facility = 20 dogs/year) and the weighted median year of facility establishment for the median dog placed in 2013 was 1946. Internationally, facilities are smaller, placing a median number per facility of eight dogs per year, with the weighted median year of facility establishment placing the median dog being 1999. Even guide dogs are relatively new in some parts of the world, and several countries focus almost entirely on placement of only guide dogs. Responding non-accredited U.S. facilities placed only three guide dogs; although placing so few dogs, the weighted median facility year of establishment for the median dog placed was 1984.

Hearing Dogs

The weighted median year of facility establishment for placing the median hearing dog in 2013 in North America was 1975; the corresponding figure internationally is 1982. International respondents placed many more hearing dogs than those in North America (**Table 1**); **Figure 1** reveals a large facility in the UK that places hearing dogs. Among the five responding, non-accredited U.S. facilities that placed hearing dogs, the weighted median establishment year of the facility placing the median dog was 1999.

Mobility Dogs

In North America, the weighted median year of facility establishment for placing the median mobility dog in 2013 was 1979, whereas, internationally, it was 1997. Among the 15

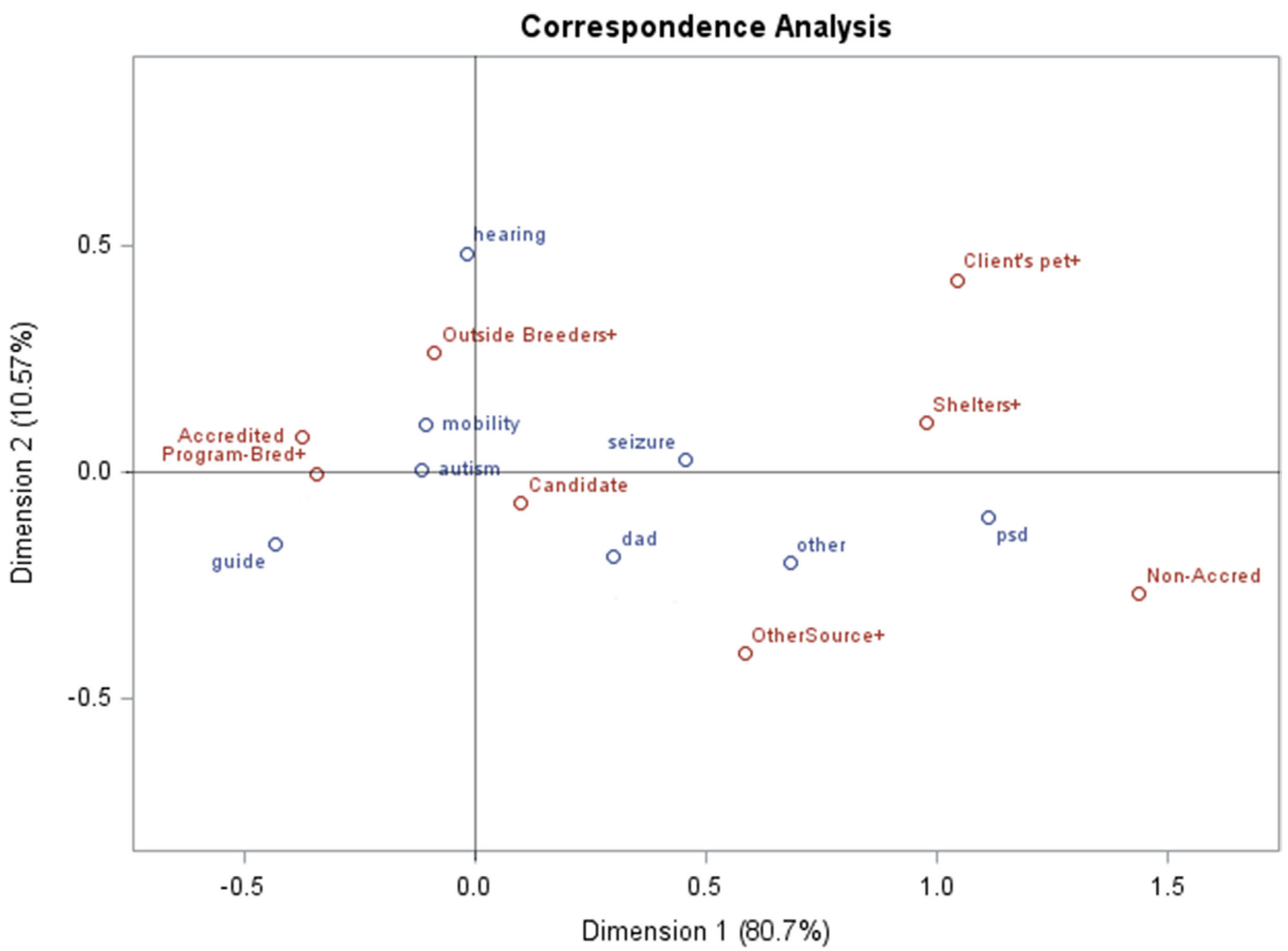


FIGURE 4 | **Canonical correspondence analysis for responding Assistance Dogs International/International Guide Dog Federation facilities: for accreditation status, counts for types of dogs placed, and sources of dogs.**

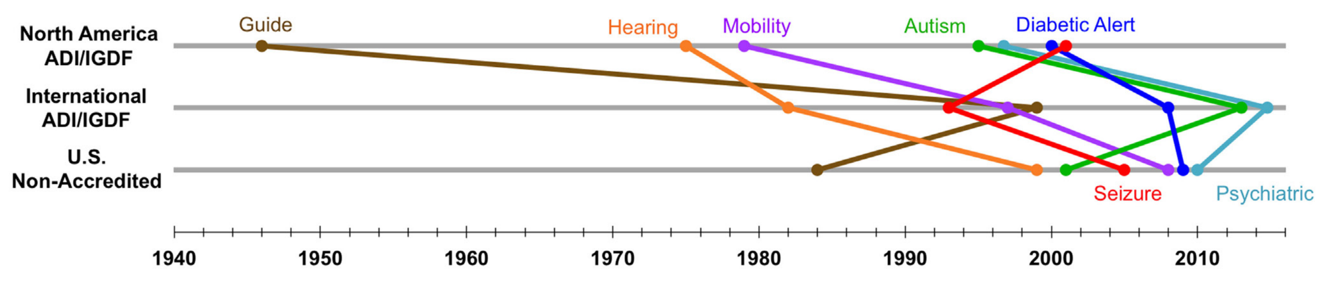


FIGURE 5 | **Weighted median years of facility establishments (weighted by the median numbers of dogs placed in the seven roles during 2013), shown for accredited North American and other international facilities, as well as non-accredited U.S. facilities.**

non-accredited U.S. facilities placing mobility dogs in 2013, the weighted median facility year placing the median dog was 2008.

Autism Dogs

The weighted median facility establishment year for placing the median autism dog in North America was 1995; internationally, it was 2013. Of eight non-accredited U.S. facilities placing autism dogs, the weighted median year was 2001.

Psychiatric Dogs

Similar to autism dogs, the weighted median facility establishment year for placing the median psychiatric dog in North America in

2013 was 1995; internationally, the corresponding year was 2013. Among 11 non-accredited U.S. facilities placing psychiatric dogs, the weighted median year for placing the median dog in 2013 was 2010.

Seizure Dogs

Among six responding facilities placing seizure dogs in 2013, the weighted median establishment year for placing the median dog in North America was 2001; the corresponding year internationally for the two facilities in 2013 was 1993. Six non-accredited U.S. facilities placed seizure dogs in 2013, and the weighted median facility year for placing the median dog was 2005.

Diabetes Dogs

Among seven responding facilities placing diabetes alert dogs in 2013, the weighted median year of facility establishment in North America placing the median dog was 2000. Internationally in 2013, no reporting facilities placed diabetes dogs, but by 2014 (figures used only in this case for comparable data), among five facilities placing a few dogs, the facility's establishment weighted median year for placing the median dog was 2008. Among six non-accredited U.S. facilities placing diabetes dogs, the weighted median year was 2009.

In general, many of North America's accredited facilities that today place high numbers of assistance dogs were established prior to 2000. Although facilities for various dogs' roles were also established in Europe prior to 2000, much of their growth in the numbers of dogs placed has come from the recent creation of facilities. In North American and non-accredited U.S. facilities, dogs for autism are being placed by long-established facilities, just following guide, hearing, and mobility dogs, but dogs for autism placements began later at international facilities. Relative to other roles of dogs, North American facilities were somewhat delayed in placing dogs for seizure detection. Dogs for diabetes primarily have arisen since 2000. In countries beyond North America and Europe, placement of dogs has proceeded more slowly with a primary emphasis continuing on the role of dogs as guides.

DISCUSSION

The past decade or two in the U.S., Canada, and Europe have seen major increases in uses of assistance dogs for improving the function, health, and well-being of their human companions. Data-based studies document benefits not just for the people with visual impairments and those using wheel chairs but for seizure alerting, hypoglycemia detection, and comforting children with autism or adults with post-traumatic stress disorder (9, 15). In the discussion below, we address the worldwide regional differences in assistance dog roles, the differences and similarities in training facilities placing assistance dogs for the ever-expanding roles they have in society. With appropriate attention to the roles of assistance dogs in public places and transportation—protecting the comfort of the public—these canine companions can add immeasurably to the health and well-being of people in an increasing number of ways.

Characteristics of Facilities Internationally, in the U.S., and in Specific Geographic Regions

Some European countries and the U.S. are increasingly welcoming to dogs in public areas. Asia is less accepting of dogs in public, which may affect the regional differences in the development of assistance dogs. Equal accommodation for people with disabilities developed in the U.S. alongside their growing expectations for individually trained dogs; the public access that was previously allowed pet dogs was insufficient for people who had assistance dogs supporting them. Therefore, DOJ (17) had to specifically differentiate the public access rights for handlers of service dogs versus pet dogs. However, in Europe, people were already allowed to accompany their pet dogs in various public locations. Thus, for some time Europeans did not need to create a special law for assistance dogs until the numbers and roles of assistance dogs expanded. In Asian countries, especially Japan, using dogs for assistance was uncommon; clear strict rules needed to be created to introduce assistance dogs into Japanese society and foster the understanding and tolerance of the public for dogs. This may explain why the expansion of assistance dogs developed early in the U.S., whereas recently, the growth has slowed and the U.S. shows a very low percentage increase from 2013 to 2014—only 3%. Some of the tapering off in the U.S. may be related to its growing number of non-accredited facilities. The highest rate of expansion—23%—is in Europe, whereas Asia remains slow in adopting varied assistance dogs, with a 5% increase from 2013 to 2014 (**Table 2**; **Figure 2**).

Characteristics of Facilities by Accreditation Status

The organizational strength of accredited facilities accounts for some of their stability, accomplishments, and growth. Not only are they proficient in their ability and infrastructure for training dogs but also they maintain the financial power and human resources that are required to be accredited by ADI and IGDF, always preparing required documents and inviting inspectors from ADI or IGDF. This helps explain why the accredited facilities are placing many more dogs of most types than the non-accredited U.S. facilities. Although there are numerous non-accredited facilities, they inevitably suffer high turnover with financial and staffing struggles as reflected in the high level of bounced back email messages, and a few of these facilities place unqualified dogs. Non-accredited facilities have no obligation to be non-profits, so occasional unscrupulous persons can exploit unwary people seeking assistance dogs. Members of the public who are seeking dogs do not necessarily know about accreditation and may pay a large fee for a dog that proves not to be useful in assisting with a specific disability.

Although guide dog facilities are established in Asia, few people acquire guide dogs. Studies of obstacles to acquiring dogs in Japan show that people with visual disabilities feel that information resources pertaining to guide dogs are limited (30). Also, despite favorable legislation and due to low cultural acceptance of dogs in public, guide dog users in Japan describe stressful experiences when taking their dogs out on a rainy day, using a lavatory, or going on an outing (31). Placement of service dogs in Japan is proceeding very slowly despite evidence of positive functional and mental effects for people partnering with service dogs (32).

Working success of dogs is a challenge: half of the IGDF facilities surveyed to assess the working success of German Shepherd Dogs, Labrador Retrievers, Golden Retrievers, and Labrador × Golden Retriever crosses found diminished working success for the Labrador × Golden crosses (33). Using external breeders and assessing the dogs in field tests were associated with greater working success. These breeds are consistent with the accredited facilities in our study and similar to those reported by the 13 GDUI (22) facilities, although Dobermans, Poodles, and Bernese crosses were also mentioned there.

A limitation of this study was the low response rate from the non-accredited facilities in the U.S. We experienced the high turnover rate and frequent difficulty in reaching these facilities: presumably, the facilities that responded represented those with greater efficacy, stability, and resources.

Lacking professional centralized guidance for assistance dogs in the U.S., the widespread lack of knowledge people have about assistance dogs creates problems for everyone involved. Businesses and landlords often are unaware of the requirements to create access for handlers with their dogs, or which questions can be asked of someone with an assistance dog. People considering acquiring an assistance dog may simply get one to self-train without realizing that the dog will not provide meaningful assistance for their particular needs. A further burden faced by the growing number of persons training their own dogs and unaccredited U.S. facilities acquiring dogs from shelters to train is the poor predictive value of screening tests to select dogs. One small study found no correlation between a dog's performance on the selection test and its ability to successfully complete a retrieval task for someone using a wheelchair (34).

Characteristics of Facilities throughout the World by the Development of Specific Roles of Dogs

Numerous historic accredited facilities continue to place large numbers of guide or service dogs, and also often account for the increasing number of placements of dogs for families with an autistic child. Arguably this is a new role for dogs that is now in the mainstream for accredited facilities, going beyond the uses of dogs for diabetes, seizure, or psychiatric needs. As studies documented in Canada and Ireland, the dogs for autism ensure the safety of the child, while also enhancing the freedom and well-being of the family (35, 36). This use poses special challenges of welfare for the dogs; the dogs are likely to bond more with a parent than the child, and the child's behavior and schedule may cause welfare burdens on the dog (37).

The placement of psychiatric dogs by accredited facilities has proceeded slowly while expanding more rapidly in the U.S. non-accredited facilities, where a majority of dogs placed assumed roles for psychiatric assistance. The distinction in the U.S. between psychiatric service dogs, emotional support dogs, and well-trained companion dogs for persons with mental illness can be confusing. Even ownership of pet dogs contributes toward the recovery from serious mental illness (38). Dogs in these roles serve as family and facilitate social connections with others, as has been well documented (39, 40).

A newer use of dogs is for medical alert, such as responding to low glycemia levels for persons with diabetes and under glucose control medication. Among 212 pet dog owners, 32% reported more than 10 incidents where the pet dog's behavior changed in relation to hypoglycemia (41). With trained alert dogs, 8 of 10 responded appropriately to their owners' blood glucose levels; they can assist with glucose control and increase the person's independence (42). Alerting to impending migraine may be another alerting role for dogs that would provide time to preemptively treat migraines (43).

Guide dogs continue as the primary assisting role of dogs around the world. Assisting with mobility is a well-established role for dogs in North America that is increasing in Europe. Hearing dogs continue to be important in Europe but are somewhat eclipsed in the U.S. by new roles for dogs. Uses of dogs for families with an autistic child are steadily increasing throughout the world, and the placements often are by large accredited facilities.

Currently, with minimal U.S. enforcement of guidelines regarding the training and placement of assistance dogs and their access to public areas, restaurants, and airplanes, assistance dog facilities have already had a period of rapid growth. Studies show that assistance dogs play an essential role in human health and welfare. Further worldwide exploration of acceptable ways to integrate such dogs and other animals into the human health realm is still another angle on the "One Health" approach to medicine.

ETHICS STATEMENT

The study included only census information on the numbers of dogs placed by assistance dog facilities. The study involved no direct involvement with the dogs or handlers, and thus no ethical review was required. We simply contacted the assistance dog facilities to acquire information on the dogs they have recently placed.

AUTHOR CONTRIBUTIONS

LH conceived the idea, oversaw data collection, and drafted manuscript. SW conducted all electronic communications with survey participants and prepared some final figures. AT prepared some final figures and edited all drafts. NW provided statistical guidance. MY participated in initial concept and survey design, assisted in initial manuscript draft, and reviewed all drafts. AG provided repeated contacts to facilities to remind them to participate and summarized data by regions.

