The Use of Spatial Analysis to Estimate the Prevalence of Canine Leishmaniasis in Greece and Cyprus to Predict Its Future Variation and Relate It to Human Disease

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Abstract. Climatic, environmental, and demographic changes favor the emergence of neglected vector-borne diseases like leishmaniasis, which is spreading through dogs, the principle host of the protozoan *Leishmania infantum*. Surveillance of the disease in dogs is important, because the number of infected animals in an area determines the local risk of human infection. However, dog epidemiological studies are costly. Our aim was to evaluate the Emerging Diseases in a Changing European Environment (EDEN) veterinary questionnaire as a cost-effective tool in providing reliable, spatially explicit indicators of canine leishmaniasis prevalence. For this purpose, the data from the questionnaire were compared with data from two epidemiological studies on leishmaniasis carried out in Greece and Cyprus at the same time using statistical methods and spatial statistics. Although the questionnaire data cannot provide a quantitative measure of leishmaniasis in an area, it indicates the dynamic of the disease; information is obtained in a short period of time at low cost.

INTRODUCTION

Leishmaniasis is a vector-borne disease presenting a serious public health concern in 98 countries and three territories worldwide.[1,2] It presents a wide range of clinical symptoms, and the visceral form is life-threatening if not treated. In the Mediterranean basin, *Leishmania infantum* is responsible for visceral leishmaniasis (VL). Dogs are the major reservoir host, and sandflies of the genus *Phlebotomus* and subgenus *Larroussius* are the vectors. In southern Europe, ~700 autochthonous human cases are reported every year, with an average of 25% seroprevalence in domestic dogs.[3] The disease in dogs (CanL) is characterized by a variety of clinical symptoms, some of which are very severe[4]; however, many infected animals are asymptomatic.[5,6] Because the higher the number of infected dogs in an area, the higher the chance of human infection, it is necessary to know the prevalence and geographical distribution of the disease in dogs to be able to assess the risk of human VL infection in that area.[7] To achieve this assessment, epidemiological surveys are required, which are costly and time-consuming and necessitate trained personnel. To overcome these problems, a veterinary questionnaire (VQ) was constructed by the Emerging Diseases in a Changing European Environment FP6 EE Project (EDEN-Leish subproject; www.eden-fp6project .net/) addressed to veterinarians to collect useful information on the disease in dogs in the area of their practice.

The aim of this study was to validate this questionnaire in the Cyprus Republic and Greece, countries where *L. infantum* infections seem to be spreading in the dog population.[8–10] To assess the questionnaire's value for epidemiological studies and monitoring practices, the data collected by the questionnaire were compared statistically and by spatial analysis with the results of two epidemiological surveys carried out at the same time as the VQ study in Cyprus[9] and Greece[10] using Geographic Information System (GIS) technology.

MATERIALS AND METHODS

Study location and dog samples. The study location was the government-controlled part of the island of Cyprus (5,896 km²; comprising five prefectures with an estimated dog population of ~100,000)[9] and Greece (131,957 km²; comprising 54 prefectures with an estimated dog population of ~700,000)[10] (Figure 1A). Epidemiological studies on leishmaniasis were conducted in Cyprus in 2005 and 2006, during which time 900 randomly selected dogs were tested. Epidemiological studies on leishmaniasis were conducted in Greece from 2007 to 2010, during which time 5,772 randomly selected dogs were tested. Seroprevalence was estimated by the indirect immunofluorescent antibody test (IFAT) with cutoff titers of 1/200 and 1/160 in the two studies, respectively. The overall seropositivity rate for Cyprus was found to be 11.8%, with some areas reaching 33%[9]; the overall seropositivity rate for Greece was 22.09%, with some areas reaching 50.2%.[10]

The dog seroprevalence data, obtained during these two epidemiological studies, were used to compare and evaluate the estimates of the incidence of the disease based on the results of the VQ collected at the same time as the epidemiological studies.

The VQ. The EDEN-Leish VQ "Veterinary survey on epidemiology and control of canine leishmaniasis," consisting of 14 questions (Figure 2), was sent to all private veterinarians in Cyprus (43 private veterinary clinics operate in the country) and Greece (~640 private veterinary clinics operate in the country) during the time that the two epidemiological studies were conducted. The questionnaire was accompanied by a letter explaining the aim of the project and a prepaid envelope for returning the questionnaire. After 1 month, a reminder was sent to the veterinarians that had failed to reply. Three months later, a telephone call was made to the veterinarians that had not responded to our request.

