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Bio-detection Dogs sniffing Leishmania infection: an efficient diagnostic tool for pet and public health

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Abstract

Canine Leishmaniasis is a multisystemic zoonotic vector-borne disease, incurable and potentially fatal, transmitted to humans and dogs by the bite of female sandflies, representing a major public health problem in the Eastern Mediterranean Region (EMR) of the World Health Organization (WHO). In the medical-diagnostic field, trained sniffer dogs have demonstrated significant potential as a non-invasive, efficient, and cost-effective screening approach for the early detection of diseases like cancer, malaria, periprosthetic joint infections or COVID-19, as well as diabetic alert service (DADs).
This paper presents a pilot study dealing with canine olfactory detection of Leishmania infection in dogs and its relevance to the topic of animal and public health. The pilot testing conducted in view of the development and execution of a larger-scale trial suggests that a broader study is likely to be successful and could produce valuable results in terms of using dogs' exceptional olfactory abilities as a rapid, efficient and reliable diagnostic tool for sniffing out infectious diseases, with specific focus on the detection of canine Leishmaniasis.

Key Words: Canine Leishmaniasis; Bio-Medical Detection Dog; VOC-profile; pathology detection; sniffer dog

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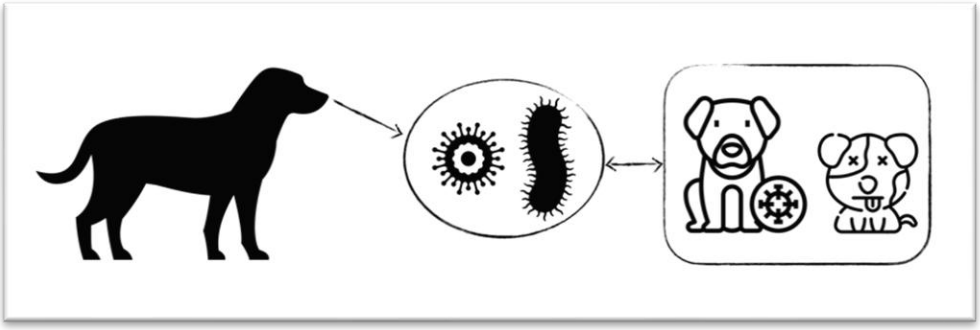
Introduction and background.

Domestic dogs' exceptional olfactory acuity allows humans to employ them to find a variety of substances (Browne, Stafford, and Fordham 2006). Dogs have an olfactory sense that is capable of detecting compounds at extremely low quantities and far more sensitive than any artificial device. Dogs' noses may possess up to 300 million olfactory receptors, compared to humans' approximately six million, their olfactory epithelium area (18 to 150 cm) is significantly larger than that of humans (3 cm), and their brains have a 40-fold larger section dedicated to smell analysis. Because of this olfactory superiority, dogs can detect scents that humans entirely miss, making what is "invisible" to us olfactorily visible and even strong to the animal. Dogs can also distinguish between different scents and independently detect each scent in a mixture of aromas. With the right training, dogs can detect drugs or explosives, track down criminals, find missing people, and even be used experimentally to carry out various challenging tasks such as the chemical detection of red palm weevil infestation (Soroker et al. 2017). Furthermore, domestic dogs gave evidence to be able to identify a variety of human diseases just by their scent (Jendrny et al. 2021), as well as being involved with medical support projects, like in the case of Glycaemia alert dogs, significantly enhancing the quality of life for owners affected by Type 1 diabetes (Rooney et al. 2019). In fact, the highly developed sense of smell of dogs, along with their capacity for operant conditioning, makes them suitable for a variety of medical detection applications. Biomedical Detection Dog (BMDD) capabilities offer a detection or screening tool only depending on the disease-state odor or the pathogen. Diseases and the presence of certain pathogens can be detected using the volatilome, which is defined as the composition of an organism's Volatile Organic Compounds (VOCs) and the VOCs reflecting its current metabolic state (including the influence of infection).

Fig.1 Bio-Medical Detection Dogs (BMDDs) can detect the odors associated with the pathogen or etiological agent, together with the altered volatilome due to disease state produced by an infected host in response to the pathogen. When sniffing out canine diseases, dog-to-dog interactions are in any case avoided by working with samples from dogs suspected of being infected.