ACKNOWLEDGMENTS

The authors appreciate the willing assistance throughout this project provided by Martha Bryant (recently deceased). They also acknowledge useful feedback offered by Benjamin Hart. Partial financial support for this project was provided by the UC Davis Center for Companion Animal Health and by a gift from Pfizer/Zoetis.

REFERENCES

1. Naderi SZ, Miklosi A, Koda A, Csanyi V. Co-operative interactions between blind persons and their dogs. *Appl Anim Behav Sci* (2001) 74:59–80. doi:10.1016/S0168-1591(01)00152-6
2. Clarke-Carter DD, Heyes AD, Howarth CI. The efficiency and walking speed of visually impaired people. *Ergonomics* (1986) 29(6):779–89. doi:10.1080/00140138608968314
3. Sanders CR. The impact of guide dogs on the identity of people with visual impairments. *Anthrozoös* (2000) 13(3):131–9. doi:10.2752/089279300786999815
4. Whitmarsh L. The benefits of guide dog ownership. *Vis Impair Res* (2005) 1:27–42. doi:10.1080/13882350590956439
5. Wiggett-Barnard C, Steel H. The experience of owning a guide dog. *Disabil Rehabil* (2008) 30(14):1014–26. doi:10.1080/09638280701466517
6. Nicholson J. The end of a partnership: the reactions of guide dog owners to the end of a working partnership with their guide dog. *Br J Vis Impair* (1993) 1:29–30.
7. *Canine Companions for Independence*. (2015). Available from: http://www.cci.org/atf/cf/%7Bd369f549-15c4-46ee-bee3-52b190502f3f%7D/CCI%20 2015%20ANNUAL%20REPORT%20PDF%20FOR%20INTERACTIVE.PDF
8. Winkle M, Crowe TK, Hendrix I. Service dogs and people with physical disabilities partnerships: a systematic review. *Occup Ther Int* (2012) 19:54–66. doi:10.1002/oti.323
9. Hart LA, Yamamoto M. Dogs as helping partners and companions for humans. 2nd ed. In: Serpell J, editor. *The Domestic Dog*. Cambridge, UK: Cambridge University Press (2016). p. 247–70.
10. Refson K, Jackson AJ, Dusoir AE, Archer DB. The health and social status of guide dog owners and other visually impaired adults in Scotland. *Vis Impair Res* (1999) 1(2):95–109. doi:10.1076/vimr.1.2.95.4411
11. *Assistance Dogs International (ADI)*. (2016). Available from: http://www.assistancedogsinternational.org/members/programs-search/
12. *International Guide Dog Federation (IGDF)*. (2016). Available from: http://www.igdf.org.uk/links/
13. Tomkins LM, Thomson PC, McGreevy PD. Behavioral and physiological predictors of guide dog success. *J Vet Behav* (2011) 6:178–87. doi:10.1016/j.jveb.2010.12.002
14. Parenti L, Foreman A, Meade BJ, Wirth O. A revised taxonomy of assistance animals. *J Rehabil Res Dev* (2013) 50(6):745–56. doi:10.1682/JRRD.2012.11.0216
15. Hart L, Yamamoto M. Recruiting psychosocial health effects of animals for families and communities: transition to practice. 4th ed. In: Fine A, editor. *Handbook on Animal-Assisted Therapy: Theoretical Foundations and Guidelines for Practice*. Amsterdam, Netherlands: Academic Press (2015). p. 53–72.
16. U.S. Department of Justice (DOJ). *Part 35. Nondiscrimination on the Basis of Disability in State and Local Government Services (as Amended by the Final Rule Published on September 15, 2010)*. (2010). Available from: http://www.ada.gov/regs2010/titleII_2010/titleII_2010_withbold.htm
17. U.S. Department of Justice (DOJ). *ADA 2010 Revised Requirements. Service Animals*. (2011). Available from: http://www.ada.gov/service_animals_2010.pdf
18. U.S. Department of Housing and Urban Development (HUD). *Joint Statement of the Department of Housing and Urban Development and the Department of Justice. Reasonable Accommodations under the Fair Housing Act*. (2004). Available from: https://www.hud.gov/offices/fheo/library/huddojstatement.pdf
19. U.S. Department of Housing and Urban Development (HUD). *24 CFR Part 5. Pet Ownership for the Elderly and Persons with Disabilities; Final Rule*. (2008). Available from: https://www.hud.gov/offices/fheo/FINALRULE/Pet_Ownership_Final_Rule.pdf
20. U.S. Department of Transportation (DOT). *Disability Issues: DOT Rule (Part 382)*. (2008). Available from: http://airconsumer.ost.dot.gov/ACAAcomplaint.htm
21. U.S. Department of Housing and Urban Development (HUD). *Service Animals and Assistance Animals for People with Disabilities and HUD-Funded Programs*. (2013). Available from: https://www.ahead.org/uploads/conference/2013/handouts/2.10%20Service%20Animals%20Ackerman/HUD_ServiceAssistanceAnimals%20April%2025%202013.pdf
22. *Guide Dog Users, Inc. (GDUI)*. (2016). Available from: http://guidedogusersinc.org/resources/gdui-school-survey/
23. Yamamoto M, Lopez MT, Hart LA. Registrations of assistance dogs in California for identification tags: 1999-2012. *PLoS One* (2015) 10(8):e0132820. doi:10.1371/journal.pone.0132820
24. Ministry of Health, Labour, and Welfare. *Act on Assistance Dogs for Physically Disabled Persons*. (In Japanese). (2011). Available from: http://law.e-gov.go.jp/htmldata/H14/H14HO049.html
25. Yamamoto M, Hart LA, Ohta M, Matsumoto K, Ohtani N. Obstacles and anticipated problems associated with acquiring assistance dogs, as expressed by Japanese people with physical disabilities. *Hum Anim Interact Bull* (2014) 2(1):59–79.
26. Miura A, Bradshaw JWS, Tanida H. Attitudes towards dogs: a study of university students in Japan and the UK. *Anthrozoös* (2000) 13(2):80–8. doi:10.2752/089279300786999860
27. Miura A, Bradshaw JWS, Tanida H. Attitudes towards assistance dogs in Japan and the UK: a comparison of college students studying animal care. *Anthrozoös* (2002) 15(3):227–42. doi:10.2752/089279302786992496
28. Koda N, Kubo M, Ishigami T, Furuhashi H. Assessment of dog guides by users in Japan and suggestions for improvement. *J Vis Impair Blind* (2011) 105(10):591–600.
29. Deshen S, Deshen H. On social aspects of the usage of guide-dogs and long-canes. *Sociol Rev* (1989) 37(1):89–103. doi:10.1111/j.1467-954X.1989.tb00022.x
30. Yamamoto M, Hart LA, Matsumoto K, Ohta M, Ohtani N. Japanese people with vision disabilities rate their experiences with information resources pertaining to guide dogs. *Int J Orient Mobil* (2013–2014) 6(1):70–82.
31. Matsunaka K, Koda N. Acceptance of dog guides and daily stress levels of dog guide users and nonusers. *J Vis Impair Blind* (2008) 102(5):295–304.
32. Shintani M, Senda M, Takayanagi T, Katayama Y, Furusawa K, Okutani T, et al. The effect of service dogs on the improvement of health-related quality of life. *Acta Med Okayama* (2010) 64(2):109–13.
33. Batt L, Batt M, Baguley J, McGreevy P. Relationships between puppy management practices and reported measures of success in guide dog training. *J Vet Behav* (2010) 5:240–6. doi:10.1016/j.jveb.2010.02.004
34. Weiss E, Greenberg G. Service dog selection tests: effectiveness for dogs from animal shelters. *Appl Anim Behav Sci* (1997) 53:297–308. doi:10.1016/S0168-1591(96)01176-8
35. Burrows KE, Adams CL, Spiers J. Sentinels of safety: service dogs ensure safety and enhance freedom and well-being for families with autistic children. *Qual Health Res* (2008) 18:1642–9. doi:10.1177/1049732308327088
36. Smyth C, Slevin E. Experiences of family life with an autism assistance dog. *Learn Disabil Pract* (2010) 13(4):12–7. doi:10.7748/ldp2010.05.13.4.12.c7758
37. Burrows KE, Adams CL, Millman ST. Factors affecting behavior and welfare of service dogs for children with autism spectrum disorder. *J Appl Anim Welf Sci* (2008) 11:42–62. doi:10.1080/10888700701555550
38. Wisdom JP, Saedi GA, Green CA. Another breed of "service" animals: STARS study findings about pet ownership and recover from serious mental illness. *Am J Orthopsychiatry* (2009) 79(3):430–6. doi:10.1037/a0016812
39. Hart L, Hart B, Bergin B. Socializing effects of service dogs for people with disabilities. *Anthrozoös* (1987) 1(1):41–4. doi:10.2752/089279388787058696
40. Lane DR, McNicholas J, Collis GM. Dogs for the disabled; benefits to recipients and welfare of the dog. *Appl Anim Behav Sci* (1998) 59:49–60. doi:10.1016/S0168-1591(98)00120-8
41. Wells DL, Lawson SW, Siriwardena AN. Canine responses to hypoglycemia in patients with type 1 diabetes. *J Altern Complement Med* (2008) 14:1235–41. doi:10.1089/acm.2008.0288
42. Rooney NJ, Morant S, Guest C. Investigation into the value of trained glycaemia alert dogs to clients with type 1 diabetes. *PLoS One* (2013) 8(8):e69921. doi:10.1371/journal.pone.0069921
43. Marcus DA. Canine responses to impending migraines. *J Altern Complement Med* (2012) 18(2):106–8. doi:10.1089/acm.2011.0773

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer GW-S declared a shared affiliation, with several of the authors and a past collaboration with one of the authors (LH) to the handling Editor, who ensured that the process nevertheless met the standards of a fair and objective review.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 11 September 2017
doi: 10.3389/fvets.2017.00144

Environmental and Physiological Factors Associated With Stamina in Dogs Exercising in High Ambient Temperatures

Patrick J. Robbins[1], *Meghan T. Ramos*[1], *Brian M. Zanghi*[2] *and Cynthia M. Otto*[1]*

[1] Penn Vet Working Dog Center, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, United States, [2] Nestlé Purina Research, St. Louis, MO, United States

This IACUC approved study was performed to evaluate the environmental, physiological, and hematological components that contribute to stamina following successive bouts of exercise that included searching (5-min), agility (5-min), and ball retrieve (<10-min). Regularly exercised dogs ($N = 12$) were evaluated on five separate occasions. The population consisted of eight males and four females ranging in age from 8 to 23 months, which included six Labrador retrievers, three German shepherds, and one each English springer spaniel, German wirehaired pointer, and Dutch shepherd. The exercise period was up to 30 min with 5 min of intermittent rest between the exercise bouts or until a designated trainer determined that the dog appeared fatigued (e.g., curled tongue while panting, seeking shade, or voluntary reluctance to retrieve). At the end of the exercise period, pulse rate (PR), core temperature, blood lactate, and venous blood gas were collected. The median outdoor temperature was 28.9°C (84°F) (IQR; 27.2–30°C/81–86°F) and median humidity was 47% (IQR; 40–57%). Median duration of exercise was 27 min (IQR; 25–29). No dog showed signs of heat stress that required medical intervention. The components used to measure stamina in this study were total activity, post-exercise core body temperature (CBT), and increase in CBT. When controlling for breed, total activity, as measured by omnidirectional accelerometer device, could be predicted from a linear combination of the independent variables: pre-exercise activity ($p = 0.008$), post-exercise activity ($p < 0.001$), outdoor temperature ($p = 0.005$), reduction in base excess in extracellular fluid compartment (BEecf) ($p = 0.044$), and decrease in TCO_2 ($p = 0.005$). When controlling for breed and sex, increase in CBT could be predicted from a linear combination of the independent variables: study day ($p = 0.005$), increase in PR ($p < 0.001$), increase in lactate ($p = 0.001$), reduction in BEecf ($p = 0.031$), increase in glucose ($p = 0.044$), increase in hematocrit ($p = 0.032$), and increase in hemoglobin ($p = 0.038$). This study suggests that the influence of outdoor temperature, pre- and post-exercise activity, and the metabolic parameters are important components of stamina associated with exertion.

Keywords: stamina, exercise, detection dogs, core body temperature, outdoor temperature, blood gas

OPEN ACCESS

Edited by:
Lynette Arnason Hart, University of California, Davis, United States

Reviewed by:
Joseph Wakshlag, Cornell University, United States
Craig Angle, Auburn University, United States

***Correspondence:**
Cynthia M. Otto
cmotto@vet.upenn.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: *13 October 2016*
Accepted: *16 August 2017*
Published: *11 September 2017*

Citation:
Robbins PJ, Ramos MT, Zanghi BM and Otto CM (2017) Environmental and Physiological Factors Associated With Stamina in Dogs Exercising in High Ambient Temperatures. Front. Vet. Sci. 4:144. doi: 10.3389/fvets.2017.00144

INTRODUCTION

Exercise, conditioning, and physical fitness are important to both working and active pet dogs. For working dogs to reach a level of field readiness, it is essential that the dog achieves and maintains top athletic performance (1–4), which includes fitness level. Working dog fitness is a combination of cardiorespiratory function, balance, strength, flexibility, proprioception, stamina, and muscle strength. Stamina is the ability to withstand high energy-demanding activity over extended periods of time. Many factors may influence or even limit stamina in dogs. These can include intrinsic factors of the athlete, such as muscular activity, fat and electrolyte metabolism, body weight and conditioning, extrinsic factors such as environmental temperature and humidity, and fixed factors including age, breed, and sex (2–4). Additional factors including acclimatization to environment and activity, such as exercise conditioning, to increase fitness may also play a role in increasing stamina in dogs.

As athletes, dogs have been used to study the multi-systemic effects of exercise and factors that limit performance. During exercise, dogs exert energy that leads to heat generation (5–7). Canine athletes have a higher cardiovascular and thermoregulatory demand that requires sport and working dogs to have a greater internal temperature and cardiac regulation. During exercise, the dog is capable of increasing its cardiac output by 74–200% (7–13) and increases its carotid blood flow by 500% (14–16), which can increase blood flow to regions of maximal heat exchange perhaps as a strategy for heat tolerance.

Temperature regulation in dogs is primarily a function of respiratory exchange and associated evaporative heat loss (17, 18). The combination of exercise and limited evaporative cooling through panting leads to several physiological changes, which include tachypnea, lactic acidosis, respiratory alkalosis, hyperthermia, increase in heart rate, and hypocapnia (2–4, 6–8, 13, 19–28). When the magnitude of these shifts occurs beyond the physical capacity or conditioning of the dog, its ability to work for prolonged durations is limited and the need for frequent rest periods is required.

Even with the existing body of research, the most important factors in limiting canine stamina have not been fully characterized.

This study evaluated the environmental, physiological, dog-specific variables that influence exercise stamina in regularly exercised dogs. We hypothesized that canine stamina (defined as a two-component assessment of activity, as measured by an accelerometer and duration of exercise) would be primarily limited by the external factors of environmental temperature and humidity. If external factors are the main predictors of exercise stamina, then strategies to increase stamina will need to focus on managing those factors or adjusting expectations of stamina with the changes in heat and humidity. By contrast, if dog-specific factors are the main predictors of stamina then training or conditioning may provide a mechanism to increase activity counts per duration of exercise.

MATERIALS AND METHODS

Animal Care and Feeding

The current study protocol (#805342) was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Twelve regularly exercised dogs (eight males and four females) of five different breeds; six Labrador retrievers, three German shepherd dogs, one Dutch shepherd dog, one Springer spaniel, and one German wirehaired pointer were tested (**Table 1**). Breed was assigned as a categorical variable for our analysis. Age of the dogs ranged from 8 to 23 months (median, 16 months).

TABLE 1 | Information on study population.

Dog name	Breed	Age	Sex
Ohlin	Labrador Retriever (LaR)	23	M
Tsunami	German Shepherd	22	F
Sirius	LaR	24	M
Ffoster	LaR	42	F
Gus	LaR	14	M
Quest	German Shepherd	8	M
McBaine	Springer Spaniel	21	M
PApa Bear	LaR	25	M
Ditto	German Wired Haired Pointer	16	M
Pacy	LaR	16	F
Logan	German Shepherd	8	M
Felony	Dutch Shepherd	7	F

The dogs' weights ranged from 31.7 to 80.0 lbs (14.4–36.4 kg). All dogs used in the exercise trial were required to have overall good general health prior to beginning the trial and were evaluated by a veterinarian at the beginning of each trial week. All dogs participating in the study had been previously trained to retrieve and perform various agility/search tasks. Exercise conditioning was defined as daily exercise of similar type and duration as used in the exercise protocol for at least 4 weeks preceding the study. Dogs were owned by the University of Pennsylvania and enrolled in the Penn Vet Working Dog Center (PVWDC) training program where each dog trains daily and lived in a foster home during evenings and weekends. Dogs were individually housed in a metal wire crate at the PVWDC when not training or exercising during the day. All field exercise and sample collection were conducted on the grounds of the PVWDC.

