Management of Canine Leishmaniasis and the Treatment Trends of Veterinarians in Aegean Region of Türkiye: A Questionnaire-based Survey

Türkiye Ege Bölgesi'ndeki Veteriner Hekimlerin Köpek Leishmaniasis Yönetimi ve Tedavi Eğilimleri: Ankete Dayalı Araştırma

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ABSTRACT

Objective: Canine leishmaniasis (CanL) is an important veterinary and public health problem in Mediterranean countries. Although CanL and vector sandflies are widespread in the study area, there are no standardized diagnostic and treatment methods followed by private clinics. This study aimed to survey (i) the treatment, diagnosis, and control measures preferred by veterinarians, (ii) compare the differences in the first-step diagnostic tests applied, and (iii) identify differences in the guidance for CanL.

Methods: This study was conducted between August 2017 and 2019, and an accessible weblink was distributed to veterinary clinics that were registered with the Veterinary Affairs of İzmir. The questionnaire was prepared by following published guidelines and several CanL guidelines and comprised 13 questions. A total of 103 veterinarians voluntarily participated in the study and completed the questionnaire.

Results: Most of the clinicians (n=99; 96.1%) reported that localized alopecia was one of the most common clinical findings in the suspicion of CanL, while only four clinician did not consider this finding in the suspicion of CanL. According to the answers received, 41 clinician (39.8%) frequently observed this finding in CanL-suspected cases, 49 clinician (47.6%) observed it occasionally, and nine clinician (8.7%) rarely observed this finding. The findings of the study indicate that a minority of clinicians employ advanced diagnostic techniques.

Conclusion: The results demonstrate that veterinarians play a unifying role in the fight against CanL. However, the preference for less advanced diagnostic methods over those employed by their European counterparts may result in insufficient detection of particularly asymptomatic patients. It is therefore necessary to validate rapid diagnostic tests, which are the most used method for diagnosing the disease. Furthermore, there is a need to increase awareness of the disease among patient owners and veterinarians in regions where it is endemic, and to recognise it as a notifiable disease in Türkiye.

Keywords: Dog, *Leishmania*, management, Türkiye

ÖZ

Amaç: Köpek leishmaniasis'i (CanL) Akdeniz ülkelerinde önemli bir veteriner ve halk sağlığı sorunudur. Çalışma bölgesinde CanL ve vektör kum sinekleri yaygın olmasına rağmen, özel klinikler tarafından takip edilen standart tanı ve tedavi yöntemleri bulunmamaktadır. Bu çalışmanın amacı (i) veteriner hekimler tarafından tercih edilen tedavi, teşhis ve kontrol önlemlerini araştırmak, (ii) uygulanan ilk adım teşhis testlerindeki farklılıkları karşılaştırmak ve (iii) CanL için rehber farklılıklarını belirlemektir.

Yöntemler: Çalışma, Ağustos 2017 ve 2019 tarihleri arasında gerçekleştirilmiş ve İzmir Veteriner İşleri Müdürlüğü'ne kayıtlı veteriner kliniklerine erişilebilir bir web bağlantısı dağıtılmıştır. Anket, yayınlanmış kılavuzlar ve çeşitli CanL kılavuzları takip edilerek hazırlanmış ve 13 sorudan oluşmaktadır. Toplam 103 veteriner hekim çalışmaya gönüllü olarak katılarak anketi değerlendirmiştir.

Türkiye PARAZİTOLOJİ Dergisi

Bulgular: Veteriner hekimlerin çoğu (n=99; %96,1) lokalize alopesinin CanL şüphesinde en sık görülen klinik bulgulardan biri olduğunu bildirirken, sadece dört veteriner hekim bu bulguyu CanL şüphesinde dikkate almamıştır. Alınan cevaplara göre, 41 veteriner hekim (%39,8) CanL şüphesi olan olgularda bu bulguyu sıklıkla, 49 veteriner hekim (%47,6) ara sıra ve dokuz veteriner hekim (%8,7) nadiren gözlemlemiştir. Çalışmanın bulguları, klinisyenlerin az bir kısmının ileri tanı teknikleri kullandığını göstermektedir.

Sonuç: Sonuçlar, veteriner hekimlerin CanL ile mücadelede birleştirici bir rol oynadığını göstermektedir. Bununla birlikte, Avrupalı meslektaşları tarafından kullanılanlara kıyasla daha az gelişmiş teşhis yöntemlerinin tercih edilmesi, özellikle asemptomatik hastaların yetersiz şekilde tespit edilmesine neden olabileceği görülmüştür. Bu nedenle, hastalığın teşhisinde özel klinikler tarafından en çok kullanılan yöntem olan hızlı tanı testlerinin doğrulanması gerekmektedir. Ayrıca, hastalığın endemik olduğu bölgelerde hasta sahipleri ve veteriner hekimler arasında hastalık açısından farkındalığın artırılmasına ve Türkiye'de bildirimi zorunlu bir hastalık olarak kabul edilmesine ihtiyaç vardır.