The oversimplified odor detection scenario (see Figure 1) just involves working with potentially contaminated samples, so that the VOC profile of the odor does not even necessarily need to be described. BMDD may be used to directly detect the pathogen or the clinical state in a wide range of disease outbreak scenarios caused by viruses, including SARS-CoV-2, bacteria, pests, and in case of disease odor directly on humans, such as Parkinson's, epilepsy, cancers, diabetes (see Maughan et al. 2022 and references therein). BMDDs can be trained to look for infected individuals, the etiological agent itself, or even smells connected to the pathogen's growth, depending on the training method and technique used. Although it has been primarily employed as a tool for human illness detection, the canine sense of smell has also been effectively employed in the field of animal disease detection, though with a far more limited scope of application (Kokocinska-Kusiak et al. 2021). In particular, one should note that, for canine screening, the training and deployment scenarios should in any case avoid dog-to-dog interactions between BMDDs and other dogs affected by potential health problems.

Visceral Leishmaniasis (VL) is a life-threatening disease caused by Leishmania infantum, a parasite of dogs and humans (the cutaneous and visceral forms of the viral infection can infect persons with weakened immune systems) transmitted by the bite of sandflies. VL represents a neglected tropical disease (NTD) that is endemic in many regions of the world, and dogs are the primary reservoir of the parasite for humans and other mammalian hosts. Infection detection in dogs is thus essential for veterinary practice, illness prevention, and epidemiological inference (Vital et al. 2022). Protozoans of the genus Leishmania, spread by the bite of female phlebotomine sandflies carrying the infection to dogs (and humans), are the cause of Canine Leishmaniosis (CanL), which manifests in dogs as a wide range of clinical symptoms. Early diagnosis is crucial for administering the right treatment and preventing the development of a more severe condition (Maurelli et al. 2020). Serological (LFT, IFAT, ELISA), parasitological (microscopic analysis of various samples), and molecular diagnostic assays (conventional, nested, and quantitative real-time PCR) are the primary, although not error-free nor inexpensive, diagnostic techniques for CanL.

Inspired by recent scientific findings based on similar methods, (Callewaert et al. 2023; Guest et al. 2019; Pellin, Malone, and Ungar 2024; Ramos et al. 2024), the idea behind this paper was that dogs, due to their excellent sense of smell, could be trained to identify Leishmania infection in other dogs with uncertain infection statuses by sniffing different types of biological samples, starting form blood serum to then move on to fur, saliva, rather than some other body fluid or tissue. In the following, we will discuss how this can be accomplished by describing a pilot study with interesting outcomes in terms of providing a novel and powerful preventive service to identify Leishmania infection in dogs through a canine olfactory detection approach whose implementation is simple, secure, minimally invasive, and cost-effective.

Current findings.

The release of stable Volatile Organic Compounds (VOCs) from tissues, which may be found in sweat, urine, feces, and exhaled breath because of metabolic changes brought on by viral and non-infectious medical conditions, is well supported by scientific evidence. VOC biomarkers fall within the canine olfaction system's detection range, since dogs can detect certain chemicals even at concentrations as low as parts per trillion, and, according to reports (Angle et al. 2016), the concentrations of potential VOC biomarkers in breath range from parts per billion to parts per trillion, while those in blood and urine range from parts per million to parts per billion.

Additionally, dogs can search and identify unique odor patterns by quickly scanning huge surface areas or evaluating hundreds of samples, which is crucial for identifying infections in large animal herds, human populations, areas of land or other kinds of setting, even when factors like temperature, humidity, wind, and thermal plumes are continually changing and field conditions include substantial odor noise. Dogs' natural ability to generalize to lower concentrations of a trained odorant (i.e., the range of lower concentrations that dogs are aware of without explicit or further training to different concentrations) represents an additional proven fact (DeChant, Bunker, and Hall 2021), which implies that samples and equipment used for detection dog training are to be prepared carefully to avoid unwanted contamination with any minimal amount of the odorant. Controlling the process of adequate odor imprinting is a challenging task, since disease detection involves volatile chemical concentrations that are hard of being monitored and little is known about the olfactory dynamics of a pathological process. In any case, during pandemic or epidemic situations, canines have proven to be an incredibly quick, highly adaptive, and accurate VOC-detection tool assisting in the isolation of infected patients who may or may not exhibit symptoms. Specifically, to contain the global COVID-19 pandemic, a number of labs worldwide have focused their efforts on training dogs to sniff biological samples from infected patients in public places and identify a SARS-CoV-2-specific VOC odor signature. This was achieved by comparing a scent detection dog test with naso-pharyngeal SARS-CoV-2 RAD immunoassays (Meller et al. 2022), and findings revealed that dogs could identify COVID-19 with a high degree of diagnostic accuracy in a matter of seconds, outperforming antigen tests in terms of detection performance (Pirrone et al. 2023). As far as the authors are aware, the occurrence of disease-associated VOC-profiles using chemical analytical methods and technical sensory devices has been demonstrated in certain cancers, respiratory diseases, and other infections; however, in the case of Leishmaniasis, this has never been documented in the literature. A peculiar finding (Staniek and Hamilton 2021) is that blood-seeking female sand flies, responsible for the transmission of the pathogen, find the smell of dogs infected with *Leishmania infantum* to be noticeably more appealing compared to what is the case for male sand flies, which are not able to spread the disease. In a natural transmission system, this is compelling evidence of parasite manipulation of the host odor, suggesting that sick canines may play a disproportionate role in sustaining infection in both the human and canine populations. For the purposes of the study presented in this paper, this result provides interesting information supporting the idea that dogs' superior sense of smell can discriminate between *Leishmania infantum* infected dogs and healthy subjects.