The questionnaire required 10–15 minutes and consisted of 14 brief multiple choice questions (Qs; Q1–Q14). They were grouped into four sections regarding (1) the type of practice (Q1–Q3), (2) epidemiological observations by the veterinarian in the area of practice relating to CanL (Q4–Q7, Q10, and Q11), (3) clinical and laboratory diagnostic methods used to confirm the disease (Q8 and Q9), and (4) measures proposed
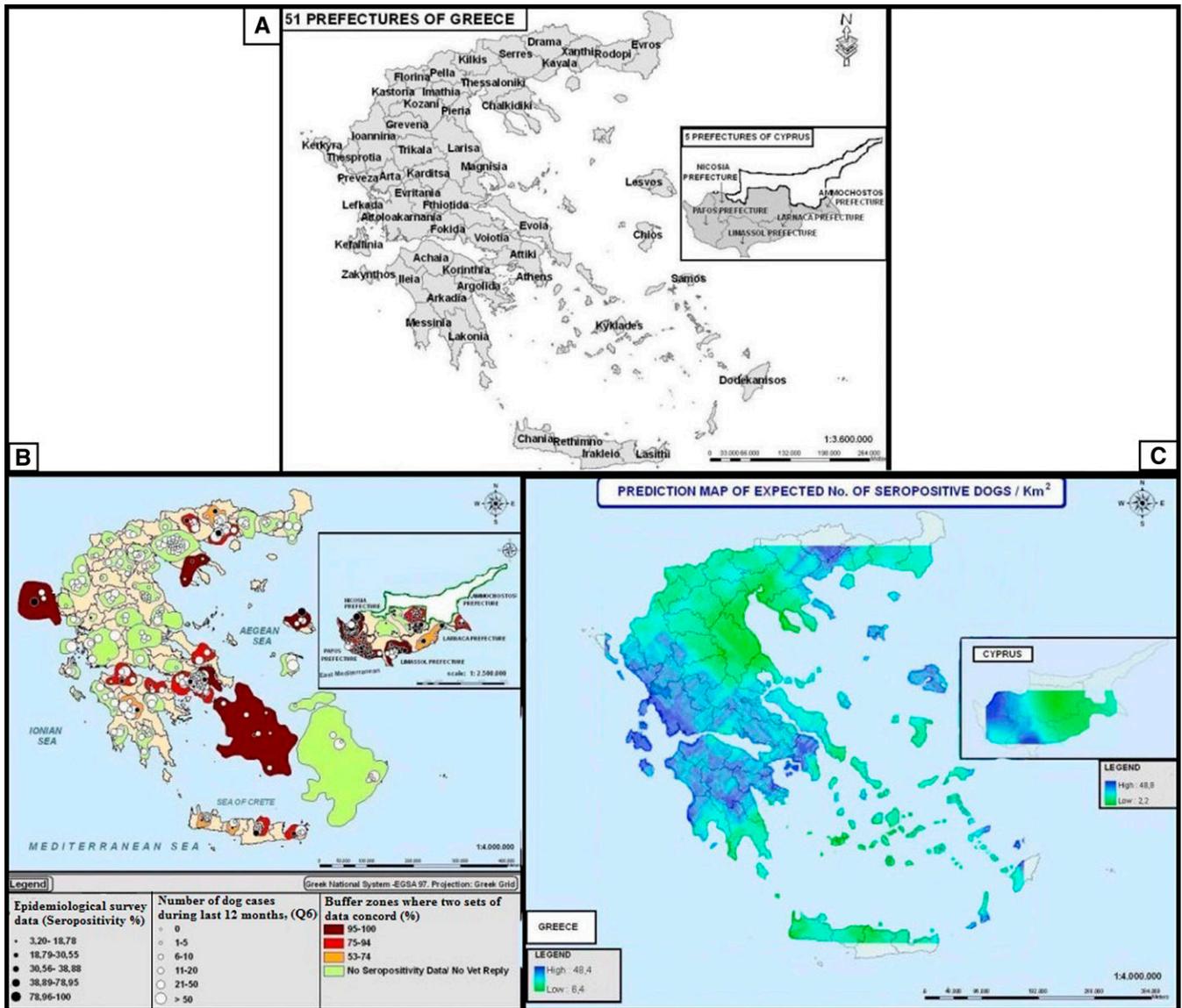

FIGURE 1. (**A**) The study area of Greece and Cyprus showing prefectures. (**B**) Comparison of the two sets of data (dog seropositivity data obtained from two epidemiological surveys[9,10] and the VQ data) on the local level by spatial analysis (GIS software). In the locations where both sets of data were available, the data matched by 53–100%, with the majority showing over 75% concordance. (**C**) Predictive maps of areas at high risk of the spread of CanL prepared with VQ data compared with those maps based on dog seroprevalence data.[9,10]

by the veterinarian to dog owners to protect their animals from sandfly bites (Q12–Q14).