All dogs were fed either two or three times daily with *ad libitum* water to maintain an optimal body condition score between 4 and 5 out of 9 (29). Dogs were maintained on commercial dry kibble (Purina Pro Plan Sport All Life Stages Performance 30/20 Formula Dog Food or Pro Plan Focus Sensitive Skin and Stomach Formula Dog Food, Nestlé Purina PetCare Company, St. Louis, MO, USA) through the duration of the trial. Dogs were given *ad libitum* water before the exercise challenge and after the 5-min post-exercise measurements. For dogs fed three times daily, the mid-day feeding was delayed until after all post-exercise measurements were taken.

Experimental Design and Exercise

The exercise field study was designed to assess a 30-min exercise challenge conducted on five separate days over a 19-day period (days 1, 7, 12, 15, and 19). The exercise challenge was conducted to assess the effect of exercise in warm ambient temperatures in a setting that reproduced various types of physical activity (scent searching/tracking, agility, and retrieving) typical of training activities for detection dogs. The study consisted of two study periods with six different dogs randomly assigned to participate in each period. The first experimental period occurred between June 12 and July 1, 2014, whereas the second was between August 7 and August 25, 2014. Temperature and humidity were measured using a wireless device (AcuRite Wireless Weather Station; Primex Family of Companies, Lake Geneva, WI, USA), which was placed at the site of the outdoor exercise challenge on each study day.

After an initial pre-exercise activity of trotting, accompanied by active stretches for 5 min, the outdoor exercise challenge was designed to be ≤30 min long; consisting of 5 min of search, followed by 5 min of rest in the shade, then 5 min of agility, followed by 5 min of rest in the shade, and a maximum 10 min of ball retrieve, and then a post-exercise activity period of light trotting/quick walking for 5 min. This duration of exercise is similar to previous studies evaluating exercise-induced physiological changes (3, 4, 28). The start time of each exercise component was documented manually. The search component of the exercise challenge consisted of each dog locating and alerting on 2–3 hidden scent sources (live humans or human cadaver remains) in a simulated rubble pile. Human cadaver remains samples was provided by Sarah Atlas of the New Jersey Task Force, who gave the sample to the PVWDC Training Manager, who is also a Canine Search Specialist with a certified Federal Emergency Management Agency (FEMA) Urban Search and Rescue Human Remains Detection Dog. The sample was 18 g of knee tissue. The sample was received on June 1, 2014 and was a 1-month old sample. The agility portion consisted of dogs climbing elevated vertical and horizontal ladders angled 0–45°, walking over unstable surfaces, cavalettis, performing distance exercises (e.g., direction and control) (**Figure 1**), and moving through tunnels, in a controlled pace set by the trainer. Following success in agility or search, dogs were rewarded with tug or ball play. For the rest components, dogs were held on leash by a stationary trainer. Dogs were allowed to sit, stand, or walk within the six-foot length of their leash. The ball-retrieve component consisted of dogs retrieving a ball or toy. Dogs were allowed up to 15 s between retrieves. Because some dogs ($n = 2$) of different breeds did not naturally retrieve, they were trotted on leash in a ball-retrieve fashion until exercise was stopped. Dogs were allotted 15 s between trots. Each exercise portion (search, rest, agility, and ball retrieve) occurred in the same designated location and order for each dog in each trial. The exercise test was stopped if dogs showed signs of curled tongue while panting, seeking shade, or slowing of retrieve (**Figure 2**). To keep the exercise termination consistent among dogs, a single experienced canine trainer (the PVWDC training manager) determined the end of exercise in each trial.

The exercise challenge started at 12:00 p.m. each day for moderate external ambient temperature challenge on the five sample collection days for each experimental period. The order of dogs in the exercise challenge was randomized on each sample collection day, except when medical reasons dictated otherwise. Tracheobronchitis, with no signs of fever or systemic illness, was first recognized in one dog on August 18th. On August 19th, two more dogs were also observed coughing. By August 26th, all but two dogs were observed with a non-productive cough.

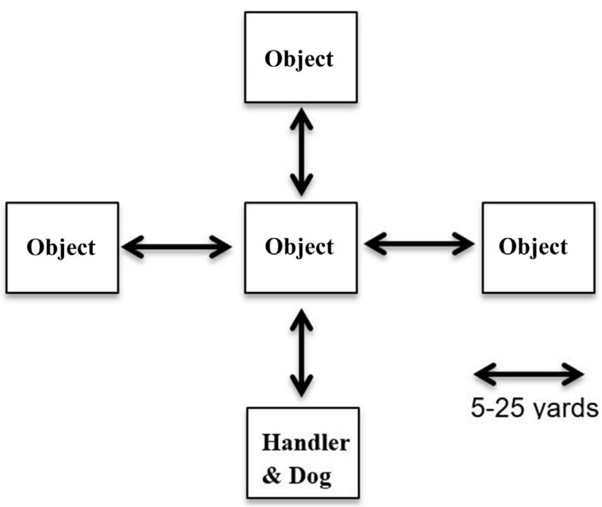


FIGURE 1 | Direction and control training is used to train disaster search dogs to safely navigate or avoid hazardous areas. Modeled after a baseball diamond, handler can direct the dog to obstacles behind, in front of, to the left or to the right of, the dog (distances ranging from 5 to 25 yards).

Sample Collection and Physiological or Biochemical Measurements

Pulse rate (PR), left (LET) and right (RET) ear temperatures, and core body temperature (CBT) were recorded for each dog on each of the five exercise challenge days either between 7 and 9 a.m. (pre-exercise), and immediately after exercise. Manual PR was obtained from the femoral artery. Left and right ear temperatures were collected using an instant ear thermometer device (Pet-Temp® Instant Infrared Ear Thermometer for Home Use, Model PT-300, San Diego, CA, USA) (30). Body temperature was collected using a telemetric core temperature monitor (CorTemp® Indigestible Core Body Temperature Sensor, HQInc., Palmetto, FL, USA) to measure CBT within the gastrointestinal tract (CBT). New CorTemp sensors were swallowed by the dogs the morning of each exercise challenge day. CBT data measured by the CorTemp sensor was collected wirelessly by the CorTemp Data Recorder (HQInc., Palmetto, FL, USA).

Similar to previous studies, the following data were collected (2–4, 6–8, 13, 19–28). Two samples of venous blood (~3 mL; jugular, cephalic, or saphenous) were collected on each exercise challenge day to evaluate pre- and immediate post-exercise analytes. Pre-exercise blood samples were collected between 7 and 9 a.m., whereas post-exercise blood samples were collected immediately (0-min) post-exercise before the cooldown period. Blood samples were taken anaerobically from a peripheral vein and analyzed immediately. Venous blood pH, gases ($PvCO_2$, HCO_3, TCO_2, PvO_2, SvO_2), electrolytes (iCa, Na, K), base excess in extracellular fluid compartment (BEecf), glucose, hemoglobin,

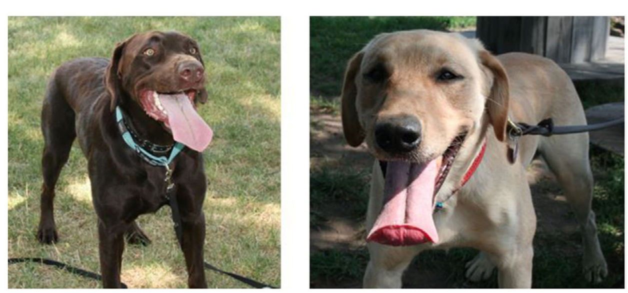


FIGURE 2 | Dogs during and after exercise demonstrating signs of exercise in the heart, include spade tongue, squinty eyes, ears retracted.

and hematocrit using point of care i-STAT CG8 + cartridge (Abaxis, Union City, CA, USA) were analyzed using a VetScan i-STAT 1 Handheld Analyzer (Abaxis, Union City, CA, USA). Blood lactate was measured using point of care analysis (Lactate Scout+, EKF Diagnostics GmbH, Magdeburg, Germany).

Locomotor Activity

Locomotor activity was measured using an omnidirectional accelerometer device (version 3.1, Actical®, Respironics, Koninklijke Philips Electronics, Bend, OR, USA) placed inside a specially designed case and attached to the dog's collar between 8 and 10 a.m., and taken off after final post-exercise 60-min measurements. The activity device was set to record activity counts with a 1-min epoch. The total activity data encompassed the warm up (5-min period of trotting/stretching before start of search exercise), exercise period, and the cooldown (5-min period of trotting/stretching after end of ball-retrieve exercise). The activity data files were batch processed using the Actical software (version 3.1, Actical®, Respironics, Koninklijke Philips Electronics, Bend, OR, USA). All epoch data were consolidated into a single Microsoft Excel file for alignment of activity count time-stamp with the manually recorded time of exercise.

Statistics

A descriptive and normality test (Kolmogorov–Smirnov) was used to determine if the data were normally distributed. Backwards-stepwise regressions were performed with stamina and change-from-baseline increase in CBT as the dependent variables; the independent variables included environmental, physiological, and hematological measurements taken for each dog on each trial day. A two-way ANOVA was used to test the effect of tracheobronchitis on post-exercise CBT and stamina. A Mann–Whitney Ranked Sum test was performed to measure the differences of temperature and humidity between the two experimental time periods dogs were tested in. Breed and gender were entered as categorical values. A linear mixed model was run where Time, Day, and Time × Day were entered in as fixed effects and animal ID was entered as a random effect. The *p*-values of least square means in **Tables 2** and **3** were generated from the linear mixed model.

RESULTS

Baseline and Post-exercise Physiological and Hematological Measures

Over the two experimental periods, outdoor temperature averaged 28.7 ± 0.7°C/83.7 ± 0.7°F, and outdoor humidity averaged 49.6 ± 1.5%. Mean duration of exercise was 27.1 min. There was no significant difference in outdoor temperature between the first (June 12–July 1, 2014) (median 29.2°C/84.5°F) and second (August 7–August 25, 2014) (median 28.9°C/84°F) experimental time periods ($p = 0.17$). There was a significant difference in humidity between the first (median 50%) and second (median 41%) experimental time periods ($p = 0.02$). Stamina, as measured by omnidirectional accelerometer device, reported 1,782.1 (1,498.4–2,028.9); total exercise activity counts per min epoch (median; IQR). Environmental and exercise measurements combined for the two experimental periods are shown in **Table 2**.

All dogs completed each trial day of the study. All pre-exercise baseline blood values were within reference ranges. Means and medians of pre- and post-exercise physiological measurements are listed in **Table 3**, whereas, means and medians of pre- and post-exercise hematological measurements are listed in **Table 4**. CBT, LET, RET, PR, pH, partial pressure of oxygen (PvO_2), oxygen saturation in peripheral venous blood (SvO_2), glucose, hematocrit, and hemoglobin all increased ($p < 0.001$) after exercise compared to pre-exercise baseline measurements. PvO_2, BEecf, bicarbonate (HCO_3^-), total carbon dioxide (TCO_2), and ionized calcium (iCal) decreased ($p < 0.001$) after exercise compared to pre-exercise baseline measurements. Mean change in pre- and post-exercise CBT was +1.8°C (4.4°F). Mean change in pre- and post-exercise LET was +2.5°C (4.9°F) and RET was +2.7°C (5.0°F).

TABLE 2 | Environmental and exercise measurements.

	Mean ± SE	Median; IQR
Temp (°C/°F)	28.7 ± 0.7/83.7 ± 0.7	28.9; 27.2/84; 81–86
Humidity (%)	49.6 ± 1.5	47; 40–57
Duration of exercise (min)	27.1 ± 0.3	27; 25–29
Average activity counts/min	1,807.8 ± 52.1	1,782.1; 1,498.4–2,028.9

Factors Related to Stamina

When controlling for breed, the following independent variables: outdoor temperature ($p = 0.005$), pre-exercise activity (p=0.008), post-exercise activity ($p < 0.001$), reduction in BEecf ($p = 0.044$), and decrease in TCO_2 ($p = 0.005$) were all significantly related to total activity (**Table 5**). The following independent variables were tested in this model, but were dropped from the model; age, sex,

TABLE 3 | Pre- and post-exercise physiological measurements.

	Pre-exercise		Post-exercise		*p*-Value
	Mean ± SE	Median; IQR	Mean ± SE	Median; IQR	
Pulse rate, beats/min	86.9 ± 3.3	84; 78–96	134.6 ± 3.3	132; 120–152	<0.001
Core body temperature, °C/°F	38.3 ± 0.3/101.0 ± 0.3	38.5; 38.3 38.8/101.3; 100.9–101.8	40.1 ± 0.1/105.4 ± 0.3	40.7; 40.3–41.4/105.3; 104.6–106.5	<0.001
Left ear temperature, °C/°F	37.3 ± 0.1/98.8 ± 0.4	37.2; 36.8–37.7/98.9; 98.2–99.9	39.8 ± 0.1/103.7 ± 0.4	39.9; 39.1–40.8/103.8; 102.3–105.4	<0.001
Right ear temperature, °C/°F	37.2 ± 0.2/98.9 ± 0.4	37.1; 36.8–37.7/98.8; 98.3–99.9	39.9 ± 0.1/103.9 ± 0.3	39.9; 39.1–40.8/103.8; 102.2–105.4	<0.001

TABLE 4 | Pre- and post-exercise hematological measurements.

	Pre-exercise		Post-exercise		*p*-Value
	Mean ± SE	Median; IQR	Mean ± SE	Median; IQR	
Lactate reading, mmol/L	1.2 ± 0.09	1.2; 1.0–1.4	1.4 ± 0.09	1.3; 1.0–1.7	0.090
pH	7.37 ± 0.02	7.37; 7.35–7.39	7.56 ± 0.02	7.54; 7.49–7.66	<0.001
$PvCO_2$, mmHg	37.5 ± 1.06	37.2; 34.2–40.2	20.0 ± 1.06	19.5; 15.1–24.5	<0.001
PvO_2, mmHg	45.4 ± 4.03	44; 34–52	70.4 ± 4.03	59; 46–78	<0.001
Base excess in extracellular fluid compartment, mmHg	−3.6 ± 0.48	(−)3.4; (−)5–(−)2	−4.8 ± 0.48	(−)5; (−)6.75–(−)3	<0.001
HCO_3, mmHg	21.5 ± 0.43	21.4; 19.7–23	17.3 ± 0.43	17.1; 15.9–18.7	<0.001
TCO_2, mmHg	22.8 ± 0.47	23; 21–25	17.8 ± 0.47	17; 16–19	<0.001
SvO_2, %	74.0 ± 2.2	78.5; 64–85	92.4 ± 2.2	94; 90–98	<0.001
Sodium, mmol/L	145.2 ± 0.32	145; 144–146	145.0 ± 0.32	145; 144–146	<0.001
Potassium, mmol/L	4.3 ± 0.04	4.3; 4.2–4.5	4.3 ± 0.04	4.3; 4.2–4.5	<0.001
Ionized calcium, mmol/L	1.4 ± 0.01	1.4; 1.4–1.5	1.3 ± 0.01	1.3; 1.2–1.3	<0.001
Glucose, mmol/L	89.9 ± 2.8	89.5; 82.3–97.0	98.3 ± 2.8	100; 93.3–105.0	<0.001
Hematocrit, %	43.1 ± 1.0	42; 40–47	45.5 ± 1.0	45; 43–48	<0.001
Hemoglobin, g/dl	14.7 ± 0.41	14; 13.6–16.1	15.5 ± 0.41	15.3; 14.6–16.3	<0.001

TABLE 5 | Regression coefficients of multiple regression for independent variables associated with total activity.