Materials and methods.

Canine detection of Leishmania-infection is thought to be mainly based on the detection of volatile organic compounds (VOCs). Dogs can detect volatile organic compounds (VOCs) with great accuracy in real time. However, environmental conditions might affect VOC detection, making it challenging to standardize. The proper range of target odors is a key component of training medical detection dogs to detect volatile organic compounds (VOCs) released from biological material because of the metabolic alterations due to the infection with Leishmania parasites.

Animals

For the study, three healthy adult female dogs of different breeds (see Table 1 and Figure 2) were recruited, and they were trained to detect Leishmania infection in serum samples. These domestic dogs had any previous operational experience with odor discrimination tasks and were trained as sniffer dogs around twice a week for about 5 months.

Table 1. Description of the selected dogs.

DOG ID	AGE	BREED	REPRODUCTIVE STATUS
Vicki	1 Yr	Miniature Poodle	Intact female
Tahlula	3 Yrs	Belgian Malinois	Spayed Female
Meg	3 Yrs	German Shepherd Dog	Intact female

Fig. 2. The three dogs that were trained during the pilot study.

Odorant.

The biological serum samples used in the study were tested by serological examination (Indirect Immunofluorescence) for the detection of anti-Leishmania antibodies in the blood, stored in the freezer at -20°C and thawed at room temperature before use. Sera from seropositive dogs with low to high anti-Leishmania antibodies detected, whose IFAT antibody titer were ranging from 1:160 to 1:1280, were considered as target odorants. From 2 to 4 ml of infected (target) and uninfected (distractor) blood serum samples were poured onto absorbent cotton pads and placed individually in various blank sniffers in aluminum, plastic or glass. All samples were prepared while wearing nitrile gloves to avoid sample handling variations that could produce odor discrepancies.

Experimental equipment and training procedure.

The training of the dogs was planned by following the learning scheme based on the imprinting of the target odor through the stimulus of food. In a first phase, the motivation to use the nose for a purpose was built, focusing only on positive work without the presence of the specific odorous target. In a second phase, the infected target odor was inserted to fix the imprinting of the target to be identified. Once the associative memorization was confirmed, first distractors were inserted, that is, opposite and similar odors, called negative. Only after a series (called repetitions) of satisfactory and clear answers on the discrimination process, the non-infected odor (blood serum negative for

Leishmaniasis) was inserted. For each trial, all target odors were randomly assigned a position on the scent containers for nose work; dogs were brought into the “theatre”, containing a range of scents, including the target scent and various distractors, and allowed to search at first position and work to last one. The imprinting was assessed when the canines demonstrated the ability to distinguish the infected target odor from the non-infected one, reaching a discriminating and decisional autonomy applied outside the comfort zone and on different theaters.

Passive signaling adoption was preferred (see Figure 3), which means that once the dogs had located the target odor, they sit and stare at the source of the odor as a pre-trained indication to communicate to the handler that they found what they were looking for without engaging in potentially disruptive or destructive behaviors. In conclusion, dogs were rewarded with food or a toy from time to time. To make sure the dogs were signaling the positive target, distractions, also known as "non-target odors," were employed to offer scents being somewhat distinct from or similar to the positive target. Dogs were never physically pushed into sampling the vapor from any specific source; instead, they were always allowed to avoid the odor’s source. Additionally, targets and distractions were positioned such that dogs couldn’t come into direct touch with or ingest the sample.