Statistical and spatial analysis of the results. Dog seroprevalence from both surveys was mapped using GIS software (Arc Map 9.3; ESRI, Redlands, CA) (Figure 1B). The postal addresses of the veterinary practices were georeferenced using the Magellan SporTrak Global Positioning System (GPS; Magellan Navigation, Santa Clara, CA) and mapped according to the number of dogs examined per week by the practice (Q3; indicating the size of practice in relation to dog clientele) (Figure 1B). Spatial analysis was used to compare VQ results with dog seropositivity data from the two previous studies. Dog seroprevalence in the Cyprus study was estimated and mapped (using GIS; ArcGIS 9.2, Redlands, CA) for each of five prefectures as well as 30 smaller areas within

the five prefectures[9]; in the Greek study, however, dog seroprevalence was estimated and mapped (using GIS; ArcGIS 9.2, Redlands, CA) for each of 54 prefectures.[10] A grouping of the numbers of infected dogs (Q6: requiring the number of confirmed CanL cases in the veterinarian's practice during the last 12 months in relation to Q3 [the number of examined dogs per week]) was compared with and related to the grouping of dog seropositivity in the area of the veterinary practice (epidemiological study data). The classification of dog seropositivity data was applied according to the method of natural breaks in the Arc map. This method was chosen (instead of a spatial clustering models method; e.g., Moran's Index), because our goal was the classification of the two sets of data (dog seropositivity data and VQ data) based on their present distribution and not to identify clusters and trends. This

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Veterinary questionnaire on the epidemiology of canine leishmaniasis

Number of questionnaire........

1. Veterinarian's name and address:..
2. Type of clientele: Small animals ☐ Rural ☐ Mix ☐ other ☐
   If other, specify:...
3. How many dogs do you examine per week?
   0 ☐ 1-3 ☐ 4-10 ☐ 11-20 ☐ more than 20 ☐
4. How many dogs with suspicions symptoms for leishmaniasis have you examined during the previous 12 months?
   0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ 21-50 ☐ more than 50 ☐
5. Which of the listed clinical symptoms do you consider valuable for CanL diagnosis?

Symptom	Frequency			Diagnostic Value (+, ++, +++)
	Rare	Frequent	Very Frequent	
Emaciation				
Hardship				
Anaemia				
Hyperthermia				
Epistaxis				
Localised alopecia				
Dermatitis furfuracea				
Onychogryphosis				
Ulcers				
Lymphadenopathy				
Ocular lesions				
Renal failure signs				
Splenomegaly				
other				

If other, specify: ....
6. How many **confirmed** CanL cases did you have during the last 12 months?
   0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ 21-50 ☐ more than 50 ☐
7. How many of the **confirmed** CanL cases were new cases?
   0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ 21-50 ☐ more than 50 ☐
8. Which methods did you use to confirm the CanL cases?
   Epidemiological data ☐   Clinical Symptoms ☐
   Serology ☐: IFAT (Indirect Immunofluorescence) ☐

   ELISA ☐
   Rapid detection Kit ☐
   PCR ☐
   Parasitological observation ☐ of: Lymph node biopsy   ☐
                                        Bone marrow biopsy   ☐
                                        Skin biopsy   ☐
                                        Other ☐ specify:..............................
9. The laboratory diagnosis took place at: In your practice ☐
                                            In a private diagnostic laboratory ☐
   In the Veterinary Services Laboratory, of the Ministry of Agriculture ☐
   Other ☐ specify:..
10. Do you believe that the dogs became infected in your area of activity? yes ☐   no ☐
11. What, in your opinion, is the trend in CanL prevalence, in your clientele, during the last 10 years?   It has increased ☐   It has diminished ☐   It is stable ☐
12. What measures do you recommend your clients to use, to avoid sandflies biting their dogs ?
    Colar ☐   Spot on ☐   Spray ☐   Shampooing ☐   none ☐
13. If you do not recommend prophylactic measures, is because :
    You do not think they are effective ☐   They are too expensive ☐
    They are not easy to find ☐   The risk of CanL is not important ☐
    Other, ☐ specify: ....
14. Do you have informative material regarding antiparasitic formulations/practices, with emphasis on sandflies?   yes ☐   no ☐

We thank you for your collaboration.