Anahtar Kelimeler: Köpek, *Leishmania*, yönetim, Türkiye

INTRODUCTION

Leishmaniases are a group of diseases caused by several species belonging to the genus *Leishmania*. Since the visceral clinical form mainly has a zoonotic character, natural reservoirs are important in the transmission cycle of the parasite. Both dogs and cats serve as reservoirs of the disease in endemic countries, and several *Leishmania* species [*Leishmania* (L.) *infantum, L. tropica*, and *L. major*] have been detected in both cats and dogs with different clinical manifestations (1-3). All infectious *Leishmania* species are capable of infecting dogs if they share the same geographic region and if the proven or possible vectors are present. A recent study revealed that the isolated *L. infantum* strains from feline leishmaniasis (FeL) cases are identical in terms of growth profile, survival capacity, and genotype to those isolated from human and canine leishmaniasis (CanL) cases (4). Canines, including domestic dogs, are the primary reservoirs of the disease in nature, and *L. infantum* is the most reported causative agent among the others (5). CanL is endemic in over 70 countries and is expanding its borders through the northern European countries due to effects of climate changes and vector spread. The disease is also reported in non-endemic countries like England, Germany, and Austria due to people traveling with their dogs. The distribution of CanL is identified as binomial, and the highest prevalence is reported between 0-3 years old and 8+ years old dogs (6-8).

Türkiye is one of the leishmaniasis endemic countries (9). Both cutaneous and visceral forms of leishmaniasis are present in Türkiye and, to date four causative agents (*L. infantum, L. tropica, L. major*, and *L. donovani*) have been reported (1,2). Visceral leishmaniasis (VL) caused by *L. infantum* is endemic in the Aegean, Marmara, Black Sea, and Mediterranean Regions of Türkiye and more than 20 cases have been reported annually. The cross-sectional surveys carried out in different endemic areas of Türkiye on CanL showed the high veterinary and public health importance of the disease (10). Field studies performed in recent years revealed that the proven vector species (*Phlebotomus tobbi*) is abundant in some endemic regions and the presence of the parasite has been demonstrated via by microscopy and molecular methods (11,12). Besides the other veterinary diseases, CanL is one of the most widespread diseases among both stray and owned dogs in Türkiye (1,13,14). According to recent studies performed in different parts of Türkiye, high molecular (46.66%) and serological (39.13%) positivity rates were reported (1). Each component of the transmission cycle (VL patient, active CanL cases, presence of vector sand fly, and detection of *Leishmania* DNA in vector species) was present in the studied areas, Kuşadası and Karaburun towns located in Ege Region of Türkiye (11,15). Also, several FeL cases were reported in Ege Region stating that there is intense parasite circulations between host and reservoirs occur (2).

Although CanL cases are prevalent in Türkiye, there is currently no mandatory standardized diagnostic or treatment/control approach used by veterinary clinicians in the country. Several diagnostic methods are used in the diagnosis of CanL for suspected cases. The inadequacy of clinical signs in infected dogs makes the use of specific tests necessary (16). Epidemiological and clinical strategies used in the diagnosis of the infection are based on serological and molecular methods (17). Serological tests such as IFA are considered the gold standard in CanL cases and should be performed regardless of clinical findings in endemic areas. As described by Baneth et al. (18), symptomatic cases are only the tip of the iceberg in CanL cases, and routine surveys may help dog owners understand the actual status of a particular region. The diagnosis power of clinician is strongly related to the laboratory infrastructure of the veterinary clinic. Surveillance studies conducted in Türkiye mostly use serological (IFAT) and molecular (ITS1 PCR) tests (1,13,14,19). Among the seven geographic regions, the Mediterranean region had the highest number of diagnosed CanL cases. Serological positivity was always higher in the studied groups compared to molecular tests. The use of molecular tools is on the rise in the diagnosis of CanL, but it is still considered expensive for suspected cases. Furthermore, the lack of trained personnel to perform serological and molecular tests is another reason for undiagnosed/misdiagnosed CanL cases in Türkiye. Since most positive cases do not exhibit any clinical findings, the reported incidence is always lower than the actual incidence (5,20,21). Due to the lack of an effective vaccine and the nature of the disease, integrated approaches such as vector control, the use of insecticide-impregnated collars to prevent sandfly bites, and the use of long-lasting insecticide-impregnated bed nets (LLINs) in endemic areas should be implemented to reduce the spread of the parasite (22).