Fig.3. Outdoor training involving natural distractors combined with infected and uninfected blood serum samples. Meg is sitting and holding her nose in the target odor port to alert the handler.

Ethical considerations.

Dogs differ in their experiences and personality. A dog’s potential as a detection dog is greatly influenced by its behavior and personality in addition to its anatomy. High levels of motivation and both mental and physical fitness are vital. Only positive reinforcement needs to be a part of the learning process. When training dogs and deploying them in the field, it is important to keep in mind that they can become tired and frustrated. Because of their wide olfactory range and the intensity with which they receive these stimuli, when dogs sniff something with curiosity, they are using all their cerebral resources to obtain information, which is a huge energy loss. Therefore, dogs may find the process of sniffing taxing and, thus, require adequate work/rest cycles.

Statistical analysis.

For evaluating training sessions, we calculated individual ac- curacy as the ratio of the number of correct trials (the dog alerts to the target stimulus and neglects all the distractors) to the total number of trials. The dogs were trained to alert the handler only when the target odor was detected; therefore, either correct alerts or "non-alerts" when distractors were detected, be they neutral substances, different specific substances or blood serum not infected by Leishmania, should be considered positive canine indications.

Preliminary results.

The dogs were trained to alert the handler only when the target odor was detected; therefore, either correct alerts or "non-alerts" when distractors were detected, be they neutral substances, different specific substances or blood serum not infected by Leishmania, should be considered positive canine indications. Dog’s preliminary performance and results for this study are shown in Figure 4 and Table 2.

Fig. 4. Dogs’ accuracy during training: individual searching performances.

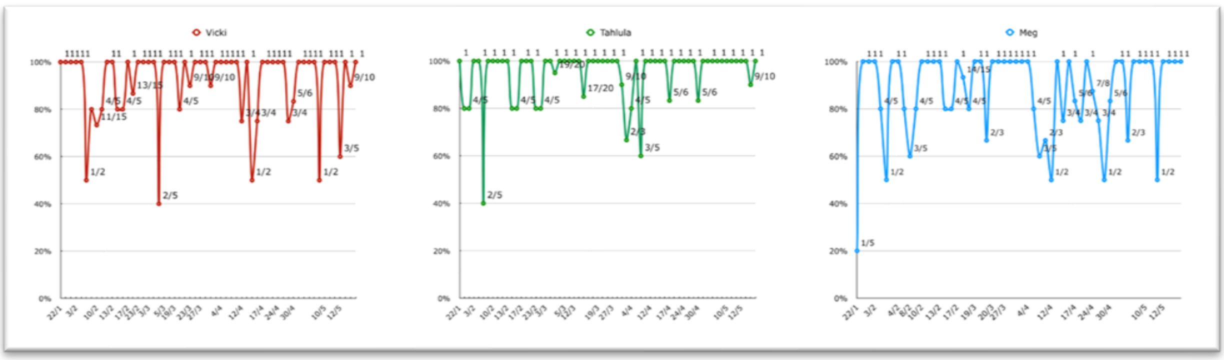


Table 2. First numbers of the study: employed sera from diverse Leishmania-infected dogs with various antibody titers and provisional outcomes from the training trial in terms of mean accuracy (m. a.).

SAMPLES	1:160	1:320	1:640	1:1280	NEGATIVE
45	9	15	7	1	13
SESSIONS	REPETITIONS	VICKI’S m.a.	TAHLULA’S m.a.	MEG’S m.a.	GLOBAL m.a.
23	548	90,85%	94,20%	90,31%	91,79%

Blood samples from 45 dogs, both infected by *Leishmania infantum* with different antibody titers and not-infected subjects, were used during the pilot trial. Kept in mind that the outcome of a pilot study is a continuous measurement, the reported data, referring to the first 23 sniffing sessions (during which several repetitions were performed) demonstrate that the dogs maintained a satisfactory percentage of global mean accuracy (91,79%) while discriminating a large number of distractors. In the absence of specific indications or references on the permanence of an odor coming from Leishmania-infected blood after the deterioration of the serum due to its storage at room temperature, a specific test over a period of 48 hours has been attempted. Specifically, the dogs sniffed three samples of infected serum, whose detected antileishmanial antibody titers were 1:160, 1:320 and 1:1280, respectively, and two samples of negative serum, directly from test tube without impregnation at a distance of 2, 5, 20, 26, 44 and 48 hours from thawing, not refrigerated during all the period. What emerged is that the dogs precisely alerted to all target odorants, ignoring the distractors each time for the entire duration of the test; after 48 hours, the propensity of the dogs to alert to the target positions was found to be reduced. We also noticed that the different antileishmanial antibody titers did not seem to be related to dogs' responses, not even in terms of shown interest over time.