FIGURE 2.   The EDEN-Leish VQ "Veterinary survey on epidemiology and control of canine leishmaniasis."

method seeks to partition data into classes based on natural groups in the data distribution. Natural breaks occur in the histogram (in which y = dog seropositivity and x = location) at the low points/valleys. Breaks are assigned in the order of the size of the valley, with the largest valley being assigned the first natural break.[11,12]

In total, 405 questionnaires and 155 sets of dog seropositivity data were collected. When relatively little data were available from an area (less than five questionnaires were returned or dog seropositivity data for < 60 dogs were obtained), the data of that area were excluded from the analysis. Using the GIS software, VQ and dog seropositivity data (from the two previous studies that were mapped according to the prefecture/area in which the dog lived) were mapped and compared; similarities and dissimilarities were identified and recorded using spatial methods and basic spatial statistics. Dog seropositivity in each area was chosen as the actual criterion for testing the reliability of the VQ (Figure 1B).

Spatial data from the EDEN database (www.eden-fp6project.net/) were used for the analysis (i.e., geodata including the coastline of Greece and Cyprus, prefecture areas, villages, and cities). Several methods were applied to test if, where, and to what degree the VQ data were consistent with the dog seropositivity data. Buffer zones for both sets of data were constructed, defining an influence area as a region (polygon) of 500-m radius (an area around a feature in a map measured in units of distance or time).[13] The buffer areas were established using Boolean algebra in the GIS environment.[14] These areas were then grouped together according to their degree of spatial concordance.[13] Three different layers were combined to establish the final buffer zones: the VQ data (Q6), the dog seropositivity data, and the buffer zones created previously for each set of data. Each area was then characterized as of strong concordance (95–100%), medium concordance (75–94%), or no concordance (53–74%). Dog seropositivity in areas where no seroepidemiological studies had been conducted was estimated based on the VQ data (Q6). According to the spatial prediction model, most of the areas represented by VQ data only were expected to have dog seropositivity levels from 3% to 55% (Table 1). Areas in which < 60 dogs were examined during the seroepidemiological studies were considered as areas of no concordance. Seroprevalence in such areas was estimated by the weighted mean[15] combined with the VQ data (Q6) (Table 1).

A prediction map was then created using VQ data (Q6) and the geostatistical method of Kriging interpolation for continuous quantitative data (a method that predicts unknown values from data observed at known locations).

Estimation of dog seropositivity in areas of no concordance, based on questionnaire data obtained from veterinarians and a small number of dogs (n < 60), estimated by the weighted mean that is based on weighted numbers of examined dogs and numbers of positive dogs per prefecture

Prefecture	Estimated seropositivity based on VQ data (optimal interval; %)	Seropositivity based on examined dogs when $N < 60$ (%)	Estimated seropositivity based on VQ data and examined dogs when $N < 60$ (%)
Greece			
Evros	10–15	14.29	13.00
Rodopi	29–30	29.17	29.00
Xanthi	50–55	59.09	54.50
Thessaloniki	38–40	39.53	38.00
Florina	5–7	6.45	6.00
Imathia	30–35	33.33	33.00
Thespotia	20–25	33.33	25.00
Trikala	10–15	13.16	13.50
Karditsa	15–20	15.79	15.50
Magnisia	25–30	100.00	35.00
Fthiotida	33–47	47.06	46.00
Aitoloakarnania	35–40	66.67	39.00
Fokida	30–35	33.33	33.00
Voiotia	3–10	8.33	7.00
Argolida	30–35	66.67	33.00
Chios	40–50	60.00	45.00
Dodekanisos	40–55	78.95	42.00
Cyprus			
Nicosia	3–8	0	

Areas in which < 60 dogs were examined during the seroepidemiological studies were considered as areas of no concordance. Seroprevalence in such areas was estimated by the weighted mean[15] combined with the VQ data (Q6 and Q3).