Some of the measures taken to fight against CanL in different countries include culling (depending on government policy), isolation of infected dogs, and medical treatment for owned dogs. Although dog culling as a strategy to reduce CanL cases is applied in some Asian and South American countries, there is no solid scientific evidence to support its effectiveness in reducing VL incidence (22). Published reports by international study groups suggest that several effective measures such as use of insecticide-impregnated dog collars that might help reduce leishmaniasis transmission from dogs to humans by 48% in endemic areas (23).

Many international foundations and study groups have worked to establish the best practices for the diagnosis and treatment of CanL. To develop diagnostic and treatment guidelines, several symposiums and workshops have been organized, and informative handbooks have been distributed to veterinarians in the study areas. This study aimed to survey (i) the treatment, diagnosis, and control measures preferred by veterinarians, (ii)

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compare the differences in the first-step diagnostic tests applied, and (iii) identify differences in the guidance for CanL. Due to the high prevalence of CanL in the Aegean and Mediterranean regions, the questionnaire was limited to registered veterinary clinician located in these areas.

METHODS

Ethically Approval

No clinical material or data were used in this study. The Helsinki Declaration criteria were taken into account in the course of the study.

Background of the Study

Türkiye Leishmaniasis study group has been working in the field for over 20 years with the aim of implementing the one health approach. The group has conducted numerous surveillance studies on vectors, reservoirs, and human leishmaniasis. Given the high risk of CanL in the Mediterranean Region of Türkiye, several meetings and workshops were held with the participation of veterinarians in the study area before the present study. We assume that survey participants have attended one of these symposiums or meetings organized by the study group. Furthermore, guidelines and informative booklets were distributed free of charge to clinician before the study.

Study Design

This study was conducted between August 2017 and 2019 in the Mediterranean Region of Türkiye, and the population of the study included private veterinarians who work on CanL in endemic areas. A total of 109 forms were returned, but six were discarded for various reasons: Three for unknown clinician, one for a duplicate form, one for not receiving dogs, and one for being located outside of the study area. The questionnaire, which comprised 13 questions (eight multiple-choice, three open-ended, and two single select), was prepared by following the published guidelines and several CanL guidelines. To ensure that no different approaches were overlooked, several multiple-choice questions were included in the "other" option. The questions were divided into three major topics: 1) the profile and number of patients, 2) observed symptoms and applied diagnostic tests, and 3) the treatment, follow-up, and protection measures suggested by veterinarians to owners. Veterinary Affairs of İzmir evaluated the questions by following the published guidelines in Türkiye and Mediterranean countries.

To ensure that the participant clinic is in the study area and eliminate the possibility of duplicate forms filled by different veterinarians working at the same clinic, an informative question was asked concerning the name, location, and contact details of the clinic. The last question was open-ended and left for participants to add anything they wanted.

The questionnaire was transferred to the Google Forms system, and an accession link was distributed to all private veterinary clinician registered with Veterinary Affairs of Aegean Region. In 2017, the number of registered clinician was 174, and the accession link was sent to the email addresses of these clinician. Additionally, the accession link was announced on the website of İzmir Veterinary Affairs. All participating clinician were confirmed to be located in Aegean regions.

Statistical Analysis

Descriptive analysis of the data was reported in terms of frequency analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Version 21.0; IBM, Armonk, NY, USA).

RESULTS

Access to the questionnaire was activated in August 2017 and deactivated in August 2020 to analyze completed results. Due to the nature of questionnaire studies, the obtained results might not reflect the actual situation. Three clinician without contact details were also discarded. The given answers regarding treatment, diagnosis, and patient profiles were visualized (Figure 1).

1) The profile and the number of the patients

This section investigated general information about clinician and the type of animals admitted, including companion animals, livestock, exotic pets, or zoo animals.

1st question: What is the distribution of animals admitted to your clinic?

Of the evaluated answers, 84 (81.6%; 84/103) of them were receiving only pet animals, such as dogs and cats, and 19 (18.4%; 19/103) were receiving stray animals, such as dogs that were either abandoned or living on the streets without an owner.

2nd question: What is the weekly number of animals admitted to your clinic?

According to the answers received, 10 clinician (9.7%) reported receiving 1-10 patients per week, 23 clinician (22.3%) reported receiving 11-20 patients per week, 31 clinician (30.1%) reported receiving 21-50 patients per week, and 39 clinician (37.9%) reported receiving more than 50 patients per week.

3rd question: How many CanL suspected cases were admitted to your clinic in the last 12 months?