	Coefficient	*p*-Value
Dog breed	−94.081	0.008
Outdoor temperature (°C/°F)	−23.824	0.005
Pre-exercise total activity	0.149	0.008
Post-exercise total activity	0.616	<0.001
Reduction in Base excess in extracellular fluid compartment	69.325	0.044
Reduction in TCO_2	−62.995	0.005

outdoor humidity, left ear temperature, right ear temperature, PR, lactate, venous blood pH, gases ($PvCO_2$, HCO_3, PvO_2, SvO_2), electrolytes (iCa, Na, K), glucose, hemoglobin, and hematocrit. A two-way ANOVA was performed with stamina as the dependent variable. When controlling for breed, stamina was not influenced if the dog was affected by tracheobronchitis ($p = 0.67$).

Factors Related to Change-from-Baseline CBT

When controlling for breed and sex, regression analysis was performed with CBT as the dependent variable. Study day ($p = 0.005$), increase in PR ($p < 0.001$), increase in lactate ($p = 0.001$), reduction in BEecf ($p = 0.031$), increase in glucose ($p = 0.044$), increase in hematocrit ($p = 0.032$), and increase in hemoglobin ($p = 0.038$) were all significantly related to an increase in CBT.

Interaction of Time, Day, and Time × Day

A linear mixed model was run where Time, Day, and Time × Day were entered in as fixed effects and animal ID was entered as a random effect. This accounts for the repeated nature of the data. The effect of study day was significant for sodium (Na) on Day −4 vs. Day 3 ($p = 0.04$), and Day 3 vs. Day 11 ($p = 0.05$), and for hemoglobin on Day −4 vs. Day 15 ($p = 0.03$), Day 3 vs. Day 15 ($p = 0.01$), and Day 8 vs. Day 15 ($p = 0.039$). For the interaction of Time × Day, there was a significant difference for lactate on Day 8 vs. Day 3 pre-exercise ($p = 0.047$), for Na on Day 3 vs. Day −4 post-exercise ($p = 0.04$), for potassium on Day 11 vs. Day 3 post-exercise ($p = 0.04$), for PR on Day 15 vs. Day −4 post-exercise ($p = 0.49$), for pH on Day 15 vs. Day −4 post-exercise ($p = 0.03$), for Glu on Day 15 vs. Day −4 post-exercise ($p = 0.028$), for Hb on Day 15 vs. Day −4 post-exercise ($p = 0.035$), for pH on Day 15 vs. Day 3 post-exercise ($p = 0.02$), for Hb on Day 15 vs. Day 3 post-exercise ($p = 0.007$), for CBT on Day 15 vs. Day 8 post-exercise ($p = 0.04$), for pH on Day 15 vs. Day 8 post-exercise ($p = 0.04$), for Hb on Day 15 vs. Day 8 post-exercise ($p = 0.013$), for SvO_2 on Day 15 vs. Day 11 post-exercise ($p = 0.039$), for Glu on Day 15 vs. Day 11 post-exercise ($p = 0.036$), and for Hb on Day 15 vs. Day 11 post-exercise ($p = 0.013$) (**Table 6**).

DISCUSSION

This study evaluated the environmental and dog-specific variables that influence exercise stamina in regularly exercised working dogs. Stamina was defined as a two-component assessment of activity, as measured by an accelerometer and duration of exercise. Selection of working dogs can be based on specific parameters that include size, conformation, gait during exercise, past and current orthopedic history, body condition score, fitness level, and behavior (31). However, environmental and dog-specific factors that are associated with stamina have yet to be studied. This study uniquely shows that pre-exercise activity, post-exercise activity, outdoor temperature, reduction in both BEecf and TCO_2 are factors related to stamina.

The exercise challenge used in this study resulted in an increase in body temperature, lactic acidosis, respiratory alkalosis, and hypocapnia. The regression model defining factors associated with stamina included two biomarkers of alkalosis; BEecf and TCO_2, as stamina increased, BEecf and TCO_2 decreased. These findings are consistent with previous studies investigating the effect of intense, short-term exercise on canine hematology and physiology (2–4, 6, 7, 24, 28, 32). In contrast to endurance, this study of stamina found significant respiratory alkalosis with pH as high as 7.89 and an associated increase in PvO_2 and decrease in HCO_3^-. Glucose and hematocrit also increased significantly.

TABLE 6 | Linear mixed model of interactions between Time, Day, and Time × Day.

	Pre-exercise (mean ± SE)	Post-exercise (mean ± SE)	*p*-Value (pre vs. post)	*p*-Value (Day)	*p*-Value (Time × Day)
Core body temperature, °C/°F	38.3 ± 0.3/101.0 ± 0.3	40.1 ± 0.1/105.4 ± 0.3	<0.001		PE 8 vs. PE 15 $p = 0.04$
Left ear temperature, °C/°F	37.3 ± 0.1/98.8 ± 0.4	39.8 ± 0.1/103.7 ± 0.4	<0.001		
Right ear temperature, °C/°F	37.2 ± 0.2/98.9 ± 0.4	39.9 ± 0.1/103.9 ± 0.3	<0.001		
Pulse rate, beats/min	86.9 ± 3.3	134.6 ± 3.3	<0.001		PE 4 vs. PE 15 $p = 0.49$
Lactate reading, mmol/L	1.2 ± 0.09	1.4 ± 0.09	0.007		Pre 3 vs. Pre 8 $p = 0.04$
pH	7.37 ± 0.02	7.56 ± 0.02	<0.001		PE −4 vs. PE 15 $p = 0.03$ PE 3 vs. PE 15 $p = 0.02$ PE 8 vs. PE 15 $p = 0.04$
$PvCO_2$, mmHg	37.5 ± 1.06	20.0 ± 1.06	<0.001		
PvO_2, mmHg	45.4 ± 4.03	70.4 ± 4.03	<0.001		
Base excess in extracellular fluid compartment, mmHg	−3.6 ± 0.48	−4.8 ± 0.48	<0.001		
HCO_3, mmHg	21.5 ± 0.43	17.3 ± 0.43	<0.001		
TCO_2, mmHg	22.8 ± 0.47	17.8 ± 0.47	0.001		
SvO_2 %	74.0 ± 2.2	92.4 ± 2.2	<0.001		PE 11 vs. PE 15 $p = 0.04$
Sodium, mmol/L	145.2 ± 0.32	145.0 ± 0.32	0.39	Day −4 vs. Day 3 $p = 0.04$ Day 3 vs. Day 11 $p = 0.05$	PE −4 vs. PE 3 $p = 0.04$
Potassium, mmol/L	4.3 ± 0.04	4.3 ± 0.04	0.38		PE 3 vs. PE 11 $p = 0.04$
Ionized calcium, mmol/L	1.4 ± 0.01	1.3 ± 0.01	<0.001		
Glucose, mmol/L	89.9 ± 2.8	98.3 ± 2.8	<0.001		PE −4 vs. PE 15 $p = 0.03$ PE 11 vs. PE 15 $p = 0.04$
Hematocrit, %	43.1 ± 1.0	45.5 ± 1.0	<0.001		
Hemoglobin, g/dL	14.7 ± 0.41	15.5 ± 0.41	0.57	Day −4 vs. Day 15 $p = 0.03$ Day 3 vs. Day 15 $p = 0.01$ Day 8 vs. Day 15 $p = 0.039$	PE −4 vs. PE 15 $p = 0.04$ PE 3 vs. PE 15 $p = 0.01$ PE 8 vs. PE 15 $p = 0.01$ PE 11 vs. PE 15 $p = 0.01$

For the interaction of Time × Day, pre- vs. post-exercise on any given day was significant for all values except sodium (Na) and potassium (K). For the interaction of Day, only Na and hemoglobin were significant, all other values did not show a significant interaction.

An increase in glucose after short-term exercise has been reported in other studies (4, 7). Increased hematocrit is commonly associated with acute exercise, rather than endurance exercise. Similar to other studies (33, 34), strenuous exercise has been shown to cause an increase in panting resulting in a loss of $PvCO_2$ and a decrease in HCO_3^-. The exercise challenge used in this study demonstrated metabolic changes associated with intense exercise in high ambient temperatures. These changes are associated with limited stamina; therefore, strategies to enhance stamina will need to consider these physiologic factors.

The data reported above indicate that outdoor temperature, but not humidity, was the environmental variable that remained in the model, and inversely related to predicting stamina; as outdoor temperature increased, stamina decreased. Others have observed that high ambient temperatures acutely induce metabolic changes in exercising dogs, which is consistent with the current study's results (4, 6). Although humidity was significantly different between the two exercise time periods, this factor did not have an impact on stamina.

Since hyperthermia can limit performance and result in serious medical consequences, the identification of predictors of increased CBT could provide useful information for proper health care and management of exercising dogs. Dogs in this study had post-exercise CBTs ranging from 38.9 to 42.4°C/102–108.3°F (average 40.1°C/105.4°F), LETs ranging from 36.3 to 42.1°C/97.4–107.7°F (average 39.8°C/103.8°F), and RETs ranging from 35.6 to 42.4°C/96–108.4°F (average 39.9°C/103.9°F). An increase in CBT and PR leads to an increase in panting, resulting in a loss of pCO_2. Dogs are capable of increasing its carotid blood flow by 500% and cardiac output by 74–200% during exercise, conceivably to cope with the energy demands required for thermoregulation. The fixed factors of dog breed, sex, and age require further study in order to better understand thermoregulatory differences, particularly in light of the low numbers of certain breeds in this study. The independent variables included in the model that relate to an increase in CBT were the metabolic factors of increase in PR, lactate, glucose, hematocrit, and hemoglobin, as well as decrease in BEecf, which is consistent with the relationship of exercise-induced changes in hematological values in similar studies investigating the physiological effect of short-term strenuous exercise on dogs (2, 4, 7, 24, 35). Splenic contraction increases during brief exercise resulting in increased hematocrit levels. As hematocrit increases, oxygen carrying capacity increases driving increased oxygen delivery. Consistent with Steiss and Wright (4), there was a significant increase in glucose after exercise. An increase in blood glucose is associated with a physiological stress response coupled with increased exercise. An increase in BEecf is a consequence of increased panting, and finally an increase in PR is an expected outcome of increased activity and the associated increase in CBT (36, 37).

When controlling for breed and sex, the fixed factors that remained in the model predicting increase in CBT was study day. The inclusion of study day (absolute study day in 19-day exercise period) in the model suggests that dogs have the capability of acclimation to consistent exercise in hot ambient temperatures over 19 days; however, temperature and humidity did not remain in the model predicting increase in CBT, suggesting absolute temperature average 28.7°C/83.7°F and humidity average 49.6% do not play a significant role affecting thermoregulation during exercise in dogs. Heat acclimation has not been well studied in working dogs, and further investigation is required to fully understand this mechanism.

Pre-exercise activity and post-exercise activity were both included in our model as predictors of stamina. Dogs with higher pre-exercise activity demonstrated higher stamina during exercise. In addition, higher post-exercise activity was associated with higher stamina during exercise. An overlooked yet fundamental aspect of exercise training for a working dog is a pre-exercise warm up and a post-exercise cooldown (3). "The warm-up mildly increases the ambient temperature of muscles and joints, and lubricates fascia to reduce risk of injury, susceptibility to trauma, as well as allowing for a greater stretch" (38). "The increased musculoskeletal temperature causes local vasodilation, shifts the oxy-hemoglobin dissociation curve, and increases muscles contraction and relaxation speeds" (39, 40). One type of warm up for human, horses, and dogs, consists of 15 min exercise intensity that increase heart rate by about 70% (41). The cool-down exercises are low intensity and ensure that blood continues to circulate from the muscles to wash out the waste products of muscle metabolism (42). Pre-exercise activity could reflect warm up activity and post-exercise activity could reflect cooldown activity or this relationship can be explained by dogs that are active are active across all time periods (sometimes referred to as the behavior of high "drive"/motivation) have higher stamina. More research is required to clarify the impact of warm-up/cool-down on stamina. Although not studied here, increased warm-up/cool-down activity may lead to fewer injuries and less medical intervention for dogs during strenuous exercise.

A few dogs used in the current study demonstrated elevated CBTs as high as 42.4°C (108.3°F) with exercise. CBT above 40.6°C (105°F) has been historically defined as heat stroke (43). However, no dogs participating in this study experienced any of the clinical manifestations of heat stroke or heat injury. Our observations were similar to other reports of working dogs (3, 4). The dogs in the current study were regularly trained to work in a hot environment, thus were accustomed to exercising with increased CBTs. In addition, CBT recovered rapidly without intervention (unpublished data). For pet dogs not accustomed to training and conditioning in a warm climate, it is ideal for exercise to occur during the cooler part of the day to minimize exposure to warmer temperatures. More importantly, communication regarding the recognition of these signs to pet owners should aid in their awareness of the pet's condition when a thermometer is likely to not be readily available.

There were several important limitations in this study. The sample population was small and included all young, healthy dogs with experience in agility and search exercises, and accustomed to strenuous activity in high ambient temperatures. Although the study population only accounted for five different breeds with unequal representation in each breed, this independent variable of breed remained in the model as a significant factor associated with stamina, requiring the need of further investigation into the effect of breed and its relation to canine exercise. Two dogs did not regularly complete the ball-retrieve exercise and instead were trotted in a ball-retrieve fashion, which may have introduced a bias. Unlike in humans where fitness can be defined by VO_{2max}, there was no readily available quantitative measure of fitness in these dogs. Further investigation into the relationship between fitness status and stamina would be beneficial to understanding the cardiovascular and respiratory systems of working dogs. The order of dogs being tested was randomized unless medical reasons dictated otherwise. Several dogs were affected by tracheobronchitis, and on a separate occasion, one dog suffered from an abrasion on the lower left hind limb (did not occur as a result of the current study) and was rested one day before being tested again. Additionally, the thermometer used for measuring left and right ear temperatures tended to be sensitive to the user and positioning of the probe, resulting in measurements that were lower than expected. Finally, the interactions of Time, Day, and Time × Day were statistically significant, but not clinically significant. Day 15 had the most significant interactions with other study days; however, the Day 15 post-exercise average of total activity (1,777.2 count/min) and outdoor temperature (82.3°F) was lower than the average total activity and outdoor temperature of the study. This frequency of Day 15 interaction significance may be due to an accumulation effect of consistent exercise throughout the study period resulting in muscle soreness or fatigue.

In conclusion, when controlling for breed, increase in pre-exercise and post-exercise activity, high outdoor temperature, and respiratory alkalosis were significantly related to stamina. Working dogs are examples of superior athletes that can withstand working in hot ambient temperatures for short periods of time that include exercising with elevated CBTs as high as 42.4°C (108.3°F), pH values reaching 7.89, and $PvCO_2$ levels as low as 8.4 mmHg. Physical signs associated with exercise in hot ambient temperatures were used to terminate exercise in order to prevent heat injury. To evaluate relevant parameters for common working dogs (search, law enforcement, etc.), activity and duration of exercise were used to define stamina. Surprisingly, CBT did not influence stamina, rather respiratory efficiency or ability to eliminate heat without creating profound acid/base disturbances was a major factor influencing stamina. This study not only defined canine stamina, but investigated the physiological, hematological, respiratory, and cardiovascular factors, fixed factors such as age, sex, breed, and environmental factors of outdoor temperature and humidity, that are associated with stamina during exercise in high ambient temperatures.

LIST OF APPROVALS

Data Set Information

Name of data set: predictors of stamina in dogs exercising in high ambient temperatures
Name of database/repository: FigShare
Url link: https://figshare.com/articles/Stamina Manuscript Data_xlsx/3581457

The data collection period was from June 12, 2014 to August 27, 2014. Data were acquired using the methods listed in the Section "Materials and Methods." No filters were applied to the data set. Readers are able to use this data set in any capacity they may choose.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Animal Welfare Act and the University of Pennsylvania, Institutional Animal Care and Use Committee. The protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee protocol (805342) on 6/5/2014.

AUTHOR CONTRIBUTIONS

PR led the study data collection, participated in study design, data analysis, and manuscript preparation. MR participated in data collection and manuscript review. BZ participated in study design, data review, and manuscript review. CO oversaw the design of the study, assisted with data collection, and performed data analysis and manuscript preparation.

ACKNOWLEDGMENTS

The authors would like to acknowledge Nestlé Purina PetCare Company for funding this research, as well as the training and business staff of the University of Pennsylvania School of Veterinary Medicine Working Dog Center.

FUNDING

Funding for this study was provided by Nestlé Purina PetCare Company, located in St. Louis, MO, USA.