Discussion and limitations.

Even though no published studies specifically address the identification of VOC from dogs infected with *Leishmania infantum* with an odor that is detectable by trained dogs, we hypothesized that dogs could detect Leishmania-infected blood samples, following several recent reports which show that different viral infections or other pathological conditions are associated with unique profiles of VOC. Preliminary results collected during this investigation suggest that trained dogs have the potential to serve as an easily portable, low-cost, and efficient sensory tool for disease detection.

Advanced search and detection of the target odor on fur, mucous membranes, secretions, tears, saliva turns out to be the next action to be considered for the project's completion and validation.

Since the initial findings presented in this paper were collected during the training phase to test the feasibility and potential of the use in field of the proposed approach, parameters such as sensitivity and specificity of dogs' indication of samples were not considered. A detailed analysis of these important conditional probabilities is part of our future plans.

The existence of specific VOC profiles associated with Leishmaniasis, beyond the study of sand flies' attraction, has never been documented in the literature using chemical analytical methods. Although dogs' searching performances provide strong indirect evidence, the characterization of a chemically identified VOC profile associated with Leishmaniasis in dogs is under investigation to provide a full understanding and independent verification of the VOC signature the dogs are specifically detecting.

So far, limitations of the current pilot study surely include the use of not a huge quantity of infected blood serum samples, the engagement of a small number of trained dogs, and the completion of the experiment using other biological samples from infected subjects. Moreover, standardizing and certifying training methods requires more effort.

Conclusions.

Canines have three times the sensory capability of many modern diagnostic tools, and it is known that trained dogs can achieve reasonable levels of diagnostic sensitivity and specificity due to the distinct smells connected to different diseases. According to our pilot study, dogs can sniff out blood samples for Leishmania infection. This discovery highlights the possibility of using canines to identify the infection in real time, which could help with prompt treatment and control of pathogen outbreaks and lessen, as a first step, the need to transport samples for testing in a lab. Based on preliminary results, which suggest the existence of a detectable odor and the dogs' ability to detect certain volatile organic compounds (VOCs) associated with Canine Leishmaniasis, further study and development are warranted.

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Conflicts of Interest: There is no potential conflict of interest, and the Authors have nothing to disclose. This work was not supported by any grant.

References:

1. Angle, T. C., Passler, T., Waggoner, P. L., Fischer, T. D., Rogers, B., Galik, P. K., Maxwell, H. S. (2016). Real-Time Detection of a Virus Using Detection Dogs. Frontiers in Veterinary Science, 2 - 2015.
2. Browne, C, Stafford, K, Fordham, R (2006). The use of scent-detection dogs. IRISH VETERI- NARY JOURNAL; 59(2).
3. Callewaert, C., Pezavant, M., Vandaele, R., Meeus, B., Vankrunkelsven, E., Van Goethem, P., Plumacker, A., Misset, B., Darcis, G., Piret, S., De Vleeschouwer, L., Staelens, F., Van Varenbergh, K., Tombeur, S., Ottevaere, A., Montag, I., Vandecandelaere, P., Jonckheere, S., Vandekerckhove, L., Tobback, E., Wieers, G., Marot, J.-C., Anseeuw, K., D'Hoore, L., Tuyls, S., De Tavernier, B., Catteeuw, J., Lotfi, A., Melnik, A., Aksenov, A., Grandjean, D., Stevens, M., Gasthuys, F., Guyot, H. (2023). Sniffing out safety: canine detection and identification of SARS-CoV-2 infection from armpit sweat. Front Med (Lausanne), 10.
4. DeChant, M. T., Bunker, P. C., Hall, N. J. (2021). Stimulus Control of Odorant Concentration: Pilot Study of Generalization and Discrimination of Odor Concentration in Canines. Animals, 11(2).
5. Guest, C., Pinder, M., Doggett, M., Squires, C., Affara, M., Kandeh, B., Dewhirst, S., Morant, S. V., D'Alessandro, U., Logan, J. G., Lindsay, S. W. (2019). Trained dogs identify people with malaria parasites by their odour. Lancet Infect Dis; 19(6), 578–580.
6. Jendrny, P., Twele, F., Meller, S., Osterhaus, A. D. M. E., Schalke, E., Volk, H. A. (2021). Canine olfactory detection and its relevance to medical detection. BMC Infectious Diseases; 21(1), 838.
7. Kokocinska-Kusiak, A., Woszczyło, M., Zybala, M., Maciocha, J., Barłowska, K., Dzieciol, M. (2021). Canine Olfaction: Physiology, Behavior, and Possibilities for Practical Applications. Animals (Basel); 11(8).
8. Maughan, M. N., Best, E. M., Gadberry, J. D., Sharpes, C. E., Evans, K. L., Chue, C. C., Nolan, P. L., Buckley, P. E. (2022). The Use and Potential of Biomedical Detection Dogs During a Disease Outbreak. Frontiers in Medicine, 9.
9. Maurelli, M. P., Bosco, A., Foglia Manzillo, V., Vitale, F., Giaquinto, D., Ciuca, L., Molinaro, G., Cringoli, G., Oliva, G., Rinaldi, L., Gizzarelli, M. (2020). Clinical, Molecular and Serological Diagnosis of Canine Leishmaniosis: An Integrated Approach. Vet Sci; 7(2).
10. Meller, S., Al Khatri, M. S. A., Alhammadi, H. K., Álvarez, G., Alvergnat, G., Alves, L. C., Callewaert, C., Caraguel, C. G. B., Carancci, P., Chaber, A.-L., Charalambous, M., Desquilbet, L., Ebbers, H., Ebbers, J., Grandjean, D., Guest, C., Guyot, H., Hielm-Björkman, A., Hopkins, A., Kreienbrock, L., Logan, J. G.,