This question in the survey was aimed to gather information on the number of dogs suspected to have CanL at the participating clinician within the last 12 months. It is important to note that the answers provided by the veterinarians only reflect the initial suspicion and do not necessarily indicate a confirmed diagnosis. Out of the 103 clinician, 13 (12.6%) reported not receiving any CanL suspected cases. The remaining 90 clinician reported receiving dogs with suspicion of CanL as follows: 37 clinician (35.9%) received 1-5 dogs, 21 clinician (20.4%) received 6-10 dogs, 19 clinician (18.4%) received 11-20 dogs, 8 clinician (7.8%) received 21-50 dogs, and 5 clinician (4.9%) received more than 50 dogs with suspicion of CanL within the last 12 months.

4th question: Which clinical findings do you base the suspicion on and how often do you encounter those findings?

Based on the answers received for this question, the clinical findings that make clinicians suspect CanL and their frequency of occurrence were recorded. The veterinarians were asked to determine the frequency of several clinical findings, and each finding was classified as occurring frequently, occasionally, or rarely. The overall answers received showed that localized exfoliative dermatitis, ulcerative and/or non-ulcerative lesions, weight loss, exfoliative periocular alopecia and/or blepharitis, and onychogryphosis were the top five clinical findings observed by clinicians in CanL suspected cases.
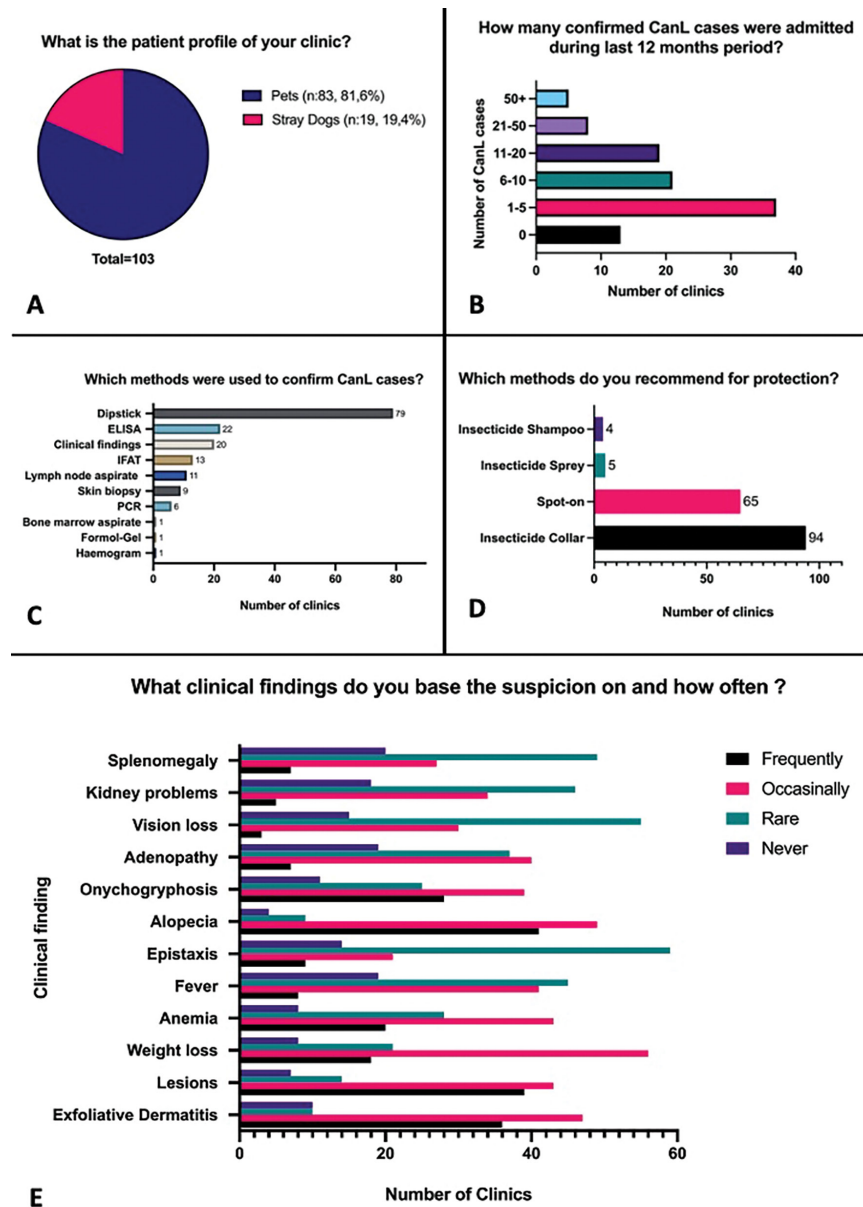
Figure 1. Several answers were visualized as; A) Patient profile of the attended clinician (Question 1), B) The number of confirmed cases during last 12 months period (Question 5), C) Methods used for CanL diagnosis (Question 7), D) Suggested methods by clinician to control CanL (Question 13), and E) Which clinical findings do veterinarians based their diagnosis and how often (Question 4). Graphics were prepared by GraphPad Prism V.9

PCR: Polymerase chain reaciton, ELISA: Enzyme-linked immunosorbent assay, CanL: Canine leishmaniasis

Most clinicians (n=99; 96.1%) reported that localized alopecia was one of the most common clinical findings in the suspicion of CanL, while only four clinician did not consider this finding in the suspicion of CanL. According to the received answers, 41 clinician (39.8%) frequently observed this finding in CanL suspected cases, 49 clinician (47.6%) observed it occasionally, and nine clinician (8.7%) rarely observed it.