This method uses variograms to express spatial variation and minimizes the error of predicted values.[15]

RESULTS

Of 683 questionnaires distributed (640 questionnaires in Greece and 43 questionnaires in Cyprus), 445 questionnaires were returned (each questionnaire represented one veterinarian and his clientele): 414 (64.7%) questionnaires from Greece and 31 (72.1%) questionnaires from Cyprus; 253 and 16 questionnaires were returned during the first month, respectively; 120 and 0 questionnaires were returned after a postal reminder, respectively; and 41 and 15 questionnaires were returned after a telephone call, respectively. Overall, 226 and 12 veterinarians refused to oblige, respectively. Most of the responders' practices were located in big cities, and the rest were in large towns (Q1) (Figure 1B). The non-responders' practices were located in cities where other veterinarians had responded. The data of the two countries will be presented as a unified area with 59 prefectures for practical reasons.

Most of the veterinarians (265 of 445) reported mainly canine clientele (Q2), and overall, 158 of 445 veterinarians saw more than 20 dogs per week (Q3). All veterinarians had examined dogs with suspicious symptoms of CanL during the previous 12 months (Q4), and 106 of 445 veterinarians examined over 50 such dogs. Most of these cases were confirmed by clinical diagnosis and laboratory tests, and only five veterinarians reported that none of their clinically suspected cases was confirmed by laboratory tests (Q6).

The most frequent clinical symptoms reported by veterinarians (Q5) were localized alopecia (373 of 445) and lymphadenopathy (364 of 445). Most CanL cases were confirmed by serology (91.1% using enzyme-linked immunosorbent assay [ELISA], 16.2% using IFAT, and 27.7% using a rapid detection kit), clinical examination (51.6%), microscopy (12.9%), epidemiological information (25.8%), polymerase chain reaction (PCR; 6.5%), or a combination of more than one method (Q8). The majority of the veterinarians (290 of 445) replied that they perform the diagnostic tests in their own clinic or serum samples are sent to a central diagnostic laboratory for testing (e.g., for ELISA; Q9). The majority of the veterinarians (301 of 445) believed that all confirmed dogs had been infected in their area of practice (Q10).

Nearly all veterinarians recommended prophylactic measures to their clients to protect dogs from sandfly bites: insecticide-impregnated collars (376 of 445), spot-on insecticides (323 of 445), insecticide sprays (308 of 445), or shampooing (34 of 445; Q12); 11 of 445 veterinarians that did not recommend prophylaxis believed that leishmaniasis is not important in their area (Q13). Despite understanding the importance of prophylactic measures, only 194 of 445 veterinarians had informative material regarding sandfly control for handing out to clients (Q14).

During the last 10 years, 227 of 445 veterinarians reported an increase in CanL cases in their area, 119 of 445 veterinarians reported a stable situation, 39 of 445 veterinarians reported a decline, and 60 of 445 veterinarians did not provide an opinion (Q11). In the χ^2 model with five or more or less than five confirmed cases per year reported by the veterinarians, the risk of a dog being infected was found to differ significantly according to the following variables: (1) geographical origin of the dog ($\chi^2 = 15.08$, degrees of freedom [df] = 2, $P = 0.001$), (2) the number of dogs examined by the veterinarians with suspicious symptoms for CanL ($\chi^2 = 17.39$, df = 1, $P < 0.001$), and (3) the number of primary infections in the dogs ($\chi^2 = 28.00$, df = 1, $P < 0.001$).

Of all areas compared using VQ and seroepidemiological data, 81% of areas were of a high degree of spatial and real concordance (buffer zones of 75–100%, 30.8% of which were buffer zones of 95–100% concordance), and 19% of areas had no concordance, which was, however, because of the lack of one set of data (Figure 1B and Table 1). The concordance map showed that, in Greece, there are higher levels of dog seropositivity in the north, on several islands, such as Chios, Kefalinia, and Evoia, and in Attiki. In Cyprus, higher levels of dog seropositivity were observed in the southwest.

It was found that predicting the variation of spread of CanL using the questionnaire data and a small number of randomly selected dogs (< 60) could provide a reliable estimate of the CanL situation in an area. According to the model, most such areas are expected to have dog seropositivity levels of 3–55% in Greece and 3–8% in Cyprus (Table 1).

The prediction map, based on the VQ data, shows the spatial variance of the estimated number of infected dogs per area of 50 km[2] for both countries (Figure 1C), which proved to be similar to the distribution of dog seropositivity data from the two epidemiological surveys (Figure 1B). The prediction map indicates the current situation with the areas of high risk (where the number of infected dogs is high or expected to rise in the near future, even in areas where no data were available) (Figure 1C).