REFERENCES

1. Pierce BJ. Physical conditioning and injury prevention for the working dog. *J Vet Behav* (2009) 4:250–1. doi:10.1016/j.jveb.2009.05.019
2. Baltzer WI, Firshman AM, Stang B, Warnock JJ, Gorman E, McKenzie EC. The effect of agility exercise on eicosanoid excretion, oxidant status, and plasma lactate in dogs. *BMC Vet Res* (2012) 8:249. doi:10.1186/1746-6148-8-249
3. Steiss J, Ahmad H, Cooper P, Ledford C. Physiologic responses in healthy Labrador retrievers during field trial training and competition. *J Vet Intern Med* (2004) 18:147–51. doi:10.1111/j.1939-1676.2004.tb00153.x
4. Steiss JE, Wright JC. Respiratory alkalosis and primary hypocapnia in labrador retrievers participating in field trials in high-ambient-temperature conditions. *Am J Vet Res* (2008) 69:1262–7.
5. Goldberg MB, Langman VA, Taylor CR. Panting in dogs: paths of air flow in response to heat and exercise. *Respir Physiol* (1981) 43:327–38. doi:10.1016/0034-5687(81)90113-4
6. Kozlowski S, Brzezinska Z, Kruk B, Kaciubauschilko H, Greenleaf J, Nazar K. Exercise hyperthermia as a factor limiting physical performance – temperature effect on muscle metabolism. *J Appl Physiol* (1985) 59:766–73.
7. Wagner J, Horvath S, Dahms T. Cardiovascular, respiratory, and metabolic adjustments to exercise in dogs. *J Appl Physiol* (1977) 42:403–7.
8. Flandrois R, Lacour JR, Osman H. Control of breathing in the exercising dog. *Respir Physiol* (1971) 13:361–71. doi:10.1016/0034-5687(71)90040-5
9. Flandrois R, Lacour JR, Echlache JP. Control of respiration in exercising dog: interaction of chemical and physical humoral stimuli. *Respir Physiol* (1974) 21:169–80. doi:10.1016/0034-5687(74)90092-9
10. Hales JRS, Dampney RAL. The redistribution of cardiac output in the dog during heat stress. *J Therm Biol* (1975) 1:29–34. doi:10.1016/0306-4565(75)90008-X
11. Maskrey M, Jennings DB. Ventilation and acid-base balance in awake dogs exposed to heat and CO_2. *J Appl Physiol* (1985) 58:549–57.
12. Saibene F, Mognoni P, Aguggini G, Clement MG. Work of breathing in dog during exercise. *J Appl Physiol* (1981) 50:1087–92.
13. Szlyk PC, Pendergast DR, Krasney JA. Sinoaortic contribution to ventilator control in exercising dogs. *J Appl Physiol* (1983) 54:400–7.
14. Baker MA, Hawkins MJ, Rader RD. Thermoregulatory influences on common carotid blood flow in the dog. *J Appl Physiol* (1982) 52:1138–46.
15. Baker M, Chapman L. Rapid brain cooling in exercising dogs. *Science* (1977) 195:781–3. doi:10.1126/science.836587
16. Sanders M, White F, Bloor C. Cardiovascular responses of dogs and pigs exposed to similar physiological stress. *Comp Biochem Physiol* (1977) 55:365–70. doi:10.1016/0300-9629(77)90156-6
17. Bernabucci U, Lacetera N, Baumgard LH, Rhoads RP, Ronchi B, Nardone A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* (2010) 4:1167–83. doi:10.1017/S175173111000090X
18. Collier RJ, Beede DK, Thatcher WW, Israel LA, Wilcox CJ. Influences of environment and its modification on diary animal health and production. *J Dairy Sci* (1982) 65:2213–27. doi:10.3168/jds.S0022-0302(82)82484-3
19. Baker MA. Cardiovascular and respiratory responses to heat in dehydrated dogs. *Am J Physiol* (1984) 246:369–74.
20. Cerretelli P, Piiper J, Mangili F, Cutica F, Ricci B. Circulation in exercising dogs. *J Appl Physiol* (1964) 19:29–32.
21. Cherniack NS, Longobardo GS. Oxygen and carbon dioxide gas stores of the body. *Physiol Rev* (1970) 50:196–243.
22. Ferasin L, Marcora S. Reliability of an incremental exercise test to evaluate acute blood lactate, heart rate and body temperature responses in Labrador retrievers. *J Comp Physiol B* (2009) 179:839–45. doi:10.1007/s00360-009-0367-z
23. Graham TE, Sinclair DG, Chapler CK. Metabolic intermediates and lactate diffusion in active dog skeletal muscle. *Am J Physiol* (1976) 231:766–71.
24. Matwichuk C, Taylor S, Shmon C, Kass P, Shelton G. Changes in rectal temperature and hematologic, biochemical, blood gas, and acid-base values in healthy Labrador retrievers before and after strenuous exercise. *Am J Vet Res* (1999) 60:88–92.
25. Piccione G, Casella S, Panzera M, Giannetto C, Fazio F. Effect of moderate treadmill exercise on some physiological parameters in untrained beagle dogs. *Exp Anim* (2012) 61:511–5. doi:10.1538/expanim.61.511
26. Proscurshim P, Russo AK, Silva AC, Piçarro IC, Freire E, Tarasantchi J. Aerobic training effects on maximum oxygen consumption, lactate threshold and lactate disappearance during exercise recovery of dogs. *Comp Biochem Physiol A Comp Physiol* (1989) 94:743–7. doi:10.1016/0300-9629(89)90627-0
27. Rovira S, Munoz A, Benito M. Hematologic and biochemical changes during canine agility competitions. *Vet Clin Pathol* (2007) 36:30–5. doi:10.1111/j.1939-165X.2007.tb00178.x
28. Rovira S, Munoz A, Benito M. Effect of exercise on physiological, blood and endocrine parameters in search and rescue-trained dogs. *Vet Med* (2008) 53:333–46.
29. Laflamme DP. Development and validation of a body condition score system for dogs. *Canine Pract* (1997) 22:10–5.
30. Angle TC, Gillette RL. Telemetric measurement of body core temperature in exercising unconditioned Labrador retrievers. *Can J Vet Res* (2011) 75:157–9.

31. Marcellin-Little D, Levine D, Taylor R. Rehabilitation and conditioning of sporting dogs. *Vet Clin North Am Small Anim Pract* (2005) 35:1427–39. doi:10.1016/j.cvsm.2005.08.002
32. Young D, Mosher R, Eerve P, Spector H. Body temperature and heat exchange during treadmill running in dogs. *J Appl Physiol* (1959) 14:839–43.
33. Hastings AB, White FC, Sanders TW, Bloor CM. Comparative physiological response to exercise stress. *J Appl Physiol* (1982) 4(52):1077–83.
34. Musch TI, Friedman DB, Haidet GC, Stray-Gundersen J, Waldrop TG, Ordway GA. Arterial blood gases and acid-base status of dogs during graded dynamic exercise. *J Appl Physiol* (1986) 61(5):1914–9.
35. Spoo JW, Zoran DL, Downey RL, Bischoff K, Wakshlag JJ. Serum biochemical, blood gas and antioxidant status in search and rescue dogs before and after simulated fieldwork. *Vet J* (2015) 206(1):47–53. doi:10.1016/j.tvjl.2015.07.002
36. Higgins EA, Iampietro PF. Thermal panting and initiation of respiratory alkalosis. *Can J Physiol Pharmacol* (1967) 45:1. doi:10.1139/y67-001
37. Hall EJ. *Guyton and Hall Textbook of Medical Physiology, Chapter 9*. Philadelphia, PA: Saunders Elsevier (2016). p. 109–22.
38. Shellock FG, Prentice WE. Warming-up and stretching for improved physical performance and prevention of sports-related injuries. *Sports Med* (1985) 4:267–78. doi:10.2165/00007256-198502040-00004
39. Franklin ME, Currier DP, Franklin RC. The effect of one session of muscle soreness-inducing weight lifting exercise on WBC count, serum creatine kinase, and plasma volume. *J Orthop Sports Phys Ther* (1991) 13:316–21. doi:10.2519/jospt.1991.13.6.316
40. Tyler CM, Golland LC, Evans DL, Hodgson DR, Rose RJ. Skeletal muscle adaptations to prolonged training, overtraining and detraining in horses. *Eur J Physiol* (1998) 436:391–7. doi:10.1007/s004240050648
41. Mukai K, Hiraga A, Eto D, Takahashi T, Hada T, Tsubone H, et al. Effects of warm-up intensity on oxygen transport during supramaximal exercise in horses. *Am J Vet Res* (2008) 69:690–6. doi:10.2460/ajvr.69.5.690
42. Takahashi T, Okada A, Hayano J, Tamura T. Influence of cool-down exercise on autonomic control of heart rate during recovery from dynamic exercise. *Front Med Biol Eng* (2001) 11:249–59. doi:10.1163/156855701321138914
43. Drobatz KJ, Haskins SC, Macintire DK, Saxon WD. *Manual of Small Animal Emergency and Critical Care Medicine*. Hoboken, NJ: Wiley-Blackwell (2012).

Conflict of Interest Statement: The study was funded by Nestle Purina Research. The final manuscript was reviewed by Nestle Purina. The manuscript does not contain any commercial product or potential for conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 26 October 2017
doi: 10.3389/fvets.2017.00174

Evaluation of Three Hydration Strategies in Detection Dogs Working in a Hot Environment

Cynthia M. Otto[1,2], Elizabeth Hare[2,3], Jess L. Nord[1,2], Shannon M. Palermo[1], Kathleen M. Kelsey[2], Tracy A. Darling[1,2], Kasey Schmidt[4] and Destiny Coleman[1]*

[1] Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, United States, [2] Penn Vet Working Dog Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, United States, [3] Dog Genetics, LLC, Sunnyside, NY, United States, [4] College of Veterinary Medicine, Washington State University, Pullman, WA, United States

OPEN ACCESS

Edited by:
Mary M. Christopher, University of California, Davis, United States

Reviewed by:
Joseph Wakshlag, Cornell University, United States
Anna Katharine Shoveller, University of Guelph, Canada

***Correspondence:**
Cynthia M. Otto
cmotto@vet.upenn.edu

Specialty section:
This article was submitted to Veterinary Humanities and Social Sciences, a section of the journal Frontiers in Veterinary Science

Received: 03 November 2016
Accepted: 02 October 2017
Published: 26 October 2017

Citation:
Otto CM, Hare E, Nord JL, Palermo SM, Kelsey KM, Darling TA, Schmidt K and Coleman D (2017) Evaluation of Three Hydration Strategies in Detection Dogs Working in a Hot Environment. Front. Vet. Sci. 4:174. doi: 10.3389/fvets.2017.00174

Physical activity in hot environments can increase the risk of heat stress or heat stroke in dogs. Heat tolerance is influenced by acclimatization to the environment, physical fitness, and hydration state. Three common strategies to promote hydration in working dogs are free access to water (W), oral electrolyte solutions (OESs), and administration of subcutaneous fluids (SQs). None of these methods have been compared for safety or efficacy in a working environment. In a cross-over design, seven vehicle-screening canines were randomly assigned to each of the three hydration strategies during working shifts at the Sarita, TX checkpoint. Physical, behavioral, and biochemical parameters were collected before, during, and after a work shift (mean 5.7 ± 0.8 h). Dogs were given 10 mL/kg oral W, 10 mL/kg chicken flavored OES, or 15 mL/kg of SQs initially followed by controlled access to W or OES. The dogs drank 15.61 ± 4.47 mL/kg/h of W and OES when in the OES group, compared to 7.04 ± 3.42 and 5.56 ± 4.40 mL of W, for the W and SQ groups, respectively. The median environmental temperature was 84.8°F (29.3°C). The median humidity was 70%. Based on mixed effects linear modeling, dogs in the OES and SQ groups had significantly higher total CO_2, and lower packed cell volume and total plasma protein at the end of the day. Creatinine increased a small but significant amount in the SQ group and decreased in the OES group. Searching behaviors were independent of hydration strategy but highly related to the dog specific factors of sex, breed, and activity level. Under conditions of controlled activity in moderate heat and humidity, dogs accustomed to the work and the environment were more likely to increase fluid consumption and hydration when provided a flavored OES. Potential benefits of OES and SQ were indirect and no adverse effects were documented for any of the hydration strategies tested.

Keywords: canine, hydration, search, behavior, sodium, electrolytes, heat stress

INTRODUCTION

Military and other working dogs (e.g., police, search-and-rescue) are critical to protect our national security and respond to disasters. Working dogs are expected to perform physically and mentally demanding tasks often in adverse environmental conditions. Many working dogs are highly motivated to perform the tasks of searching or criminal apprehension and may exert themselves to the point of severe dehydration, collapse, heat stress, and even heat stroke (1–4). One of the most common potentially preventable causes of death in military working dogs is heat stroke (2). The risk of heat stroke increases with dehydration (5). Dehydration can be a complication of these intense working environments. Even in the moderate temperatures that occurred in September of 2001, handlers reported dehydration in the search dogs working at the World Trade Center and Pentagon (6). Dehydration was the most common medical finding in dogs deployed to the 2010 earthquake in Haiti (7). Austere environments, such as war zones in the Middle East and border regions in the southern US, further increase the risk of dehydration.

Since dogs have minimal sweating capacity, thermoregulation relies primarily on evaporative mechanisms through panting (8). Heat, humidity, and hydration are thought to impact the dog's ability to thermoregulate (4, 9, 10). High ambient temperatures decrease the heat gradient from the dog to the environment and add external heat to the dog. Humid conditions impair effective evaporative cooling. Humid environments, such as those experienced in response to Hurricane Katrina and in US Border regions along the Gulf Coast, can contribute to heat stress. In addition to the environmental factors of heat and humidity, the dog's adaptation to environmental conditions (also called acclimatization), overall fitness/conditioning and state of hydration are thought to be major factors that impact heat tolerance (11). Canine thermoregulation involves the inhalation of cooler, dryer air through the nose and mouth, which causes evaporative heat loss from the nasal and oral mucosa and tongue, and the exhalation of hotter, moister air. In conditions in which the rate of heat generation is greater than the rate of heat dissipation, increased salivation and lingual blood flow allow for convection and evaporative cooling (12). Evaporative cooling results in electrolyte-free water loss (estimated at ~10 mL/kg/h) which can contribute to dehydration (13). Most studies of working or hunting dogs have shown small or no electrolyte changes following exercise (4, 14, 15). In one study of sled dogs, sodium was significantly decreased following 10 days of endurance racing (16, 17). Because dogs do not sweat, any electrolyte loss would be through saliva or urine. Salivary Na and Cl are lower than the serum, and salivary bicarbonate, K, and Ca are higher (18). In hydrated dogs, salivary loss is estimated at 7 mL/kg/h during exercise (13). In dehydrated dogs, the amount of water lost to cooling was approximately 7% lower than in hydrated dogs, but salivation was reduced by over 90% (13). Increased urinary sodium loss was hypothesized in hyponatremic sled dogs despite an inability to document it at the sampling time after the race (15). As dehydration increases, the availability of water for evaporation, salivation, and circulation will be diminished; heat tolerance and physical, mental, and olfactory performance will be reduced.

Conditioned human athletes are better able to tolerate heat than non-athletic humans (19). Oxygen utilization (VO_2max) is a classic measure of cardiovascular fitness. In dogs, VO_2max measurement has been limited to research studies (20, 21). Lactate threshold has been used as a surrogate for VO_2max, but is not widely utilized in canine exercise physiology (21). A 6-min walk test has been used to differentiate obese dog cardiopulmonary performance from lean dogs, but did not discriminate between overweight and lean dogs (22). In an exercise conditioning study of police dogs, conditioned dogs had higher post exercise temperatures and lower creatine kinase and aspartate aminotransferase (AST) (23). There is no standard test to define fitness in canine athletes in the field or evaluate the impact of physical conditioning on heat tolerance in dogs. Both acclimatization and conditioning are long-term management strategies that should be considered, though in the acute setting, manipulating hydration is the most appealing approach to prevent dehydration and increase heat tolerance and workability.

Hydration strategies employed may vary with the location and the nature of the mission. Currently, dog handlers commonly use oral water (W), subcutaneous fluids (SQs), and oral electrolyte solutions (OESs) to prevent dehydration. Neither the safety nor efficacy of these hydration strategies has been previously evaluated. The aim of this study was to evaluate the effects of three hydration strategies on hydration status and performance in Border Patrol dogs screening vehicles on the Texas border in the summer. We hypothesized that there would be no difference between the OES, W, and SQ groups for hydration parameters or adverse effects.