Ulcerative and non-ulcerative lesions were other findings stated in the question, and of the 103 participants, 96 clinician (93.2%) reported that the presence of cutaneous lesions is one possible clinical finding in the suspicion of CanL, while seven clinician (6.8%) do not consider this finding in the suspicion of CanL.

Of the received answers, 39 clinician (37.9%) frequently noted cutaneous lesions in CanL suspected cases, 43 clinician (41.7%) occasionally noted them, and 14 clinician (13.6%) rarely noted them.

Weight loss was reported frequently by 18 clinician (17.5%), occasionally by 56 clinician (54.4%), and rarely by 21 clinician (20.4%). A total of 95 clinician reported that loss of appetite and weight is one of the findings that make them suspect CanL in suspected cases, while eight clinician (7.8%) do not take this finding into account in the suspicion of CanL.

Exfoliative dermatitis, another clinical finding listed in this question, was notably detected by clinicians (n=93; 90.3%),

while 10 clinician did not report exfoliative dermatitis in CanL suspected cases. According to the received answers, 36 clinician (35%) frequently, 47 clinician (45.6%) occasionally, and ten clinician (9.7%) rarely noted exfoliative dermatitis in CanL suspected cases.

Lastly, 92 clinician (89.3%) reported that they observed onychogryphosis in CanL suspected cases. Eleven clinician did not report onychogryphosis in CanL suspected cases. Twenty-eight clinician (27.2%) frequently, 39 clinician occasionally (37.9%), and 25 (24.3%) clinician rarely noted onychogryphosis in suspected cases.

2) Observed symptoms and applied diagnostic tests

5th question: How many confirmed CanL cases were admitted to your clinic in the last 12 months?

Thirteen clinician (12.6%) reported that they did not have any confirmed cases of CanL during the last 12 months. The remaining clinician (n=90; 87.4%) had at least one confirmed case of CanL. The responses were grouped as follows: 37 clinician (35.9%) had received 1-5 cases, 21 clinician (20.4%) had received 6-10 cases, 19 clinician (18.4%) had received 11-20 cases, eight clinician (7.8%) had received 21-50 cases, and finally five clinician (4.9%) had received more than 50 confirmed cases of CanL during the last 12 months period.

6th question: How many of the confirmed cases were new? (diagnosed over 12 months ago).

Twenty-two of the attended clinician (21.4%) stated that none of the confirmed cases were new. Of the reported new cases, 52 of the clinician (50.5%) stated 1-5, 21 clinician (20.4%) were stated 6-10, four clinician (3.9%) stated 11-20, three clinician (2.9%) stated 21-50, and lastly one clinic (1%) stated more than 50 of the confirmed cases were new.

7th question: Which methods were used to confirm CanL cases?

Several options were provided in the question, and multiple answers were recorded. The three most applied methods were as follows: Rapid diagnosis kit (dipstick) (n=79; 76.7%), ELISA tests (IgG Commercial Kits) (n=22; 21.4%), and clinical findings only (n=20; 19.4%).

8th question: Where did you diagnose the CanL case?

The majority of the attending veterinarians (n=83; 80.6%) stated that they applied diagnostic methods in their clinician. Twenty-two of the veterinarians (21.4%) stated that they sent obtained samples to a private laboratory, and ten clinician (9.7%) sent obtained samples to the nearest veterinary faculty.

9th question: Have these confirmed cases been infected in the area where you work?

According to the received answers, the majority of the clinician (n=72; 69.9%) believed that CanL cases were infected in the area where they worked, while 24 clinician (23.3%) believed that positive cases were infected in another region. Additionally, seven clinician (6.8%) declared that they had no idea about this question.

10th question: Where are those positive dogs kept?

The response to the question was provided in three options. Fifty-two of the clinician (55.3%) stated that diagnosed dogs are kept indoors, 12 clinician (11.7%) stated that dogs are kept outdoors, and 30 clinician (29.1%) stated that diagnosed dogs are kept both indoors and outdoors. Additionally, four clinician declared that they have no idea about this question.