DISCUSSION

With autochthonous cases of CanL and VL emerging in new foci in Europe, such as northern Italy[16] and southern

Germany,[17] it is essential that epidemiological studies are carried out frequently to follow the spread of the disease in dog populations. However, the cost of such surveys in money, time, and trained personnel can be an obstacle to the surveys being carried out frequently enough.

The EDEN-Leish VQ was created to overcome this problem. The results of two epidemiological surveys, carried out in Cyprus[9] and Greece,[10] were used to test and evaluate a survey based on the EDEN-Leish VQ carried out in these countries at the same time using spatial analysis. In the locations where data were available from both the VQ and dog seroprevalence studies, the two sets of data matched by 53–100%, with the majority showing over 75% concordance (Figure 1B). Predictions as to the spatial and temporal patterns of expansion of CanL in the two countries as a whole based on the VQ data indicated high-risk areas (Figure 1C) that matched areas with high dog seroprevalence.[9,10] Such high-risk areas are the areas where CanL and VL pose the highest risk and were found to be Attiki, Peloponisos, Kavalla, Kerkira, and the western regions of Greece and Cyprus as well as some areas in southern Cyprus. They are the areas reported to have the highest current number of infected dogs and human cases.[9,10] The prediction map shows that leishmaniasis will be a more intense phenomenon in the future in both countries, because numbers of seropositive dogs are incrementally escalating[8–10] (Figure 1C). Predictive maps are useful, because they indicate the spread of the disease and its establishment in new areas; therefore, control measures can be planned at an early stage and implemented in good time to control the dispersal of the parasite and its vectors. Because of the complexity of the factors governing the distribution of this disease, the predictive map shows a high degree of local variation, which GIS analysis is able to take into account.

Although the VQ may not provide a quantitative measure of the problem in an area, it gives a strong indication of the spatial and temporal dynamics of the prevalence of the disease locally and in the whole country. Such information is important for veterinary and public health and useful for reservoir monitoring. However, such a tool has its limitations, especially regarding veterinarians' responses to Q10 and Q11, which may be prone to bias. For this reason, areas of interest that emerge from an epidemiological study using the VQ should be further investigated by a cross-sectional survey.

The VQ offers a two-way exchange of information between the research team and the veterinarians. By providing the information requested, the veterinarians become alert and aware of what is considered important by the specialists. The information and contact are valuable, especially in areas where no studies on CanL are taking place and where the veterinarians may not be aware of the seriousness of the problem or the risk of CanL being introduced in their area. Given that the veterinarians play a key role in the fight to control zoonoses, the network developed through the questionnaire can prove useful in such efforts. The specialists, however, gain valuable information from the veterinarians' experiences on the means available to them in the laboratory diagnosis of CanL and the correctness of the clinical symptoms that they use for diagnosis. It is an important fact, because it has been shown that dogs with at least three clinical symptoms have the highest risk of being PCR-positive for *Leishmania*.[18] Another important piece of information provided by the VQ is the value and effectiveness of the preventive measures used in the area of the veterinarian's practice. Thus, valuable information can be obtained in a relatively short time with very little cost.

With climatic and other changes favoring the emergence of leishmaniasis in new areas, the application of a tool, such as the VQ, combined with GIS technology facilitates examination of risk factors and explores endemicity in areas where dog surveys have not previously been carried out, as was shown in Murcia, Spain.[19] The VQ can be used as an early warning system and a screening tool to provide the portrait of leishmaniasis in a country/region and follow-up changes to safeguard public health. If needed, dog seropositivity rates can be estimated more precisely in the areas of interest using a small number of randomly selected dogs (= 60) combined with the questionnaire data. It is evident that this combination of information can provide a cost-effective and reliable indication of seroprevalence in a particular area (Table 1).

This approach is also likely to be cost-effective in monitoring the course of the disease in human populations as well, because dogs, the main reservoirs of *L. infantum* and *L. chagasi*, play a key role in the transmission of the parasite to humans.[20] As a consequence of interventions for reducing the number of infected dogs in different areas, human VL incidence was shown to drop considerably.[21,22]

The EDEN-Leish VQ proved to be a reliable, cost-effective tool for locating, recording, and even predicting the spread of CanL in an area. Our findings support the view that the VQ approach can be integrated into health services to safeguard public health.

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