MATERIALS AND METHODS

Animals

Seven Border Patrol dogs working for Customs and Border Protection at the Sarita, Texas border station (Rio Grande Valley Sector) were selected based on canine and handler availability during the experimental period. All dogs were trained to screen vehicles and to alert to both narcotics and concealed live humans. All dogs worked, trained, and were kenneled at the same facility, and all lived with their handlers. All dogs were deemed healthy and in good condition based on physical examinations. The age, breed, sex, neuter status, physical examination parameters, and history were collected from the handlers on all dogs.

Experimental Design

All protocols and the study were approved by the University of Pennsylvania and US Army Medical Research and Materiel Command Institutional Animal Care and Use Committees (U Penn IACUC protocol 804293, USAMRMC proposal SO120002) and all experimental procedures were conducted in accordance with the recommendations of the committees. In this field study where dogs were working their normal assigned shifts, each dog was randomized independent of the other dogs to account for differences in work schedules. Dogs were randomly assigned to one of three treatment protocols on each of 3 days of study participation. Study days were limited to Monday, Tuesday, Wednesday, and Thursday to eliminate the uncontrolled variable of higher traffic on weekends. Only dogs scheduled for duty between 7:00 a.m. and 2:00 p.m. or between 9:00 a.m. and 3:00 p.m. were included. During this time period, dogs typically worked 30 min then rested 30 min. Dogs were studied in groups of 2 or 3 dogs on the same day, depending on handler schedule. Based on a pilot study performed with the Philadelphia Police Canine Unit, dogs initially received 10 mL/kg oral W, 10 mL/kg OES (Hydrolyte, Advanced Nutritional Support, Elka Park, NY, USA), or 15 mL/kg of SQs (Plasmalyte A, Abbott Laboratories, North Chicago, IL, USA) initially. See **Table 1** for the electrolyte composition of the supplements. Every 30 min, whether they were working or resting, dogs in each treatment group were offered 10 mL/kg of W. If the dogs in the OES group drank less than 3 mL/kg of water, they were offered 10 mL/kg of OES. W and OES were kept in an air-conditioned room prior to offering it to the dogs. The dogs in the W and SQ groups were only offered water. If the dogs consumed the entire 10 mL/kg of water before the end of the 30-min time interval, they were offered a second 10 mL/kg bowl.

The time of each hydration interval and study activity was recorded for each dog. If any dog exhibited signs of physical distress (i.e., anxiety, aggression, lethargy, unwillingness to work) and/or was unable to maintain adequate hydration, it was to be removed from the study and treated appropriately.

Data Collection

Timing of Data Collection

A physical examination was performed on each dog at the beginning of each day. Body weight, temperature, pulse, respiratory rates, urine, and blood samples were obtained at the beginning, middle, and end of each work shift.

Fluid volume consumed, internal body temperature, heart rate and rhythm, qualitative assessments of activity, urination, and defecation, as well as ambient temperature, percent humidity, and wind speed, were measured every 30 min.

Dog Fluid and Food Intake and Urination/Defecation

The fluid consumption was measured based on the remaining fluid left in the bowl. The bowls were held as each dog drank to minimize spillage, however there was no way to account for small amounts of fluid that was splashed by the dog. The total fluid volume, fluids administered for the SQ group, water intake for the SQ and W groups, and water plus OES for the OES group, was recorded for each dog for each study day and normalized to the body weight and duration of work (mL/kg/h). Sodium load was calculated based on the sodium content of each fluid (**Table 1**) and the volume of each fluid. A subjective measure of urine volume (increased, decreased, or normal) and fecal characterization based on the handler's knowledge of his dog's normal elimination patterns and fecal scoring using the 1–7 score with accompanying photographs of examples (24, 25) was recorded for each dog every 30 min during each study day. A veterinarian was on site to confirm any reported abnormal fecal scores during the work shift. Dogs were maintained on their normal feeding schedule; they were not fed during the work day, except for a small amount of canned dog food associated with the ingestion of the internal temperature sensing capsules of the CorTemp® system (HQInc Wireless Sensing Systems & Design; Palmetto, FL, USA).

TABLE 1 | Measured and reported electrolyte composition of oral electrolyte solution (OES) and subcutaneous fluids (SQ).

Ingredient	OES measured	SQs reported
Sodium (mmol/L)	152	140
Potassium (mmol/L)	7.1	5
Chloride (mmol/L)	109	98
Buffer	Bicarbonate 25 mmol/L	Acetate 27 mEq/L Gluconate 23 mEq/L
Magnesium (mmol/L)	2.3	1.5
Glucose (mmol/L)	13.7	0
Osmolality (mOsm/L)	332[a]	294
Effective strong ion difference (mEq/L)[b]	50	47

[a]*Osmolality was calculated as 2([Na^+] + [K^+]) + ([glucose]/18), where brackets represent concentration.*

[b]*Effective strong ion difference = [Na^+] + [K^+] − [Cl^-].*

Dog Activity

Quantitative activity counts were monitored using omni-directional accelerometers (ActiCal, Philips Respironics; Murrysville, PA, USA) (26). During the work day, each dog wore an activity monitor programmed with its identity. The activity monitors were secured to standard flat buckle collars upon arrival onsite each morning and removed after the end of each day's final search. At the end of each study day, data were transferred from the monitors to a computer using the ActiReader data-downloading device. Total activity counts were normalized for hours of work.

Qualitative activity levels and location of activity were recorded every 30 min based on an expected cycle of search and rest. If any other activity, such as a secondary search, was performed, or a subject performed more than one activity in the 30-min interval, the activities were noted, accompanied by the approximate time spent performing each activity.

Internal Body Temperature

Internal (gastrointestinal) body temperature was monitored using the CorTemp® system (27). On the first day of participation in the study and after morning weight was obtained, each dog was administered a non-toxic ingestible core body thermometer pill placed in approximately 5 tablespoons of canned dog food (Pedigree, Mars Global Pet Care, Franklin, TN, USA). In the mornings of subsequent study days, dogs were scanned for the CorTemp® capsule prior to being offered another capsule. If the original capsule was present in the dog and transmitting appropriately, a new capsule was not administered until the old capsule passed. Body temperatures were recorded every 30 min with a handheld CorTemp® Data Recorder. If a reading could not be obtained due to capsule or recorder error, rectal temperatures were taken instead. At the end of the study, CorTemp® data were downloaded from the Data Recorder onto a computer using the CorTrack™ II software. For analysis, the difference between the values collected at baseline and at the end of the day was utilized.

Heart Rate and EKG

Heart rates and EKGs were monitored using the AliveCor iPhone 4 s phone case and iPhone app (AliveCor, Inc., San Francisco, CA, USA) as validated for cats and dogs (28). Heart rates were recorded every 30 min with the AliveCor device. The electrodes on the phone made contact via isopropyl alcohol with the thorax between rib spaces 3–7 for approximately 10–60 s to obtain an accurate heart rate and EKG recording. Any cardiac abnormalities were noted. If an EKG reading was unable to be obtained, pulse or heart rates were obtained by femoral pulse palpation and/or cardiac auscultation. For analysis, the difference between the values collected at baseline and at the end of the day was utilized.

Body Weight, Blood Samples, and Urine Measurements

Weight in kilograms was obtained using a walk-on electronic scale (Jorvet J0825PM, JorVet Walk on Scale 36″; Jorgensen Labs, Loveland, CO, USA) that was calibrated twice daily. Blood samples (3 mL) were obtained from a peripheral or jugular vein and were directly analyzed or anticoagulated in a Li heparin vacutainer for use in an ISTAT CHEM8+ to measure sodium (Na^+), potassium (K^+), chloride (Cl^-), ionized calcium (iCa), total CO_2 (TCO_2), glucose (Glu), blood urea nitrogen (BUN), creatinine (Creat), hematocrit (Hct), hemoglobin (Hb), and anion gap (Abaxis veterinary research laboratories, Union City, CA, USA). Lactate blood levels were analyzed directly using a handheld lactate meter (Lactate Scout, EKF Diagnostics, Penarth, Cardiff, UK). All remaining blood was placed in an EDTA tube and four samples were removed and placed in microhemotocrit tubes and centrifuged at 11,000 rpm for 3 min (LW Scientific ZIPocrit Centrifuge, Lawrenceville, GA, USA) to obtain packed cell volume (PCV). A handheld refractometer, was used to determine total protein (TP). All remaining blood in the EDTA tube was centrifuged at 3,150 rpm for 5 min and the plasma was collected and frozen for future analysis. Urine specific gravity (USG) was measured onsite with a handheld refractometer and urine microalbumin was measured with a commercially available semiquantitative test strips (Heska E.R.D. Healthscreen Canine Urine Tests, Heska, Loveland, CO, USA) on free catch midstream urine samples. Urine samples were stored at 4°C for sodium and Creat analysis by the Vitros 4600 Chemistry System (Ortho Clinical Diagnostics, Raritan NJ). Briefly, Creat is hydrolyzed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sacrosine, in the presence of sarcosine oxidase, is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of leuco dye to produce a colored product. The resulting change in reflection density is measured at two time points and the difference in reflection density is proportional to the concentration of Creat present in the sample. For Na, the slide consists of two ion-selective electrodes, each containing methyl monensin (an ionophore for sodium), a reference layer and a silver layer and a silver chloride layer coated on polyester support. A drop of patient sample and a drop of VITROS reference fluid on separate halves of the slide results in migration of both fluids toward the center of the paper bridge. The liquid enters the slides and a stable liquid junction is formed that connects the reference electrode to the sample electrode. Each electrode produces an electrochemical potential in response to the activity of Na. The potential difference between the two electrodes is proportional to the Na concentration in the sample. For analysis, the difference between the values collected at baseline and at the end of the day was utilized.

Fractional excretion of sodium was calculated as described by Hinchcliff (16)

$$FC_{Na} = \left(U_{Na} * S_{creat}\right) / \left(S_{Na}\, U_{creat}\right) * 100,$$

where U_{Na} and S_{Na} are the concentrations of sodium in urine and serum, respectively, and S_{creat} and U_{creat} are the concentrations of Creat in serum and urine, respectively.

Odor Detection Performance

A standardized search problem was set up at the beginning and end of each workday (see **Figure 1**; Video S1 in Supplementary Material depicting a training session in which a dog and a handler team conduct a detailed vehicle search). A training aid containing a scent that the dog was trained to find or a live human was hidden in a vehicle. The training aid/human was in the same location for all dogs at a single time point on a given day, but the location was different on each day and different in the morning and the evening to minimize handler influence. The search was set up to mimic the format of a secondary road search of a parked vehicle at the checkpoint. The distance from the starting point to the middle of the car in which the training aid was hidden was 30 feet, and this information was not provided to the handlers. Starting points were adjusted based on the location of the training aid. The number of cars and/or other obstacles varied from day to day but contained no less than 1 vehicle and no more than 3 vehicles. The handler was not informed of the location of the training aid. The time from initiation of search at the starting point to the time of indication was recorded. Any false alerts or behavior changes were recorded. The location of the training aid was selected by the canine instructor, and the training aid was placed at least 5 min prior to the search start. Each search was recorded using both a fixed and a handheld video camera (Sony Handicam Camcorder HDR-CX380/B, Sony Corp., New York, NY, USA). Ambient temperature, humidity, wind speed, and wind direction were recorded with both a handheld and wireless device (Extech Anemometer/Thermometer/%Humidity, Extech Instruments, Nashua, NH, USA; and AcuRite Wireless Weather Station; Primex Family of Companies, Lake Geneva, WI, USA). The video recordings were reviewed using Noldus Observer Software (Noldus Information Technology, Leesburg, VA, USA) by a single reviewer that had not participated in the field study. The duration of behaviors was recorded. The behavioral ethogram (see **Table 2**) was derived from different ethograms evaluating stress in dogs under various environments (29–31). Each behavior was reported as the proportion of the search time in which it was observed. For analysis, the frequency of behaviors in the morning search for each dog was subtracted from the frequency of that dog's behaviors in the afternoon search.

Statistical Analysis

Descriptive data were visually inspected and tested for normality using Kolmogorov–Smirnov test in a commercial statistics package (SigmaPlot 11.0, Systat Software; Chicago, IL, USA). Data were reported as mean and SD for normally distributed data or median and range for nonparametric data.

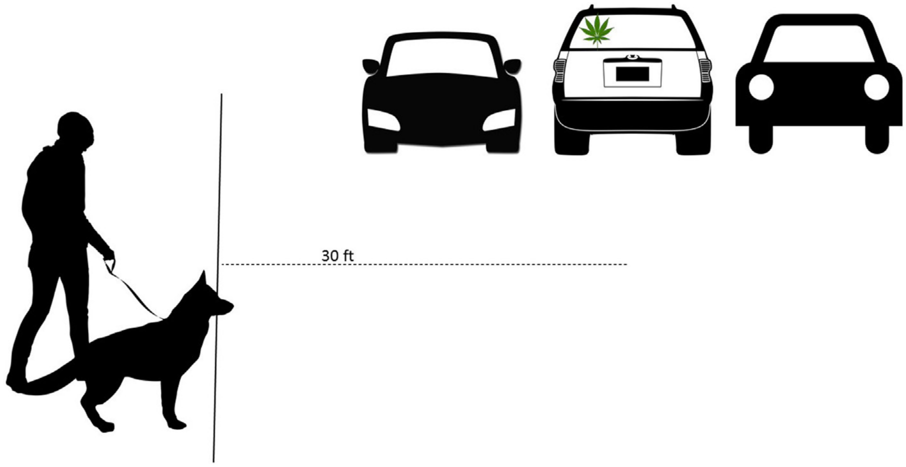


FIGURE 1 | A diagram representing the standardized search activity. The handler and the dog were positioned 30 feet from the vehicle containing the concealed training aid (signified by the marijuana leaf). The dog searched in a detailed fashion on leash until it alerted to the training aid.

TABLE 2 | Behaviors recorded.

Ear position	Up Down
Ear state	Relaxed Alert
Tail position	Tail up Tail down
Tail movement	Wagging Stiff Relaxed movement
Mouth state	Relaxed Taut
Mouth position	Open Closed
Mouth activity	Licking Panting
Tongue position	In mouth Out to side Out straight
Canine focus	On handler On search
Post-search reward	Tug play Other reward
Interactions with canine	Cueing the location of the aid No reward for alert Successful alert and reward Dog jumping up on handler during reward Placing feet up on handler during reward Loose grip on the reward tug toy Strong grip on the reward tug toy

Mixed-effects linear models were fit using the lmer function in the lme4 (32) package in the open-source R statistical software package which is available, along with supplementary packages, at https://cran.r-project.org. This function fits mixed models to data with crossed factors and is robust to deviations from the normal distribution. It estimates variance parameters for a data set using an iterative maximum likelihood procedure that searches for a set of variance estimates that have the highest probability (likelihood) of producing the data (33).

The serum chemistry dependent variables tested were lactate, sodium, potassium, chloride, iCa, total bicarbonate, Glu, Creat, and anion gap. The hematology-dependent variables tested were hematocrit, PCV, TP, and Hb. Other dependent variables tested were the change in weight, change in pulse, and fractional excretion of sodium over the day of work. For the full model, fixed factors included hydration strategy, sex, and breed and individual ID was a random factor. Covariates included activity count (counts/h), dog's change in weight (kg), age in months, change in pulse (maximum–minimum), liquid intake (mL/kg/h), change in USG (morning–afternoon), average daily ambient temperature (F), and average daily humidity. Age was fitted as a covariate to account for variation between dogs and because covariates provide more statistical power than age categories fitted as fixed effects. For the dependent variable fractional excretion of sodium, an additional covariate, sodium load, was included in the model, and intake of liquid including SQ fluids was used (mg/kg/h). To address warnings from the maximum likelihood procedure about variables being of different scales, activity counts/h was divided by 10,000, age in months was divided by 10, and USG was multiplied by 100.

The drop1 function was used to assess the significance of each fixed factor and covariate in contributing to the fit of the model. drop1 fits a set of models with each model missing one of the variables present in the original model. The significance of a variable is determined by a chi-square test of the likelihoods of the new model (missing the relevant variable) relative to the original model. Fixed factors and covariates with $p < 0.05$ for the chi-square test were included in a refined model. This process was continued until all the variables were significant according to drop1, with a final model fitted including the significant drop1 variables from the previous model. The reported p-values for variables were obtained using the ANOVA function in the "car" package. The pairs function of the lsmeans package in R was used to obtain contrasts of hydration methods and confidence intervals for hydration strategies when they contributed significantly to the fit of the model. The baseline value for the contrasts of hydration strategies was W so that both OES and SQ strategies could be compared with the method most commonly in used. R's summary function was used for each refined model to determine the regression coefficient and significance of factors such as hydration strategy.