Lorenzo, H., Maia, R. d. C. C., Mancilla-Tapia, J. M., Mardones, F. O., Mutesa, L., Nsanzimana, S., Otto, C. M., Salgado-Caxito, M., Santos, F. de los, Silva, J. E. S. da, Schalke, E., Schoneberg, C., Soares, A. F., Twele, F., Vidal-Martínez, V. M., Zapata, A., Zimin-Veselkoff, N., Volk, H. A. (2022). Expert considerations and consensus for using dogs to detect human SARS-CoV-2-infections. Frontiers in Medicine Volume, 9.
11. Pellin, M. A., Malone, L. A., Ungar, P. (2024). The use of sniffer dogs for early detection of cancer: a One Health approach. American Journal of Veterinary Research; 85(1).
12. Pirrone, F., Piotti, P., Galli, M., Gasparri, R., La Spina, A., Spaggiari, L., Albertini, M. (2023). Sniffer dogs performance is stable over time in detecting COVID-19 positive samples and agrees with the rapid antigen test in the field. Scientific Reports; 13(1), 3679.
13. Ramos, M. T., Chang, G., Wilson, C., Gilbertie, J., Krieg, J., Parvizi, J., Chen, A. F., Otto, C. M., Schaer, T. P. (2024). Dogs can detect an odor profile associated with Staphylococcus aureus biofilms in cultures and biological samples. Front Allergy, 5.
14. Rooney, N. J., Guest, C. M., Swanson, L. C. M., Morant, S. V. (2019). How effective are trained dogs at alerting their owners to changes in blood glycaemic levels: Variations in performance of glycaemia alert dogs. PLOS ONE; 14(1), 1–16.
15. Soroker, V., Suma, P., Pergola, A. L., Llopis, V. N., Vacas, S., Cohen, Y., Cohen, Y., Alchanatis, V., Milonas, P., Golomb, O., Goldshtein, E., Banna, A. E. M. E., Hetzroni, A. (2017). Surveillance Techniques and Detection Methods for Rhynchophorus ferrugineus and Paysandisia archon. John Wiley & Sons; 10, 209–232.
16. Staniek, M. E., Hamilton, J. G. C. (2021). Odour of domestic dogs infected with Leishmania infantum is attractive to female but not male sand flies: Evidence for parasite manipulation. PLoS Pathog; 17(3).
17. Vital, T., Teixeira, A. I. P., Silva, D. M., Carvalho, B. C. de, Dallago, B., Hagström, L., Hecht, M. M., Nitz, N., Abad-Franch, F. (2022). Detecting Leishmania in dogs: A hierarchical-modeling approach to investigate the performance of parasitological and qPCR-based diagnostic procedures. PLoS Negl Trop Dis; 16(12).

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