3) The treatment, follow-up, and protection measures suggested by the veterinarian

11th question: What do you use first in treatment?

This question was open-ended, and clinician were able to write any combination of treatments used in their clinician. Of the collected answers, 85 clinician (92.4%) claimed that their first choice of treatment for CanL is Allopurinol. Ten clinician (7.8%) reported using Miltefosine as their first choice of treatment.

12th question: What do you think about the rate of CanL among your patients over the last 10 years?

According to the answers collected from this question, 59 clinician (57.3%) stated that they believe CanL cases have been on the rise over the last 10 years, while 33 clinician (32%) did not report any changes in the number of CanL cases. Only six clinician (5.8%) reported that there has been a decline in CanL cases over the last 10 years period. Five clinician (4.9%) did not answer this question.

13th question: Which methods do you recommend to dog owners for protection?

This question was prepared with multiple choices, and more than one answer was recorded for each clinic. The vast majority of veterinarians (n=94; 91.3%) who answered this question recommended the use of insecticide-impregnated dog collars. Out of 94 clinician recommending the use of insecticide-impregnated dog collars, 61 clinician (59.2%) suggested the use of both a collar and spot-on for protection. Only four clinician did not suggest the use of insecticide-impregnated dog collars for protection.

DISCUSSION

Canine leishmaniasis, caused by *Leishmania infantum*, is endemic in the Aegean, Marmara, Black Sea, and Mediterranean Regions of Türkiye (13). It is of the greatest importance to be aware of the prevalence of the disease in endemic sites in order to gain an understanding of its epidemiology, given the close relationship between dogs and humans and the life cycle of the parasite (24). Various studies conducted in different districts of Türkiye have shown the presence of the parasite in different hosts and vector sand flies (2,10,25,26). In line with these studies, 31.1% of the veterinarians who participated in the survey detected more than 10 CanL cases annually, and 57.3% of them emphasized that the CanL cases had increased in the last 10 years. One possible reason for the increase in CanL cases in the Aegean region might be the changing vector sand fly density, as previously reported by our study group (27,28). According to a recent report by the Aegean Region Municipality, it is estimated that 400,000 stray dogs were living in either indoor or outdoor conditions, and the number of stray dogs annually increased by almost 5,000 (29). Of the 103 veterinarians who participated in the survey, 84 (81.6%) provided consulting services to adopted pet animals, while the remainder worked for stray animals in kennels. In the present study, 57 (55.3%) and 30 (29.15%) of the dogs were staying in outdoor and indoor/outdoor conditions, respectively. Therefore, it can be assumed that 87 (84.4%) of the dogs might stay in suitable outdoor conditions during twilight, evening, and night-active biting times of sand flies. Considering all the factors mentioned above, the rise in CanL cases is not a surprising result.

CanL is a chronic disease that manifests with symptoms such as weakness, reluctance, anemia, generalized lymphadenopathy, dermatitis, alopecia, onychogryphosis, epistaxis, and

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asymptomatic proteinuria (17,18). However, the number of dogs showing clinical signs of the disease is believed to be only the tip of the iceberg, especially in endemic areas, as dogs can be infected without showing any symptoms (1,15). In the survey, the veterinarians reported that localized hair loss, weakening, and the presence of persistent wounds in various parts of the body were often associated with CanL in their detailed physical examinations. This finding is consistent with similar studies conducted in other countries (30,31). *Leishmania* invades its host viscero-cutaneously and can cause skin problems such as dermatitis, hair loss, non-healing wounds in various parts of the body, and scaling. However, it should be noted that other vector-borne parasitic diseases, bacteria, neoplasia, metabolic or autoimmune diseases can also cause similar symptoms. Epistaxis was reported as a rare symptom found in only 4% of the cases in the literature (32), but according to the survey results in Slovenia (33), 54% of the veterinarians reported that they frequently observed epistaxis. Veterinarians often suspect CanL with abnormal nail growth and anemia, but fever and poor renal prognosis are rarely attributed to CanL by the participants. Immunocomplex deposition in the renal glomeruli is a symptom with high prognostic value together with ocular disorders, providing information about the poor progression of the disease. Suspicious clinical findings are widely reported in the literature, and even a single concordant sign in endemic regions could be a strong indicator for CanL (31,32,34-37).

The clinical findings of the infection are mostly insufficient in the diagnosis of the disease, and advanced diagnostic methods are needed for the correct diagnosis (16). In the management of CanL, it is important to perform a differential diagnosis to exclude other vector-borne or non-vector-borne diseases that may cause skin lesions (32). Although direct microscopy of the parasite is a definitive diagnostic method, it is not commonly used in clinician due to several reasons, such as the invasive nature of skin and lymph puncture sampling procedures required to visualize the causative agent, patient owners' preference, low sensitivity (38), and dependence on the skill of the user (39). Therefore, veterinarians participating in the study are unlikely to choose this method for diagnosis in their clinician. The flexibility of dog owners in allowing diagnostic tests to be performed in veterinary clinician can significantly affect the frequency and number of detected cases (30). In the diagnosis of CanL, it is known that the interpretation of serological or molecular techniques such as indirect fluorescent antibody test (IFAT) and polymerase chain reaction (PCR), which have been frequently used in recent years, along with clinical findings and blood biochemistry, will increase the accuracy of diagnosis. However, ELISA-based rapid diagnostic kits, which take less time than other methods in diagnosis and allow for fast therapeutic measures, are insufficient for diagnosis alone, and are frequently used by veterinarians as a preliminary diagnostic method in clinician. Serological testing is used in up to 95% of symptomatic dogs which often have high antibody levels, and the tests are easy and inexpensive to perform. However, most assays fail to detect low antibody titers and to diagnose asymptomatic cases (17,40). The fact that serological testing is the preferred first diagnostic method by veterinarians may provide inaccurate information about the actual infection rate (20). Therefore, the disease should be followed up with a quantitative serological or molecular method (31,41). When evaluating the diagnostic methods used by the veterinarians in the study, it was found that 86.4% and 93.8% did not prefer the IFA test and PCR methods, respectively. Furthermore, it was determined that 99% of the attending physicians did not use blood biochemistry values, which are considered valuable for clinical staging in CanL and recommended by LeishVet guidelines (https://www.leishvet.org/) as an aid in diagnosis. Although IFAT is described as the gold standard method for diagnosing clinical cases and ELISA is a highly sensitive and specific test for clinically expressed CanL, they are not sufficient for detecting asymptomatic cases. In Spain, Slovenia, Italy, and France, studies have shown that IFA and ELISA tests are used more frequently to detect CanL than in Türkiye. Therefore, it is important to consider the limitations of these methods and use quantitative serological or molecular tests to monitor the disease progression accurately (20,31,33,42,43). While most veterinarians diagnose the disease in their clinician, it is noticed that private laboratories and laboratories of veterinary faculties, where more equipped and advanced diagnostic tests can be performed, are much less preferred. Hence, it should not be overlooked that the number of animals found to be sick or infected with *Leishmania* spp. might be underreported.

Despite the high sensitivity and specificity of ELISA, IFA, and PCR methods, 76.7% of the veterinarians who participated in the study reported that they frequently use rapid diagnostic tests due to their ease of use in field conditions. While high specificity and sensitivity are essential for veterinarians to make an accurate diagnosis of the disease, numerous rapid diagnostic kits have been developed for this purpose. One of the rapid kits developed for this aim is the rk39 immunochromatographic test strip, which is produced based on the rk39 antigen. The rk39 antigen is a 39 amino acid repeat B-cell epitope in a protein conserved between *L. infantum* and *L. donovani* (44). Among the rapid diagnostic kits, the rk39 test is widely used. Courtenay et al. (45) determined the sensitivity of the rk39 test to be 78% in their study and evaluated this value as low for use in effective prevention programs. The low overall sensitivity of rk39 to detect infection suggests that it is not an effective tool for estimating the prevalence of the disease or for identifying infected dogs in control programs (46). Dual-Path Platform (DPP®), another rapid diagnostic kit used in the field, detects antibodies against *L. infantum* rk26/rk39 fusion protein by colloidal gold-based immunochromatography. Grimaldi et al. (47) stated that the DPP kit is effective in detecting dogs with severe disease and may be a diagnostic marker for active disease, but it has a very low sensitivity in detecting asymptomatic dogs. SNAP® Canine Leishmania antibody test, which is prepared by purifying antigens of *L. infantum* promastigotes, is another rapid diagnosis kit frequently preferred by veterinarians. It was reported by Souza et al. (48) to have higher sensitivity and specificity, and it was stated in their study that it would be a good alternative to DPP®. Despite the low sensitivity to detect infected dogs, the rapid kit's high specificity and the brief time between sampling and results make it a preferred choice for veterinarians. Encouraging the use of further diagnostic tests in addition to the use of rapid kits will contribute to more effective detection of the disease and thus control programs. Moreover, further research is needed to develop more sensitive rapid kits using different antigen combinations and to increase the validation of currently used kits. Increasing the diagnostic accuracy will contribute to the fight against the CanL by ensuring that the agents to be selected in the treatment of the disease are used appropriately and effectively.

The most used antileishmanial agent by the surveyed veterinarians was allopurinol (either alone or in combination with other drugs) (79.6%). Although a synergistic effect was reported for the combination of drugs (49), only 4.9% of veterinarians in our study used a combination of antimony and allopurinol in treatment, possibly due to the route of administration and high cost. When examining the results of surveys conducted in other countries, it is observed that the majority of veterinarians in Portugal and Slovenia, as well as Türkiye, prefer to use allopurinol alone in treatment. However, the combined use of allopurinol and antimony is very widespread in France, Italy, and Spain (31). In the present study, only 3% of veterinarians used allopurinol and domperidone, which were reported to be more effective when used together as a CanL treatment protocol (50). A recently published study conducted in Spain (51) shows that Spanish veterinarians use this treatment protocol more often than their Turkish counterparts. Fortunately, amphotericin B, which is the first choice in human leishmaniasis cases but poorly tolerated by dogs and not suitable for veterinary use (31), has not been included in any practitioner's treatment protocol in this study. Miltefosine, which is not licensed in Türkiye yet, is included in the treatment protocols of 7.71% of our survey participants, who use it together with allopurinol. Obtaining a license for Miltefosine in Türkiye in the future will ensure that the combination of Miltefosine and Allopurinol, which is recommended by LeishVet and ESCCAP, will be used more and may increase the success of the treatment (5,52). While some similarities in practices exist, the different treatment protocols used in various countries underscore the need to standardize CanL treatment, particularly considering the parasite's drug resistance, to effectively control both human and canine leishmaniasis.

In all guidelines concerning public and veterinary health, the importance of preventive measures against CanL is emphasized (30). However, the results of this study indicate that a significant proportion of veterinarians (50.5%) who responded to the questionnaire did not have access to any presentations, brochures, or guidelines regarding the management of the disease published by the World Organization for Animal Health or other organizations such as LeishVet. This highlights the need for improved dissemination and accessibility of information and guidelines related to the prevention and management of CanL among veterinary professionals (53). The majority of veterinarians who participated in the study preferred the use of insecticide-impregnated collars as a preventative measure, as they can reduce the likelihood of vector flies feeding on the animal and increase their mortality rate (54,55). The use of insecticide-impregnated collars as a prophylactic measure is crucial in interrupting the biological cycle of *Leishmania* species by preventing vector-host communication. The majority of veterinarians participating in the study preferred this control method, as it reduces the blood-sucking of vector flies and their chances of survival, thereby contributing to the prevention of both canine and human leishmaniasis cases. Recommending or using insecticide-containing collars to prevent dogs from encountering vector flies, regardless of their health status, is an important step in preventing a disease that poses a threat to public health (32,56). In addition, a majority of veterinarians (66.3%) in the study favored using topical spot-on insecticides as a preventive measure to protect dogs from the disease. A recent study has reported that the combined use of topical insecticides and insecticide collars gave more effective results in the fight against sand flies. However, some veterinarians (5.1%) recommended alternative, non-specific control measures such as shampoos, sprays, and other insect repellents (57). Besides this, 2.91% of the participants expressed apathy towards the risk of CanL and cited the cost of treatment as a factor. In contrast, studies conducted in European countries have shown that most veterinarians are concerned about the disease and prefer using topical insecticides or repellents as a preventive measure (30,58,59).

The risk of leishmaniasis is closely tied to public and animal health, so further research should focus on increasing awareness of guidelines and promoting standardization in practice through greater cooperation between the two professions, utilizing the one health approach (42). Additionally, guidelines on the disease should be translated into native languages to make them more accessible, and more frequent updates incorporating current ecological and epidemiological data can lead to more effective disease control (20). The questionnaire responses suggest that quantitative serological and molecular methods, which are critical in CanL diagnosis, are underutilized. Therefore, veterinarians should be encouraged to perform these tests more frequently, whether in endemic regions or not. This can help identify unnoticeable cases, provide more accurate information on seroprevalence and epidemiology, and indirectly improve public health. Veterinarians should also inform pet owners about the health effects of CanL, which is closely related to public health.

In addition to chemical or biological agents, traditional methods such as keeping doors-indoors at night when flies are active and protecting pets with mosquito nets should be recommended in the fight against CanL. Advising pet owners on these measures can increase awareness and aid in disease prevention.

CONCLUSION

This article provides an overview of the current clinical management of CanL in endemic regions of Türkiye based on data reported by private veterinarians. While the study reveals that many veterinarians follow similar practices in preventing and managing CanL, the lower preference for advanced diagnostic methods compared to their European counterparts may lead to a lack of detection of asymptomatic patients. Therefore, it is crucial to emphasize the following points: (i) CanL should be a notifiable disease in the veterinary field, (ii) private veterinarians' knowledge about the disease should be increased, and (iii) commercial rapid diagnostic tests should be validated in different endemic regions of Türkiye.

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