The same model selection method was used to model fractional excretion of sodium except that total fluid, including liquid administered subcutaneously replaced total liquid intake, and sodium load was included as an additional covariate.

Mixed effects models were also used to determine whether hydration strategy was associated with scores on behavior measures such as ear, tail, mouth position, attention, and interactions with the handler. Models for behavior scores included hydration method, sex, and breed as fixed factors, change in body weight, activity level, ambient temperature, and humidity as covariates, and individual as a random factor. The same procedure of dropping variables from the models and retaining significant ones was used to arrive at final models.

Based on the mixed model, fractional excretion of sodium was not influenced by dog or by treatment strategy; therefore, data were combined for all dogs and all groups. The effect of time of day was evaluated using Mann–Whitney rank sum test.

RESULTS

Three females and four males participated. Two females were spayed Belgian Malinois, and one female was a spayed German Shepherd Dog. Of the males, two were neutered Belgian Malinois (one short coated, one long coated), and the intact males included a Belgian Malinois and a German Shepherd Dog. All dogs were between the ages of 3 and 8 years of age, with a mean age of 5.5 ± 1.9 years. Mean weight was 30.6 ± 4.3 kg.

The mean internal body temperature across all test days was 101.9 ± 1.3°F (38.8 ± 0.7°C). The maximum temperature recorded was 104.5°F (40.3°C) and only five recordings (representing three dogs) were ever greater than 103.0°F (39.4°C). The median heart rate was 100 beats/min with a range of 96–141. No arrhythmias were detected. During the majority of examinations, dogs were panting.

Daily examinations did not find any medical problems that precluded work for any of the dogs. All dogs completed each study day. One dog ended one study day early due to the handler's personal emergency. No dog had abnormal stool consistency. Of the 97 reports on urine production, the majority (54) described normal volume, 32% were decreased volume and 11% were increased volume. Each of the three hydration strategies was represented in each of the subjective assessments of urine volume. Mean USG prior to commencing with work was 1.031 ± 0.014; whereas mean USG at midday was 1.022 ± 0.013 and at the end of the day median USG was 1.027 (range 1.003–1.042). No microalbumin was detected in any urine sample at any time. Other than the food associated with delivery of the internal temperature sensing capsule, no dog was fed during the work shift. The mean and SD of the chemistry, hematologic, urine, and physical variables as a function of time and group can be found in **Tables 3–6**.

The dogs worked a mean of 5.7 ± 0.8 h per day and their mean activity was 44,975 ± 10,552 counts/kg/h. The dogs spent 15.6% of their time with activity levels consistent with rest (<204 counts/min), 66.7% of their time consistent with walking (activity counts > 204 and <1,751) and 17.7% of their time at a trot or more intense level of activity (>1,751 counts/min) (34) (**Figure 2**). During the study days the median environmental temperature was 84.8°F (29.3°C) (range 74.0–99.9°F; 23.3–37.7°C). The median humidity was 70% (range 39–100%) with a median wind speed of 5.6 mph (range 0–18°mph).

TABLE 3 | (A) Plasma chemistry results as a function of time and treatment group in the water (W) group, (B) plasma chemistry results as a function of time and treatment group in the oral electrolyte solution (OES) group, and (C) plasma chemistry results as a function of time and treatment group in the subcutaneous fluids (SQs) group.

	AM		Midday		PM	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
A	**W**					
Lactate (mmol/L)	0.119	0.033	0.095	0.038	0.088	0.018
Na^+ (mmol/L)	146.14	1.95	146.00	0.82	146.57	2.15
K^- (mmol/L)	4.04	0.45	3.96	0.3	3.91	0.46
Cl^- (mmol/L)	117.14	2.34	117.57	2.07	117.57	2.64
iCa (mmol/L)	0.348	0.023	0.343	0.023	0.338	0.023
TCO_2 (mmol/L)[a]	19.6	2.6	18.7	1.7	17.4	1.3
Glucose (Glu; mmol/L)	5.10	0.52	5.04	0.56	5.06	0.30
BUN (mmol/L)	6.32	3.78	5.71	3.55	5.20	3.01
Creat (μmol/L)[b]	79.56	15.91	84.86	18.56	87.52	11.49
Anion gap	14.6	1.5	14.6	1.9	16.4	3.6
B	**OES**					
Lactate (mmol/L)	0.127	0.037	0.133	0.072	0.108	0.030
Na^+ (mmol/L)	145.43	4.04	146.71	1.11	145.83	4.26
K^- (mmol/L)	4	0.4	3.71	0.43	4.72	2.13
Cl^- (mmol/L)	118.29	2.87	117.14	2.48	116.83	2.86
iCa (mmol/L)	0.348	0.015	0.333	0.030	0.335	0.013
TCO_2 (mmol/L)[a]	19.6	1.4	19.3	2.8	20.7	1.6
Glu (mmol/L)	5.35	0.49	5.14	0.34	4.87	0.65
BUN (mmol/L)	6.38	3.22	5.15	2.02	5.12	2.66
Creat (μmol/L)[b]	80.44	16.80	80.44	10.61	83.98	12.38
Anion gap	14.7	1.4	14.7	2.3	15.2	0.8
C	**SQs**					
Lactate (mmol/L)	0.110	0.046	0.100	0.041	0.110	0.067
Na^+ (mmol/L)	146.29	2.29	145.86	3.53	145.43	1.13
K^- (mmol/L)	4.07	0.4	3.7	0.41	3.84	0.48
Cl^- (mmol/L)	117.71	1.7	117.71	1.89	118.14	2.27
iCa (mmol/L)	0.340	0.025	0.328	0.035	0.338	0.035
TCO_2 (mmol/L)[a]	20.0	1.8	19.1	0.9	18.3	2.2
Glu (mmol/L)	5.10	0.54	5.13	0.29	5.02	0.44
BUN (mmol/L)	5.97	2.56	5.05	1.78	4.64	1.64
Creat (μmol/L)[b]	80.44	14.14	84.86	9.72	88.40	12.38
Anion gap	0.110	0.046	0.100	0.041	0.110	0.067

All values are reported as the mean and SD in international units.
iCa^+, ionized calcium; tCO_2, total carbon dioxide; BUN, blood urea nitrogen; Creat, creatinine.
[a]For TCO_2, the change over the day was significantly different for W versus OES ($p < 0.0001$) and W versus SQ ($p < 0.0001$).
[b]For Creat, contrasts were significant between all three pairs of hydration types: W and OES ($p = 0.015$), W and SQ ($p = 0.010$), and OES and SQ (0.001).

TABLE 4 | (A) Hematology results as a function of time and treatment group in the water (W) group (B), hematology results as a function of time and treatment group in the oral electrolyte solution (OES) group, and (C) hematology results as a function of time and treatment group in the subcutaneous fluids (SQ) group.

	AM		Midday		PM	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
A	**W**					
Hct (%)	45.3	4.5	42.9	2.9	41.3	4.8
PCV (%)[a]	49.3	4.8	47.4	3.1	45.0	3.7
TP (g/L)[b]	71.3	3.1	69.4	2.9	68.9	3.2
Hgb (g/L)	152.4	16.1	145.9	9.8	140.3	16.2
B	**OES**					
Hct (%)	45.4	3.5	41.0	2.7	38.3	1.8
PCV (%)[a]	48.7	3.0	46.1	3.0	43.5	2.2
TP (g/L)[b]	71.0	2.5	66.9	2.5	66.5	4.4
Hgb (g/L)	150.6	12.8	139.3	9.1	130.3	6
C	**SQs**					
Hct (%)	44.3	3.9	43.1	3.3	41.3	3.7
PCV (%)[a]	48.6	3.8	47.1	3.5	45.3	2.9
TP (g/L)[b]	70.6	3.7	70.4	3.6	70.0	3.4
Hgb (g/L)	150.6	13.2	146.7	11.2	140.4	12.4

All values are reported as the mean and SD in international units.
Hct, hematocrit; PCV, packed cell volume; TP, total protein; Hgb, hemoglobin.
[a]For PCV, contrasts were significant between W and OES (p = 0.007), and OES and SQ (p = 0.015).
[b]For TP, contrasts were significant between W and SQ (p = 0.0009) and between OES and SQ (p = 0.013).

TABLE 5 | (A) Urinalysis results as a function of time and treatment group in the water (W) group, (B) urinalysis results as a function of time and treatment group in the oral electrolyte solution (OES) group, and (C) urinalysis results as a function of time and treatment group in the subcutaneous fluids (SQ) group.

	AM		Midday		PM	
International units	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
A	**Water**					
Urine specific gravity (USG)	1.029	0.016	1.025	0.015	1.026	0.012
FE Na	0.39	0.42	NA	NA	1.06	0.34
Urine Creat (μmol/L)	13,371	7,541	NA	NA	13,296	8,921
Urine Na (mmol/L)	91.29	97.95	NA	NA	239.17	101.4
B	**OES**					
USG	1.034	0.013	1.017	0.01	1.015	0.01
FE Na	0.16	0.18	NA	NA	5.02	3.84
Urine Creat (μmol/L)	15,367	7,284	NA	NA	5,293	6,131
Urine Na (mmol/L)	43.43	52.63	NA	NA	231.33	77.58
C	**SQs**					
USG	1.029	0.014	1.027	0.015	1.029	0.014
FE Na	0.24	0.21	NA	NA	1.01	0.32
Urine Creat (μmol/L)	13,721	9,056	NA	NA	14,940	9,408
Urine Na (mmol/L)	42.43	31.25	NA	NA	227.81	103.9

All values are reported as the mean and SD in international units.
FE, fractional excretion; Creat, creatinine.

Effect of Hydration Method

Fluid consumption was influenced by the hydration strategy ($p < 0.001$) but not by the individual dog ($p = 0.088$). Mean fluid consumption in mL/kg/h was 7.04 ± 3.42 for W, 15.61 ± 4.47 for OES, and 5.56 ± 4.40 for SQ. The average additional sodium provided for the OES group was 416 mmol, for SQ was 65 mmol, and for W was 0 mmol. Only dogs in the OES group had the opportunity to consume OES. For those dogs, the ratio of OES to total fluid consumed ranged from 19 to 94%.

When controlling for other variables in the refined models, hydration method had a statistically significant impact on the change in blood TCO_2 ($p < 0.0001$), Creat concentration ($p < 0.0001$), PCV ($p = 0.001$), and TP ($p < 0.0001$), but had no significant impact on the change in lactate, Na, K, Cl, iCa, BUN, Glu, Hct, TP, and fractional excretion of Na. For TCO_2, contrasts were significant between W and OES ($p < 0.0001$) and between W and SQ ($p < 0.0001$) with regression coefficients of 0, −2.07, and −1.92 for W, OES, and SQ, respectively. The TCO_2 in dogs receiving OES or SQ was significantly higher at the end of the day when compared to the W group. For Creat, contrasts were

TABLE 6 | Physical parameters as a function of time and treatment group.

	AM		Midday		PM	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Water						
Body weight (kg)	30.29	4.13	29.86	4.15	30.6	4.27
Pulse (beats/min)	95	10	98	26	100	9
Oral electrolyte solution						
Body weight (kg)	30.74	4.35	31.43	4.67	30.56	4.32
Pulse	102	17	96	25	100	15
Subcutaneous fluids						
Body weight (kg)	30.56	4.2	30.27	4.1	30.56	4.32
Pulse	100	14	95	22	100	12

All values are reported as the mean and SD in international units.

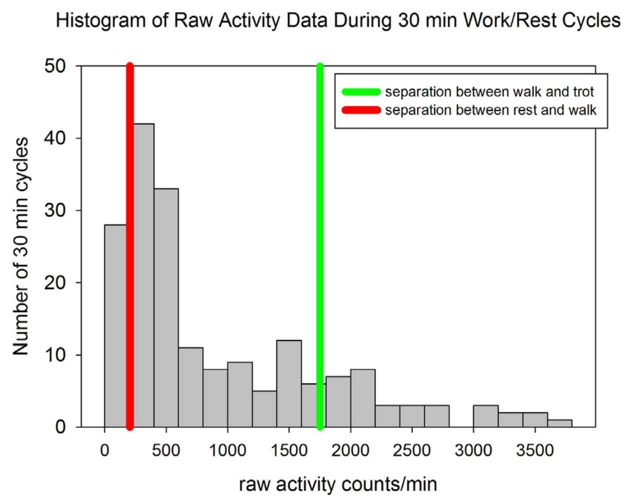


FIGURE 2 | A histogram of the raw activity counts for each 30 min period of either activity or rest for all dogs. The red line represents the activity count of 204 counts/min which has been shown to distinguish sedentary activity from walking activity with 100% specificity and 100% sensitivity in pet dogs. The green line represents the activity count of 1,751 counts/min which has been shown to distinguish walking from trotting activity with 92% specificity and 92% sensitivity in pet dogs (34).

significant between all three pairs of hydration types: W and OES ($p = 0.015$), W and SQ ($p = 0.010$), and OES and SQ (0.001). Regression coefficients for hydration types were 0, −0.13, and 0.12 for W, OES, and SQ, respectively. The Creat in OES although mildly increased from baseline at the end of the day was lower compared to W, whereas in SQ it was higher. For PCV, contrasts were significant between W and OES ($p = 0.007$), and OES and SQ ($p = 0.015$), with regression coefficients of 0, 0.64, and 0.04, respectively. Both OES and SQ treatments resulted in lower PCV at the end of the day compared to W. For TP, contrasts were significant between W and SQ ($p = 0.0009$) and between OES and SQ ($p = 0.013$), with regression coefficients of 0, –0.07, and –0.31 for W, OES, and SQ, respectively. Compared to W, TP decreased more in OES, and SQ groups at the end of the work period.

When evaluating all groups, there was a relationship between fractional excretion of sodium and sodium load ($p = 0.0001$). None of the other parameters tested in the model were significant. When evaluating the effect of time on fractional excretion of sodium for all dogs and all treatment groups, there was a significant increase over the working day (median 0.125 in the AM versus 1.240 in the PM; $p < 0.001$).

Odor Detection Performance

The variability in duration of search was a function of the difficulty of the search, the environmental conditions, and the type of training aid. The duration of search did not provide data that could be compared across dogs or dates since not all dogs performed the same search. In order to evaluate for fatigue or stress, the behavioral variables were analyzed. Hydration strategy had no effect on any of the behavior scores. Higher activity level was associated with decreased frequency of ears down ($p < 0.003$), stiff tail ($p < 0.0001$), proportion of time the mouth was coded as either fully open or fully closed ($p < 0.05$), panting ($p < 0.05$), and jumping up ($p < 0.0001$). Sex was associated with relaxed ears ($p < 0.05$), stiff tail movement ($p < 0.005$), canine focus on search ($p < 0.001$), lack of reward upon indication ($p < 0.01$), and placing feet up ($p < 0.01$). Females were less relaxed, less focused and less interactive. The breed was associated with duration tail wagging ($p < 0.05$), and canine jumping up ($p < 0.001$) with German shepherds having longer duration of tail wagging and less frequent jumping up than Belgian Malinois. An increased ambient temperature was associated with the canine placing its feet up during reward ($p < 0.001$) and a loose grip on the reward ($p < 0.01$).

DISCUSSION

In this study of low to moderate activity in detection dogs screening vehicles for narcotics or concealed humans in the Rio Grande Valley Sector (Sarita, TX checkpoint) of the southern US border, the three different hydration strategies had minor effects. There was no detectable effect on internal body temperature or activity. The most dramatic difference between the groups was fluid intake. When dogs were offered the chicken flavored OES, they drank significantly more liquid than when they were in the W or SQ groups. In a previous study of a different OES formulation in search dogs, dogs consumed minimal amounts of OES (35). The OES in this study was readily consumed by the dogs that could have been influenced by the chicken flavoring, the glucose, or the sodium content of the OES.

The rationale for providing OES in humans is based primarily on the electrolyte loss from sweating during exercise. In dogs, sweating is not a major mechanism of heat regulation and sweat glands are primarily localized to their feet (36). This aspect of canine physiology has led some authors to suggest that OES is not appropriate and may not be safe for use in dogs (37).

Dehydration could be expected to increase both PCV and TP through hemoconcentration secondary to water loss; however, both the OES and SQ groups had a significant decrease in PCV and TP. Steiss (38) documented a decrease in PCV over the course of a field trial competition in Labradors. In sled dogs (16), reductions in both PCV and TP have been reported. Expansion of plasma volume during exercise has been proposed to explain the reductions in PCV and TP (16). The TCO_2 in dogs when receiving either OES and SQ group was significantly higher than when they received W. Both OES and SQ included a buffering agent which may have contributed to the effect on TCO_2. Alternatively, plasma volume expansion may have resulted in increased perfusion and heat dissipation contributing to less panting (loss of CO_2) or less of a metabolic acidosis. Blood pH and partial pressure of CO_2 were not available.

Compared to W, the OES treatment was associated with a smaller post exercise increase in blood Creat; whereas SQ treatment was associated with an increase in Creat. These results suggest that either dehydration or muscle damage over the day was occurring in the SQ group; whereas the OES group had evidence of less dehydration or alternately underwent diuresis. Most Creat originates from muscle stores of phosphocreatine. Small transient increases in Creat may be seen after consumption of a meat based diet, but this should not have been influenced by the hydration strategy as all dogs received the same amount of canned food with the temperature monitoring capsule. In a study of exercising search and rescue dogs provided ad lib water for rehydration, the dogs also had a small but significant increase in Creat (26). Increases in Creat can accompany physical conditioning, exercise, muscle damage (e.g., rhabdomyolysis) or dehydration. In a study of sled dogs with exertional rhabdomyolysis, Creat was not different between affected and controls (39). Rhabdomyolysis is unlikely, although creatine phosphokinase (CPK) was not measured in these dogs. These data suggest that the OES dogs had less muscle injury, were either more hydrated, or were undergoing diuresis.

Although hydration strategy was not a significant factor influencing the difference of fractional Na excretion, the OES group did have the highest Na load. There were no significant changes in blood sodium as a function of time of the day or hydration strategy in the dogs in this study; however, there was a significant increase in fractional sodium excretion in the urine as a function of time of day. Although these dogs were not performing endurance activity, there are not studies of fractional sodium excretion in moderate exercise. The significantly reduced sodium in sled dogs following 10 days of endurance racing (16, 17) raises the possibility of increased sodium loss with prolonged exertion. In one study (16), arginine vasopressin (AVP) was decreased at 2 h after completing the race. In that study, fractional excretion of sodium was decreased post-race, however the authors hypothesized that there was increased urinary sodium loss from activation of the renin–angiotensin–aldosterone system and increased muscle catabolism leading to increased urea filtration (16). In one study of sled dogs, sodium supplementation was associated with improved sodium homeostasis (17). Dogs in all groups had an increase in fractional excretion of sodium over the working day, but maintained their blood sodium concentrations. These findings suggest that despite some sodium loss with work, the normal homeostatic mechanisms in healthy dogs can maintain sodium and handle the additional sodium load from a sodium rich OES. Despite the high consumption, there were no electrolyte abnormalities, suggesting that the OES solution tested was safe in these healthy dogs. Electrolyte supplementation in small quantities may be beneficial in exercising dogs, but further studies are needed.

The influence of dog specific factors (i.e., fluid intake, sex, and breed) as well as environmental factors (i.e., ambient temperature) demonstrates the complexity of hydration regulation. The application of these findings to dogs that perform high intensity work in hot and humid environments may be limited for a number of reasons. The dogs were accustomed to both the environment and steady work load. The mean activity of these dogs was 44,975 counts/h or 750 counts/min; this is 41% of what was observed in working dogs participating in a 30 min exercise protocol (unpublished data). In a study of pet dogs using this accelerometer, rest resulted in a median activity count of 20 counts/min, walking resulted in a median activity count of 1,196 counts/min and trotting resulted in 3,027 counts/min (34). The dogs screening vehicles spent approximately half of the time working but only 16% of their time "at rest," so throughout the day were active > 80% of the time. Extrapolation of these results to dogs from cool climates responding to a disaster in hot climates or being shipped to a military installation in regions with higher heat and humidity may not be accurate. The hydration demands and response to different strategies may also be influenced by the duration and intensity of exertion by the dogs. The variability in the body weight measurements could have been influenced by fluid intake, loss of feces, or urine or variation in the scale. The temperatures and humidity did not reach the extremes that might be present in the most adverse working environments. In addition, the dogs worked under a shaded cover when screening vehicles; therefore, they did not have the effect of direct heat. The mean working temperature in these dogs was 101.9 ± 1.3°F (38.8 ± 0.7°C) which is lower than temperatures typically observed in working, and hunting dogs during exercises which averages over 40.6 C (105.0 F) (6). The inability to document a functional impact of the different hydration strategies on olfaction was limited by the practical nature of a field study in which the dogs were required to perform their normal tasks of screening vehicles. It was not possible to conduct a standardized odor detection assessment with all dogs under the same conditions. The behaviors of the dogs while searching were not affected by the hydration strategy, which may be an indirect measure of performance.

In summary, under these field conditions of controlled activity in moderate heat and humidity, dogs that are accustomed to the work and the environment were more likely to increase fluid consumption when provided a flavored OES. It is unknown if the electrolytes in the OES were beneficial or the effects were simply a function of increased consumption. Under these conditions, indirect measures suggest improved hydration with OES and SQ; however, Creat was increased to a significantly greater extent with SQ. Searching behaviors were independent of hydration strategy but highly related to the dog specific factors of sex, breed and activity level.

Future, laboratory studies under controlled environments may be valuable in addressing the effect of hydration strategy on total body water, the mechanisms of increased fractional sodium excretion with moderate exercise and the impact of hydration strategy on olfactory performance. A laboratory study would also allow for a Latin square design to better control variables. Future field studies are needed to evaluate more extreme conditions, the influence of flavoring versus electrolyte supplementation, and a larger cohort of dogs; but based on the current study, no adverse effects were documented for any of the hydration strategies tested. The use of other OES formulations or the impact of electrolyte-free flavorings was not evaluated and, therefore, the safety and efficacy of these strategies remains unknown. The decision on which strategy is used to best maintain hydration in dogs working in adverse environments will be influenced by the local limitations and demands of the environment and mission.

ETHICS STATEMENT

All protocols utilized were reviewed and approved by the University of Pennsylvania and US Army Medical Research and Materiel Command Institutional Animal Care and Use Committees (U Penn IACUC protocol 804293, USAMRMC proposal SO120002).

AUTHOR CONTRIBUTIONS

CO designed the study, executed data collection, reviewed the data, and participated in the manuscript preparation. EH performed the data analysis and participated in manuscript preparation. JN, SP, KK, TD, and KS participated in data collection and manuscript preparation. DC participated in data analysis and manuscript preparation.

FUNDING

Supported by the Department of Defense-Special Operations Command USSOCOM W81XWH-13-1-0038, with special thanks to the Sarita Texas checkpoint Border Patrol agents and their dogs; funding for TD provided by the American Veterinary Medical Foundation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fvets.2017.00174/full#supplementary-material.

VIDEO S1 | A video demonstrating a dog performing a systematic search of vehicles and the reward on identification of the odor source. This is a dog in training and with permission of the Penn Vet Working Dog Center. No videos from the study are included in order to preserve the anonymity of the CBP agents.

REFERENCES

1. Bruchim Y, Klement E, Saragusty J, Finkeilstein E, Kass P, Aroch I. Heat stroke in dogs: a retrospective study of 54 cases (1999-2004) and analysis of risk factors for death. *J Vet Intern Med* (2006) 20(1):38–46. doi:10.1892/0891-6640(2006)20[38:HSIDAR]2.0.CO;2
2. Evans RI, Herbold JR, Bradshaw BS, Moore GE. Causes for discharge of military working dogs from service: 268 cases (2000-2004). *J Am Vet Med Assoc* (2007) 231(8):1215–20. doi:10.2460/javma.231.8.1215
3. Baker JL, Hollier PJ, Miller L, Lacy WA. Rethinking heat injury in the SOF multipurpose canine: a critical review. *J Spec Oper Med* (2012) 12(2):8–15.
4. Steiss JE, Wright JC. Respiratory alkalosis and primary hypocapnia in Labrador Retrievers participating in field trials in high-ambient-temperature conditions. *Am J Vet Res* (2008) 69(10):1262–7. doi:10.2460/ajvr.69.10.1262
5. Cheung SS, McLellan TM. Heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensable heat stress. *J Appl Physiol* (1998) 84(5):1731–9.
6. Slensky K, Drobatz K, Downend A, Otto C. Deployment morbidity among search and rescue dogs from 9/11. *J Am Vet Med Assoc* (2004) 225(6):868–73. doi:10.2460/javma.2004.225.868
7. Gordon LE. Injuries and illnesses among urban search-and-rescue dogs deployed to Haiti following the January 12, 2010, earthquake. *J Am Vet Med Assoc* (2012) 240(4):396–403. doi:10.2460/javma.240.4.396
8. Goldberg MB, Langman VA, Richard Taylor C. Panting in dogs: paths of air flow in response to heat and exercise. *Respir Physiol* (1981) 43(3):327–38. doi:10.1016/0034-5687(81)90113-4
9. Baile EM, Guillemi S, Pare PD. Tracheobronchial and upper airway blood flow in dogs during thermally induced panting. *J Appl Physiol* (1987) 63(6): 2240–6.
10. Horowitz M, Nadel ER. Effect of plasma volume on thermoregulation in the dog. *Pflügers Archiv Eur J Physiol* (1984) 400(2):211–3. doi:10.1007/BF00585045
11. Hemmelgarn C, Gannon K. Heatstroke: thermoregulation, pathophysiology, and predisposing factors. *Compend Contin Educ Vet* (2013) 35(7):E4.
12. Crawford EC Jr. Mechanical aspects of panting in dogs. *J Appl Physiol* (1962) 17:249–51.
13. Baker MA, Doris PA, Hawkins MJ. Effect of dehydration and hyperosmolality on thermoregulatory water losses in exercising dogs. *Am J Physiol* (1983) 244(4):R516–21.
14. Spoo JW, Zoran DL, Downey RL, Bischoff K, Wakshlag JJ. Serum biochemical, blood gas and antioxidant status in search and rescue dogs before and after simulated fieldwork. *Vet J* (2015) 206(1):47–53. doi:10.1016/j.tvjl.2015.07.002
15. Robbins PJ, Ramos MT, Zanghi BM, Otto CM. Environmental and physiological factors associated with stamina in dogs exercising in high ambient temperatures. *Front Vet Sci* (2017) 4:144. doi:10.3389/fvets.2017.00144
16. Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA. Effect of racing on serum sodium and potassium concentrations and acid-base status of Alaskan sled dogs. *J Am Vet Med Assoc* (1997) 210(11):1615–8.
17. Ermon V, Yazwinski M, Milizio JG, Wakshlag JJ. Serum chemistry and electrolyte alterations in sled dogs before and after a 1600 km race: dietary sodium and hyponatraemia. *J Nutr Sci* (2014) 3(e26):1–5. doi:10.1017/jns.2014.39
18. de Beer EJ, Wilson DW. The inorganic composition of the parotid saliva of the dog and its relation to the composition of serum. *J Biol Chem* (1932) 95:671.
19. Lisman P, Kazman JB, O'Connor FG, Heled Y, Deuster PA. Heat tolerance testing: association between heat intolerance and anthropometric and fitness measurements. *Mil Med* (2014) 179(11):1339–46. doi:10.7205/MILMED-D-14-00169
20. Marconi C, Pendergast D, Krasney JA, Rennie DW, Cerretelli P. Dynamic and steady-state metabolic changes in running dogs. *Respir Physiol* (1982) 50(1):93–110. doi:10.1016/0034-5687(82)90010-X
21. Proscurshim P, Russo AK, Silva AC, Piçarro IC, Freire E, Tarasantchi J. Aerobic training effects on maximum oxygen consumption, lactate threshold and lactate disappearance during exercise recovery of dogs. *Comp Biochem Physiol A Comp Physiol* (1989) 94(4):743–7. doi:10.1016/0300-9629(89)90627-0
22. Manens J, Ricci R, Damoiseaux C, Gault S, Contiero B, Diez M, et al. Effect of body weight loss on cardiopulmonary function assessed by 6-minute walk test and arterial blood gas analysis in obese dogs. *J Vet Int Med* (2014) 28(2):371–8. doi:10.1111/jvim.12260
23. Leschnik M, Heidrich C, Thalhammer JG, Bubna-Littitz H. Evaluation of physical fitness in Austrian police dogs. *Wien Tierarztl Monatsschr* (2007) 94(7–8):175–9.
24. Laflamme DP, Xu H, Cupp CJ, Kerr WW, Ramadan Z, Long GM. Evaluation of canned therapeutic diets for the management of cats with naturally occurring chronic diarrhea. *J Feline Med Surg* (2012) 14(10):669–77. doi:10.1177/1098612X12446906
25. Wennogle SA, Martin L, Oleo-Popelka FJ, Xu H, Jean-Phillipe C, Lappin MR. Randomized trial to evaluate two dry therapeutic diets for shelter dogs with acute diarrhea. *Int J Appl Res Vet Med* (2016) 14(1):30–7.
26. Yam PS, Penpraze V, Young D, Todd MS, Cloney AD, Houston-Callaghan KA, et al. Validity, practical utility and reliability of Actigraph accelerometry for the measurement of habitual physical activity in dogs. *J Small Anim Pract* (2011) 52(2):86–92. doi:10.1111/j.1748-5827.2010.01025.x
27. Angle TC, Gillette RL. Telemetric measurement of body core temperature in exercising unconditioned labrador retrievers. *Can J Vet Res* (2011) 75(2):157–9.
28. Kraus MS, Gelzer AR, Rishniw M. Detection of heart rate and rhythm with a smartphone-based electrocardiograph versus a reference standard electrocardiograph in dogs and cats. *J Am Vet Med Assoc* (2016) 249(2):189–94. doi:10.2460/javma.249.2.189
29. Beerda B, Schilder MBH, Van Hooff JARAM, De Vries HW, Mol JA. Behavioural and hormonal indicators of enduring environmental stress in dogs. *Anim Welfare* (2000) 9(1):49–62.
30. Ng ZY, Pierce BJ, Otto CM, Buechner-Maxwell VA, Siracusa C, Werre SR. The effect of dog-human interaction on cortisol and behavior in registered animal-assisted activity dogs. *Appl Anim Behav Sci* (2014) 159:69–81. doi:10.1016/j.applanim.2014.07.009
31. Haverbeke A, Diederich C, Depiereux E, Giffroy JM. Cortisol and behavioral responses of working dogs to environmental challenges. *Physiol Behav* (2008) 93(1–2):59–67. doi:10.1016/j.physbeh.2007.07.014
32. Bates D, Maechler M, Bolker B, Walker S, Bojesen Christensen RH, Singmann H, et al. *lme4: Linear Mixed-Effects Models Using Eigen and S4. R Package Version 1.0-6* (2014). Available from: http://CRAN.R-project.org/package=lme4
33. Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* (2015) 67. doi:10.18637/jss.v067.i01
34. Michel KE, Brown DC. Determination and application of cut points for accelerometer-based activity counts of activities with differing intensity in pet dogs. *Am J Vet Res* (2011) 72(7):866–70. doi:10.2460/ajvr.72.7.866
35. Mazin RM, Fordyce HH, Otto CM. Electrolyte replacement in urban search and rescue dogs: a field study. *Vet Ther* (2001) 2:140–7.
36. Machida H, Giacometti L, Perkins E. Histochemical and pharmacologic properties of the sweat glands of the dog. *Am J Vet Res* (1966) 27(117):566–73.
37. Wakshlag J, Shmalberg J. Nutrition for working and service dogs. *Vet Clin North Am Small Anim Pract* (2014) 44(4):719–40. doi:10.1016/j.cvsm.2014.03.008
38. Steiss J, Ahmad HA, Cooper P, Ledford C. Physiologic responses in healthy Labrador Retrievers during field trial training and competition. *J Vet Int Med* (2004) 18(2):147–51. doi:10.1111/j.1939-1676.2004.tb00153.x
39. Piercy RJ, Hinchcliff KW, Morley PS, DiSilvestro RA, Reinhart GA, Nelson SL Jr, et al. Vitamin E and exertional rhabdomyolysis during endurance sled dog racing. *Neuromusc Disord* (2001) 11(3):278–86. doi:10.1016/S0960-8966(00)00199-1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership