Veterinary Parasitology 333 (2025) 110350

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Updating the epidemiology of canine leishmaniosis in the United Kingdom through the use of electronic health data

Rocío Checa [a,*], Fernando Sánchez-Vizcaíno [b,1], Guadalupe Miró [c], Gina Pinchbeck [d], Hayley Jones [d,2], Peter-John Noble [d], Alan David Radford [d]

[a] Microbiology and Parasitology Department, Faculty of Pharmacy, Universidad Complutense of Madrid, Pl. de Ramón y Cajal s/n, Madrid 28040, Spain
[b] Bristol Veterinary School, University of Bristol, University of Bristol, BS40 5DU Langford Campus, Bristol, United Kingdom
[c] Animal Health Department, Veterinary School, Universidad Complutense of Madrid, Avda. Puerta de Hierro s/n, Madrid 28040, Spain
[d] Small Animal Veterinary Surveillance Network (SAVSNET) and Institute of Infection, Veterinary and Ecological Sciences, Veterinary School, University of Liverpool, Leahurst, Neston CH64 7TE, United Kingdom

ARTICLE INFO

Keywords:
Dog
Electronic health records
Leishmania infantum
United Kingdom
SAVSNET

ABSTRACT

Dogs infected with *Leishmania infantum* have been increasingly reported in the UK mostly related to imported/travelled dogs. Up-to-date epidemiologic data are essential for a better control of this zoonotic disease in such emerging areas. This study aimed for the first time, to estimate the percentage and temporal variation of dog and cat samples testing positive for *L. infantum* infection at commercial diagnostic laboratories, and to describe the travel history of positive dogs and cats positive to leishmaniosis in a network of UK veterinary practices. *Leishmania infantum* serology and PCR data were collected by the Small Animal Veterinary Surveillance Network (SAVSNET) from five UK national veterinary diagnostic laboratories between 2010 and 2022 and were analysed. In addition, electronic health records (EHRs) were collected from 251 veterinary practices across the UK between March 2014 and September 2022. Text mining tools were used to identify cases compatible with clinical leishmaniosis as recorded in the clinical narratives; these were subsequently manually validated. Data from a total of 25,327 diagnostic samples (25,201 from dogs and 126 from cats) were analysed including 20,517 sera tested by either quantitative ELISA or IFAT, and 4810 by PCR. *Leishmania infantum* antibodies were detected in 39.7 % of tested dog samples and 1.07 % of cat samples. In dogs, seropositivity increased from 2013 to 2022. *Leishmania* DNA was only detected by PCR in samples from dogs (11.8 %). A total of 368 dogs with canine leishmaniosis (CanL) were identified from clinical narratives. Of these, 189 had either visited, or were rescued/imported from, Spain, Greece, Cyprus and other southern European countries. Among factors associated with CanL in the UK canine population, dogs between 3 and 6 years of age were 4.71 times more likely to have CanL than those two years or younger. In addition, there was an increased risk of having recorded CanL clinical cases from 2017 to 2022, compared to 2014. Southeast of England was the UK region that accounted for the highest number of CanL cases (34.51 %). This study provides recent trends in *Leishmania* infection in dogs in the UK, identifies risk factors and countries likely associated with imported cases, and provides important insights to help plan and monitor national intervention strategies.

1. Introduction

Canine leishmaniosis (CanL) is a widespread zoonotic endemic disease in Europe, primarily at the Mediterranean basin, where *Leishmania infantum* is its causative agent and dogs the main peridomestic reservoir for human infection (Baneth et al., 2008; Miró et al., 2008). Moreover, *L. infantum* may infect a wide range of mammals, including cats (Alcover et al., 2021), horses (Leonel et al., 2021), wild carnivores (red foxes, badgers or wolves) (Del Río et al., 2014) and other wild mammals living in zoo parks (Miró et al., 2018). The parasite is transmitted to humans

* Corresponding author.
E-mail addresses: rocichec@ucm.es (R. Checa), f.svizcaino@bristol.ac.uk, fsanchezvizcaino@ucam.edu (F. Sánchez-Vizcaíno), gmiro@ucm.es (G. Miró), ginap@liverpool.ac.uk (G. Pinchbeck), Hayley.Jones3@liverpool.ac.uk (H. Jones), rtnorle@liverpool.ac.uk (P.-J. Noble), alanrad@liverpool.ac.uk (A.D. Radford).
[1] Veterinary School, UCAM Catholic University of Murcia, 30107 Murcia, Spain
[2] Department of Public Health, Policy and Systems, Institute of Population Health, University of Liverpool, L69 3GF, Liverpool, UK

https://doi.org/10.1016/j.vetpar.2024.110350
Received 11 July 2024; Received in revised form 8 November 2024; Accepted 9 November 2024
Available online 10 November 2024

and animals by blood-sucking phlebotomine sandflies (Killick-Kendrick, 1990). Additionally, other non-vectorial routes (e.g. blood transfusion, vertical, venereal) can be involved in the *Leishmania* transmission (Ferreira-Silva et al., 2018; Naucke et al., 2016). In the United Kingdom, the presence of a competent sandfly vector species for CanL has not been reported so far; hence, this parasitic infection is not considered an endemic disease.

Leishmania infantum infection in dogs can cause an often fatal disease with a wide range of general clinical manifestations including generalized lymphadenomegaly, lethargy and weight loss. Following the infection, various cutaneous, ocular, vascular and neurological disorders may develop. In most cases, clinicopathological disorders compatible with this disease are present (LeishVet, 2024). Usually, sick dogs need long-term therapy and monitoring throughout life (Solano-Gallego et al., 2009). This implies that for the correct clinical management of CanL both committed owners and veterinarians with knowledge about this disease are needed.

Specific tests are available for the diagnosis of *L. infantum* infection in dogs showing clinical signs suggestive of CanL. In addition, they may also be carried out to detect infection of apparently healthy dogs travelling to, or from, endemic CanL areas (blood donors, breeding dogs, prior to CanL vaccination, imported dogs or as part of the annual check of leishmaniosis in dogs residing in an endemic area). Direct CanL tests include the microscopical identification of the intracellular form of the parasite (amastigotes) and the molecular detection of parasite DNA from infected tissues by PCR (e.g., bone marrow, lymph node, skin, spleen, etc.). PCR is a more sensitive method than microscopy (Solano-Gallego et al., 2009). Indirect tests are based on detecting anti-*L. infantum* IgG in serum samples. Among these, quantitative techniques are the method of choice, with the enzyme-like immunosorbent assay (ELISA) and immunofluorescence antibody test (IFAT) being most common (World Organisation for Animal Health (WOAH), 2021).

In non-endemic areas such as the United Kingdom, CanL may represent a challenge for practitioners due to the wide range of clinical manifestations and a relative lack of clinical familiarity. However, both globalization including increasing travel of dogs as well as climate change may promote the emergence of canine vector-borne diseases in new geographical areas. Most *L. infantum* infected dogs found in non-endemic areas have been relocated from, or travelled with their owners, to endemic regions (Maia and Cardoso, 2015; Teske et al., 2002). Indeed, over recent years, *Leishmania* infected dogs showing clinical signs or clinicopathological abnormalities have been reported in the UK (Shaw et al., 2009; Silvestrini et al., 2016); in large part, these dogs have been infected in CanL endemic countries, being either dogs "rescued" from continental Europe and re-homed in the UK or dogs that accompanied their owner on holidays to CanL-endemic countries. In addition, a recent study found that 14.8 % of overseas rescue dogs in the UK tested positive for *L. infantum* (Norman et al., 2020).

Regarding dogs travelling in Europe, the non-commercial movement of companion animals (dogs, cats, and ferrets) is regulated under the European Union (EU) Pet Travel scheme (PETS; EU Reg. 576/2013), which include rabies vaccination, treatment against *Echinococcus multilocularis*, EU Pet Passport with Animal Heath Certificate and microchip. A more restrictive scheme governs the movement of companion animals from a shelter between Member States when it implies the transfer of ownership of the animal following the Balai Directive (EU 92/65/ECC). This regulation includes a veterinary health check within 48 hours before travel and a health certificate issued by an Official Veterinarian. Interestingly, 89 % of imported rescue dogs came to the UK under PETS (Norman et al., 2020).

Diseases such as CanL may be more easily controlled in countries like the UK, where competent vectors are absent. However, additional non-vectorial routes of transmission may be involved both in endemic and non-endemic areas, such as blood transfusion, vertical, venereal, and dog-to-dog transmission (Duprey et al., 2006; Ferreira-Silva et al., 2018; Naucke et al., 2016; Naucke & Lorentz, 2012; Silva et al., 2009; Vida et al., 2016). In fact, between 2019 and 2020, three autochthonous cases (dogs with no travel history) were reported in the UK. Two of these cases were most likely associated with dog-to-dog transmission and the other with vertical transmission (McKenna et al., 2019; Wright and Baker, 2019; Wright and Moral-Gant, 2020). Nevertheless, updated information on the scale of *Leishmania* infection in resident dogs within the UK and their geographical distribution is lacking.

Using health data from a large sentinel network of UK veterinary practices and laboratories, this study aimed, for the first time, to estimate (i) the percentage and temporal variation of dog and cat samples testing positive for *L. infantum* infection at participating commercial diagnostic laboratories, and (ii) to describe the clinical picture, treatment and travel history of dogs with recorded leishmaniosis in veterinary practices. Such national level statistics are fundamental if control of this pathogen is to be achieved.

2. Material and methods

This study used data collected between 2010 and 2022 by the Small Animal Veterinary Surveillance Network (SAVSNET) on leishmaniosis in pet dogs and cats from two different sources: commercial veterinary diagnostic laboratories and veterinary practices in the UK (Fig. 1). Both practices and laboratories are recruited by convenience and represent a sentinel network for data collection across the UK (Tulloch et al., 2017). The selected time for the two datasets was the maximum possible for which records were available. SAVSNET has been collecting EHRs from veterinary practices since March 2014, while electronic data from veterinary diagnostic laboratories were available from 2010 onwards. Data collection and use by SAVSNET is ethically approved by the University of Liverpool Research Ethics Committee (RETH000964).

2.1. Laboratory data collection

Leishmania infantum serology and PCR data from five veterinary diagnostic laboratories were collected electronically between June 2010 and October 2022 and were analysed. Within that period, 25,327 relevant test results were recorded from 25,201 dog and 126 cat samples as follow: 20,424 dog serology results obtained by ELISA (n=15,275) or IFAT (n=5149); 4777 dog PCR results; 93 cat serology results obtained by ELISA (n=63) or IFAT (n=30) and 33 cat PCR results (Fig. 1).

Test result data were collected from canine and feline samples submitted by UK veterinary surgeons specifically for the diagnosis of *L. infantum* infection to the laboratories participating in SAVSNET. Therefore, most of the samples may correspond to a dog with a clinical suspicion of leishmaniosis, either due to presenting compatible clinical signs or because they have travelled or are going to travel to an endemic area for CanL. Duplicated and discordant results were removed when repeated tests were conducted on the same sample. However, due to the sensitivity of these data, each tested sample could not be linked to the individual dog of origin, therefore samples taken from the same animal at different time points cannot be identified and may have been included in the analysis.

For samples tested, the following electronic health data were captured: species, breed, sex and age of the animal, date of the diagnosis, diagnostic technique, test result and postcode area of the submitting veterinary practice.

Specific serological and molecular tests were interpreted according to the reference range established by the corresponding laboratory. Results from quantitative ELISA and IFAT assays were collected to detect specific serum antibodies (IgG) against *L. infantum*. The serology dataset was analysed by classifying the dogs as positive or negative, as the quantitative data were not comparable between laboratories. Molecular diagnostic methods (PCR and qPCR) were used to detect *Leishmania* spp. DNA. For DNA positive samples, the cycle threshold (Ct), number or number of parasites per ml of blood or, number of parasites per million cells were also collected where available. The samples tested by PCR

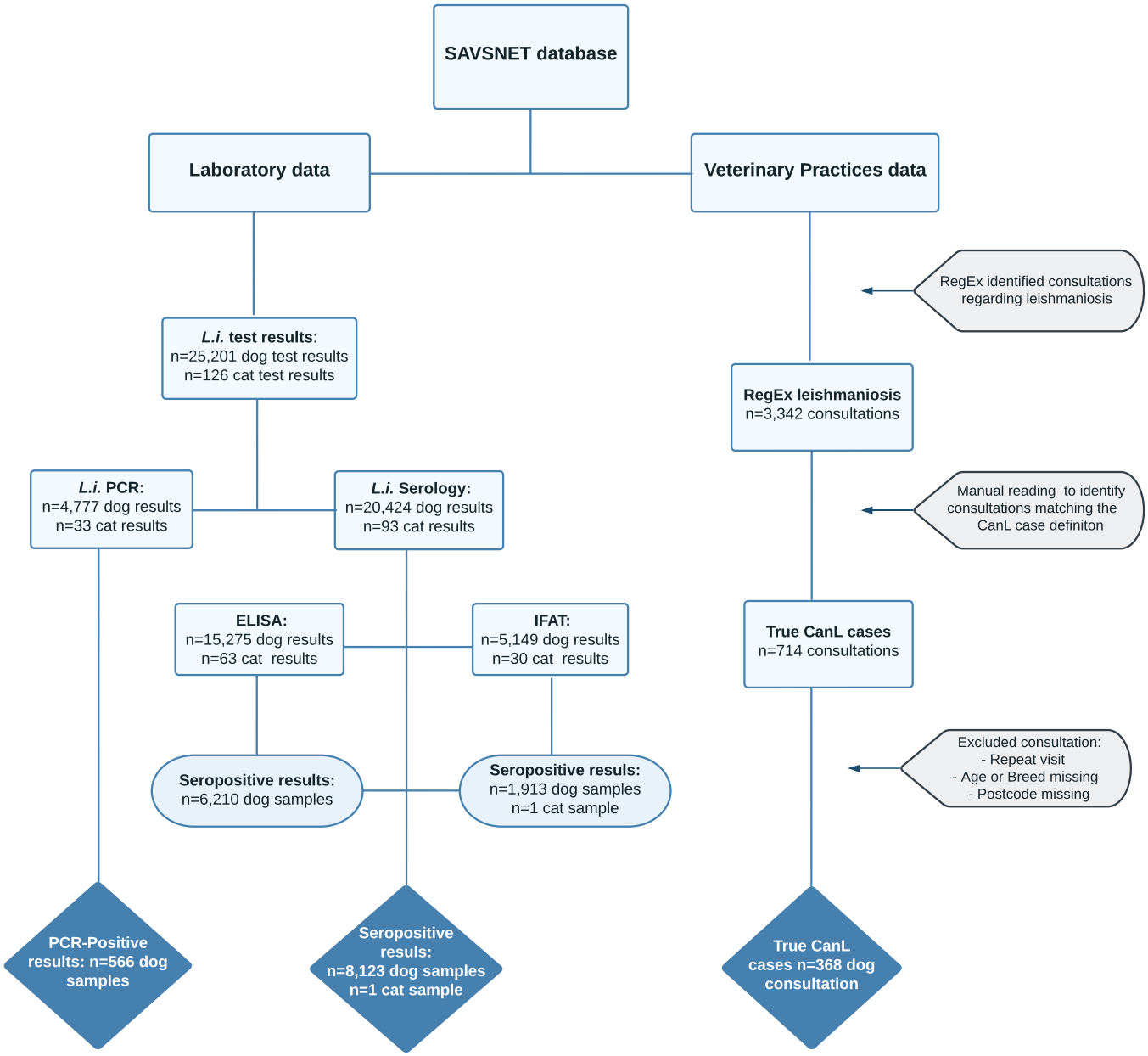

Fig. 1. Diagram of the data extraction process from SAVSNET. Classification of the data from veterinary diagnostic laboratories according to the diagnostic technique used and the positive or negative result, and the criteria used to extract consultations where a case of CanL was identified in the clinical narratives.

included blood, skin biopsy, bone marrow or lymph node aspirates and conjunctival swabs; this information was not recorded for all samples.

2.2. Veterinary practice data collection

Electronic health records (EHRs) were collected in real-time through SAVSNET from 251 volunteer veterinary practices across the UK between March 2014 and September 2022 (Brant et al., 2021). Briefly, SAVSNET collects data on consultations including the age, sex, species and breed of the patient, any treatments prescribed, the postcode of the owner, and the free text clinical narrative written by the attending practitioner.

Text mining tools based on regular expressions (regex) were used to identify suspect leishmaniosis clinical cases based on text in the clinical narratives (supplementary material 1); retrieved consultation narratives were subsequently read by a veterinarian to identify those consultations that met the case definition (Fig. 1), namely (i) a sick animal tested positive for leishmaniosis using microscopy, serology and/or PCR, or (ii) an animal on treatment for CanL (in which case we presumed a previous diagnosis), or (iii) an animal that had received treatment for leishmaniosis and was being monitored. For each unique consultation that met the case definition additional data were retrieved including sex, neuter status, breed, age, date of the consultation, treatment, and the owner's post-code. Using each unique animal identification number, all consultations from the same animal were extracted to generate a more complete picture of leishmaniosis cases in this population including clinical signs, any treatment administrated, outcome, and any recorded travel history.

To determine risk factors for recorded leishmaniosis cases, a retrospective case-control study was carried out. Control consultations that were not identified by the regex were randomly selected from the entire canine SAVSNET database at a ratio of 1 case: 5 controls.

2.3. Statistical analysis

Data captured for dog samples tested (the diagnostic laboratory dataset) were analysed using the software package R (version 4.2.2). Descriptive proportions of positive dog samples tested by serology and/or PCR (number of positive samples divided by the total dog samples submitted for *Leishmania* infection/disease diagnosis) and the confidence intervals were calculated (Wilson method). Seven categorical variables were included: breed, sex, reproductive status and age of the dog, geographical region of submitting veterinary practice (NUTS 1 level), month and year of the sample submission. Due to the large number of dog breeds, this categorical variable was summarised to standardised breed terms according to the *Fédération Cynologique Internationale* (FCI) (https://www.fci.be/en/) and then categorised into thirteen breed groups: ten groups according to breed aptitude, crossbreeds, unclassified (breeds not yet classified) and unknown (breed not recorded). When samples from the same dog and day were submitted for diagnosis by both PCR and serology (samples with the same identification number), a single sample was included in the analysis: a dog was considered positive when it had a positive result either by PCR or IFAT; and negative when both tests were negative. Subsequently the chi-square test was used to assess associations between categorical variables and the *L. infantum* positive result. Significance was set at $P < 0.05$. The standardized residuals were calculated to assess the strength of the difference between observed and expected values. When the standardized residual was ≥ 2, then a strong significant difference was considered.

For the retrospective case-control study (the veterinary practices dataset), univariable and multivariable logistic regression models were performed, using R version 4.2.2 software. Significance was also set at $P < 0.05$. The same seven explanatory categorical variables as for the analysis of the diagnostic laboratory dataset were included, but in this case with recorded data from the CanL cases (sex, neutered status, age, FCI breed group, NUTS 1 level region where the dog lived, month and year of the consultation). An initial multivariable regression model included all variables (Ireland (NUT 1 level: UKN) was removed from the multivariable regression due to there being no cases); a backward selection method was used. The variable chosen to remove was decided from computing an Analysis of Variance (ANOVA) on the regression coefficients after each logistic regression model to assess the significance of the variables left in the model and removing the variable with the highest p-value. This process was repeated until all variables left in the model were significant. Interaction terms of the variables were assessed separately. However, none were found to be significant and therefore were not included in subsequent models. The Akaike information criterion (AIC) scores for each model were then calculated (for the initial model, and every model produced until the final reduced model). The reduced model had the lowest AIC score, suggesting it has a good fit to the data. This reduced model and the initial model were then compared using ANOVA to see if the fit of the reduced model is good enough at capturing the data compared to the initial model. The reduced model had a statistically significant ($P = 0.041$) fit to the data. This reduced model was then used to calculate the odds ratios and their 95 % confidence intervals (CI).

The software QGIS (version 3.22.) was used for spatial analysis. For the laboratory and practice datasets, the postcode of the submitting vet or owner respectively was grouped at NUTS1 level (*Nomenclature des Unités territoriales statistiques*).

3. Results

3.1. Laboratory data

During twelve years (2010–2022), 25,327 samples were submitted to five veterinary diagnostic laboratories in the UK for *Leishmania* diagnostic testing (combined ELISA, IFAT, or PCR; 25,201 dogs and 126 cats).

Anti-*Leishmania* antibodies were detected in 39.7 % (95 % CI: 39.1 % – 40.4 %) of dog samples tested (8123/20,424) by either ELISA or IFAT over the twelve-year in which the data were collected (2010–2022; Fig. 1). The results using ELISA were as follows: 40.6 % of samples were positive (6210/15,275), 53.0 % negative (8096/15,275) and 6.3 % borderline (969/15,275); for the seropositive samples, 39.3 % (2439/6210) showed antibodies levels (measured as ELISA values) four times higher than the cut-off established by the testing laboratory. The IFAT results were similar with 37.1 % testing positive (1913/5149) of which 61.2 % (1171/1913) showed low-medium antibody titres (1:100 – 1:400), while 38.7 % (742/1913) showed high titres (1:800 – 1:12,800).

In contrast to dogs, data were only available for 93 cat samples tested by either ELISA or IFAT giving only one seropositive result (1.07 %: 95 % CI: 0.05 %–6.6 %) by IFAT with a low titre level (1/50).

Leishmania DNA was detected by PCR in 11.8 % (95 % CI: 10.9 %–12.8 %) of dog samples tested over the time of this study (566/4777; Fig. 1). In 371 of the 566 dog PCR-positive samples, the level of *Leishmania* DNA was quantified as low (21.0 %: 78/371), moderate (59.5 %: 221/371) or high (19.4 %: 72/371) based on either the Ct number (n=123) or the number of parasites per ml of blood (n=103), or the number of parasites per million cells (n=8), depending on the units used by the veterinary diagnostic laboratory where the assay was performed. Parasite DNA was not found in any of the 33 cat samples submitted.

The total number of samples submitted for *Leishmania* infection/disease diagnosis increased during the study period (Fig. 2) due to the growing number of laboratories participating in the SAVSNET network over the years. From 2010–2015, diagnostic data were available from one veterinary laboratory, while since 2015, data were collected from four laboratories, and finally, since 2019 data were collected from five laboratories. However, regardless of the number of laboratories contributing data, the number of samples submitted and recorded at each individual laboratory also exhibited an increasing trend during the study period.

Temporally the proportion of submitted samples positive for *L. infantum* infection showed an increase over the years of the study. This trend is especially strong due to increased seropositivity of *L. infantum* observed in tested dogs, from 31.5 % in 2013 (100/317) to 42 % (1529/3642) in 2022 (Fig. 3). In contrast, the PCR results showed a modest decrease in the proportion of PCR-positive samples from its peak in 2015 (16.1 %; 48/229) until 2022 (13.4 %, 54/403) (Fig. 3). Furthermore, there was a slight decrease in the proportion of positive samples observed during the COVID-19 pandemic (in 2020 and 2021) in both PCR-positive and seropositive samples.

The positivity of the samples did not show any monthly seasonal pattern with positive samples observed throughout the year. February and June (59/395 and 64/429, respectively) were the months with the highest number of PCR positive samples recorded, whilst the maximum number of seropositive samples were observed in February, May and July (Fig. 4).

The majority of positive results were found in samples from dogs attending veterinary practices located in the East and South of England (Fig. 5), showing significant statistical differences from the North East (UKC) and London (UKI) regions ($p<0.001$) (Table 1).

3.1.1. Epidemiological data on positive dogs

The 25,201 dog samples submitted for either PCR or serological leishmaniosis diagnosis included 965 dogs for which samples were submitted for both analyses simultaneously, leaving a final population of 24,236 tested dogs. Of these, 7989 were female (32.9 %), 9190 male (37.9 %) and 7057 unknown (29.1 %); 4255 were entire dogs (17.6 %) while there were 11,563 neutered dogs (47.7 %) and 8418 unknown (34.7 %); 8321 were from pure-breed dogs (34.3 %), 10,368 from crossbreed dogs (42.8 %) and 5547 without breed recorded (22.9 %).

A significantly higher proportion of positive dogs were Pointing dogs (FCI breed group 7: continental pointing dogs, Pointer, and Setters) and

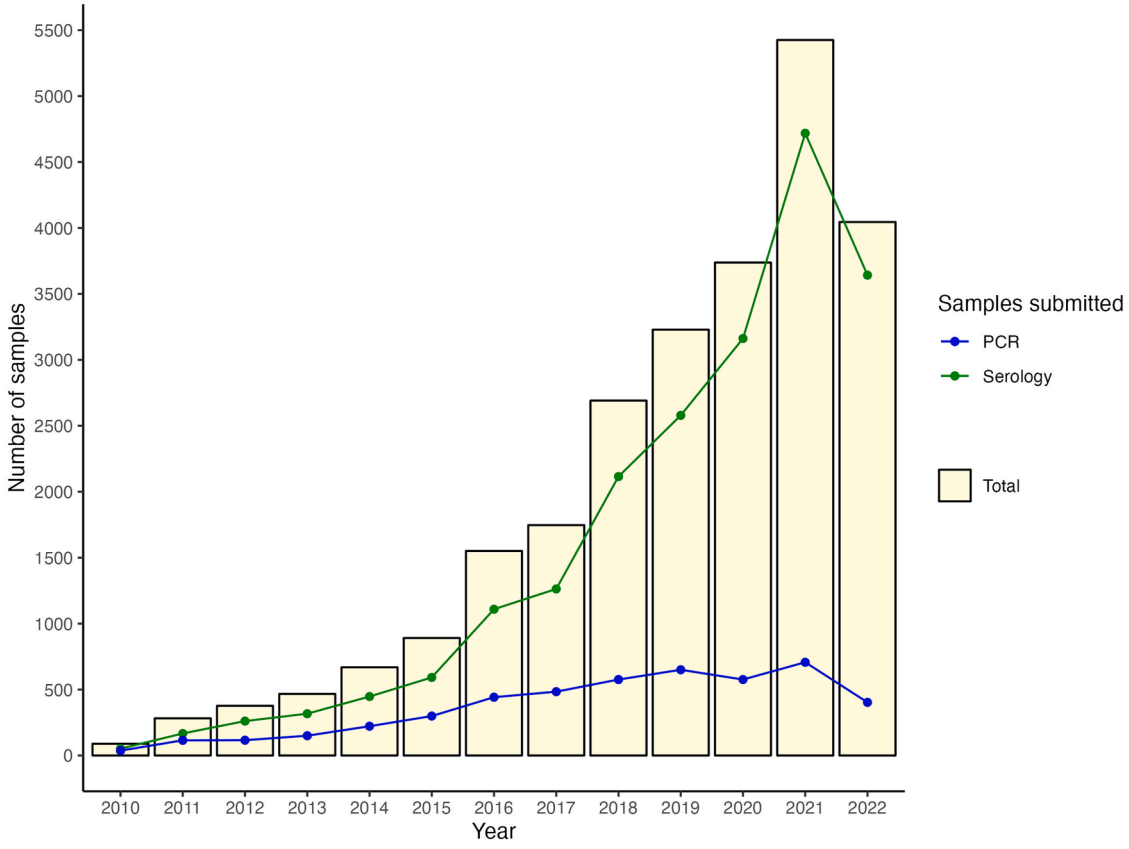

Fig. 2. Number of UK canine samples submitted annually to participating laboratories for a diagnosis of leishmaniosis between 2010 and 2022.

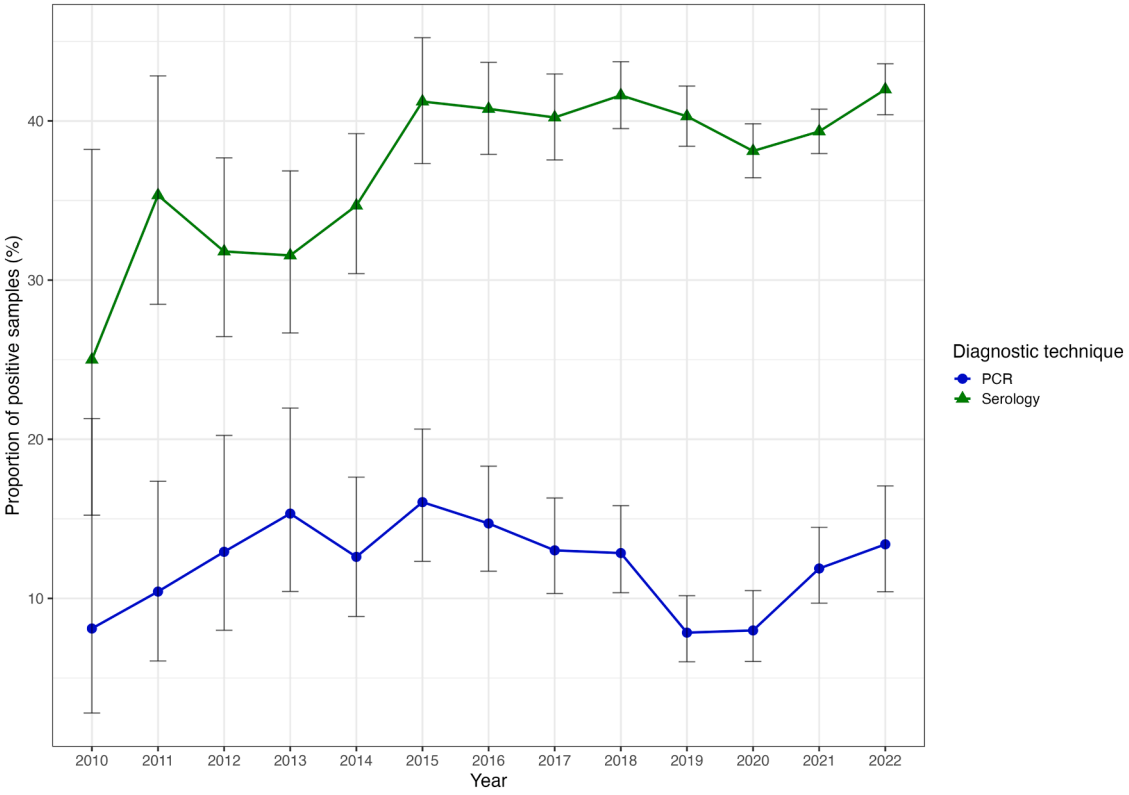

Fig. 3. Proportion of yearly UK canine samples testing positive by PCR and serology for *L. infantum* between 2010 and 2022. Confidence intervals (95 %) are represented with errors bars.

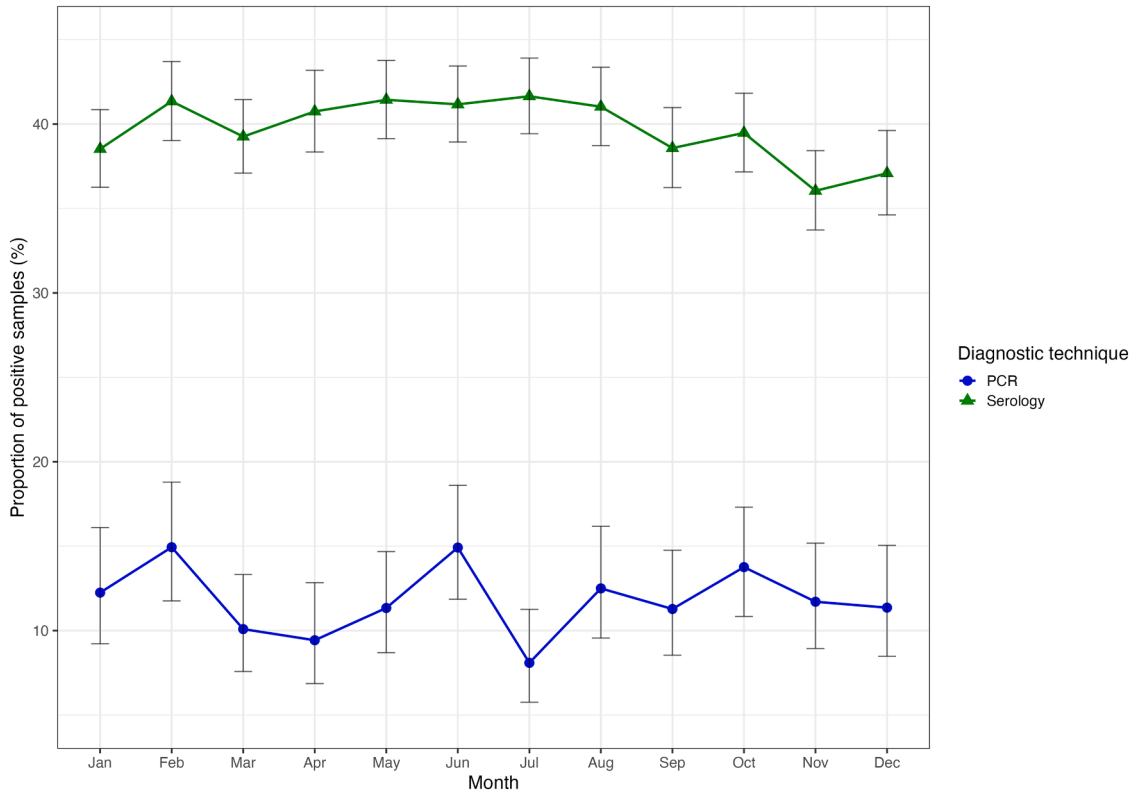

Fig. 4. Proportion of monthly UK canine samples testing positive by PCR and serology for *L. infantum* between 2010 and 2022. Confidence intervals (95 %) are represented with errors bars.

Crossbreed dogs ($P \leq 0.001$). In addition, a significant statistical association was found in both males (35.8 %; 3289/9190) and neutered dogs (36.5 %; 4218/11,563) and the *L. infantum* positivity ($P = 0.002$ and ≤ 0.001 respectively). Regarding age, dogs between 3 and 8 years old were significantly more likely to test positive ($P \leq 0.001$) (Table 1).

3.2. Veterinary practice data

Using text mining tools 3342 leishmaniosis related consultations were found in the SAVSNET database, which after reading by a domain expert, identified 714 consultations meeting the case definition (21.4 %) from 386 dogs. 66.5 % (n=257) of the dogs were represented by a single consultation, while 33.4 % (n=129) had multiple visits. When a dog had multiple visits, only the first visit was included in the subsequent statistical analysis. Finally, 18 EHRs were removed from the final dataset due to incompleteness: in three dogs the owner's postcode was missing, in four the age was not recorded and in 11 the breed was not recorded.

This final veterinary dataset therefore consisted of 368 cases (Fig. 1). Of these, 165 were female (44.84 %) and 203 males (55.16 %), 94 were entire (25.54 %) while 274 were neutered (74.46 %), 124 were pure breed (33.69 %) while 244 crossbreed (66.30 %) (Table 2). The mean age was 5.53 (SD ±3.10), ranging from 9 months to 16 years, with a median age of 4.8.

Results of the final multivariable logistic regression model are shown in Table 2. Neutered dogs were significantly more at risk for CanL than entire dogs (OR 1.73, CI 1.28 – 3.35, $P < 0.001$). When compared with Retrievers (group 8), a greater risk was found in Pointing dogs (group 7) and crossbreed dogs which were 13.86 and 4.75 times more likely to present with CanL respectively ($P < 0.001$). Dogs aged 3–6 years were also at increased risk of having recorded CanL cases (OR 4.71, CI 3.36 – 6.67) compared to dogs less than or equal to 2 years of age.

The UK region with more recorded CanL cases (34.41 %; n=127) was the Southeast of England (NUT1 level: UKJ) (Fig. 6). The Northeast, Northwest, Yorkshire and the Humber and Scotland regions (NUT1 level: UKC, UKD, UKD and UKM) had significantly lower risk of having recorded CanL cases compared to the UKJ region.

CanL cases were recorded in all months, but as with the laboratory dataset, no seasonality was observed. However, there was a significant higher probability of having recorded CanL in dogs from 2017 to 2022 ($P < 0.001$) compared to 2014.

3.2.1. Clinical sings, treatment, and travel history

Regarding the clinical status of dogs with leishmaniosis presenting at the time of the consultation, 50.5 % (186/368) were sick dogs whilst 45.9 % (169) were apparently healthy dogs under monitoring and 3.5 % (13) were dogs whose clinical status could not be determined through the clinical narratives. The most common clinical signs reported in sick dogs were skin disorders (n=87) followed by poor general condition and lymphadenopathy (n=30), ocular disorders (n=27), articular disorders (n=23) and disorders in the renal function (n=21). For 199 out the 368 cases, information about treatment was also recorded. The most common treatment was allopurinol either alone (150/199; 75.4 %) or in combination with miltefosine (31; 15.6 %), pentavalent antimonial drugs (5; 2.5 %) or domperidone (8; 4.0 %). The use of domperidone alone (4; 2.0 %) or its combination with miltefosine (1; 0.5 %) were more rarely recorded.

Concerning the dog's travel history, 189 of the 368 (51.3 %) cases had evidence of travel history overseas recorded in the clinical narratives. In 98 of the 189 cases the reason of the travel was recorded: 88 were rescued dogs, 4 imported dogs from and 6 had visited overseas countries. Predominantly from Spain (n=83), Greece (n=43), Cyprus (n=30) and other European countries including France (n=6), Italy (n=5), Portugal (n=6) and Romania (n=3). Less frequently recorded were Belgium (n=1), Denmark (n=1), Germany (n=2), Morocco (n=1), Netherlands (n=1), Republic of Macedonia (n=1), Serbia (n=1), Tunisia (n=1) and Türkiye (n=2). There were seven dogs which had been in

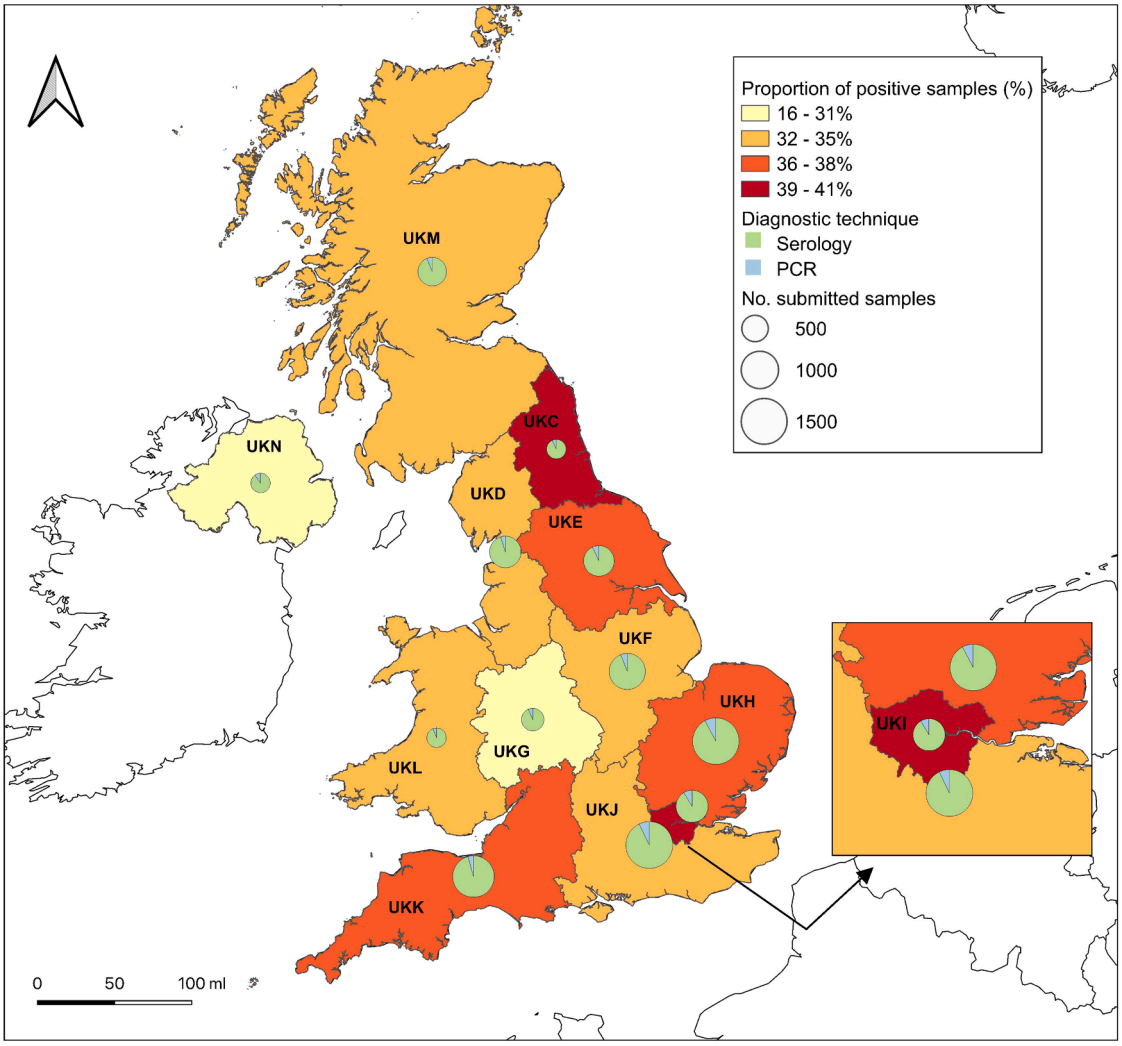

Fig. 5. Geographical distribution of the proportion (%) of canine samples tested positive by serology and/or PCR for *L. infantum* within each UK NUTS1 region based on the laboratory dataset (n=24,236).

more than one overseas country. In addition, the type of travel was uncertain, and the location was not mentioned in the clinical narratives of 91 dogs, despite evidence of travel being recorded in their EHR. For the remaining 179 CanL cases (48.6 %) there was no recorded travel history; none of these specified the animal had not travelled so these cases could not be considered synonymous with autochthonous infection.

4. Discussion

It is often difficult to understand disease epidemiology in data poor populations such as companion animals. In this study, we took a novel approach based on the analyses of two largely independent yet complementary datasets to generate the most comprehensive overview of the CanL situation in the UK to date. This creates a baseline against which future patterns can be evaluated, identified potential control points, as well as risk factors for infection in UK dogs.

Data from veterinary diagnostic laboratories showed a considerable increase in the total number of samples submitted for diagnosis of leishmaniosis in dogs since 2010. This may indicate veterinarians in the UK have developed a greater awareness of leishmaniosis as a differential diagnosis for dogs (i.e., dogs showing cutaneous lesions), suggesting increased awareness of the presence of this emerging disease in the UK canine population. It is also possible some of this increase is due to the change in recruitment of laboratories submitting to SAVSNET.

Our results suggested an increase in CanL detection by serology in dogs since 2013 in this study population. The reasons for this are unknown but may be driven either by an increase in the translocation of infected dogs from endemic areas to the UK, either with their owner or following rescue/rehoming (Maia & Cardoso, 2015), or a higher level of overseas travel by UK dogs to CanL-endemic areas (e.g. UK residents traveling for work or vacation with their dogs). Indeed, since the quarantine regulations were changed and the Pet Travel Scheme (PETS) was introduced in 2000 in the UK, there has been an increase in the number of dogs travelling into the UK (dogs imported or travelled) (Shaw et al., 2009; Trees and Ridge, 2016). Other non-vectorial routes of transmission such as vertical, venereal, blood transfusion or dog-to-dog (Duprey et al., 2006; Naucke and Lorentz, 2012; Silva et al., 2009; Vida et al., 2016; Wright and Moral-Gant, 2020) may also be involved causing additional autochthonous infections, although none of these were specifically recorded in our analyses of EHRs. Whilst almost 45 % of the cases in the veterinary visiting dataset lacked a recorded travel history, they also lacked recorded evidence of not travelling overseas. Better understanding of the travel history of these cases could represent a vital way for the early identification of regions should new autochthonous infection become established.

Alternatively, the growing detection of *Leishmania* infection may also reflect increased diagnostic sensitivity by veterinary surgeons who, over

Table 1
Epidemiological variables recorded in positive dog samples (by serology and/or PCR) from the UK diagnostic laboratories. The Chi-square test was used to assess associations between categorical variables and the seropositive and/or PCR positive animal for *L. infantum* infection.

Data origin		Laboratory data						
		Dog samples (n=24,236 samples; 8555 positives, 15,681 negatives)						
Category		No. Total samples	No. Positive samples	%	IC 95 %	χ^2	df	*P*-value
Sex	Female	7989	2682	33.57	32.54–34.61	9.17	1	0.002
	Male	9190	3289	35.79*	34.81–36.77			
	Unknown	7057	2584	36.62	35.50–37.75			
Neuter status	Entire	4255	1318	30.98	29.6–32.38	41.16	1	<0.001
	Neutered	11563	4218	36.48*	35.61–37.36			
	Unknown	8418	3019	35.86	34.85–36.89			
Breed	G1. Sheepdogs	691	195	28.22	24.99–31.69	253.43	11	<0.001
	G2. Schnauzer	1337	415	31.04	28.62–33.57			
	G3. Terriers	911	297	32.6	29.64–35.71			
	G4. Dachshunds	123	36	29.27	21.95–37.84			
	G5. Spitz	338	96	28.40	23.86–33.43			
	G6. Scent hounds	314	111	35.35	30.27–40.79			
	G7. Pointing dogs	2017	892	44.22*	42.07–46.4			
	G8. Retrievers	1458	407	27.91	25.67–30.27			
	G9. Toy Dogs	478	87	18.20	15.00–21.91			
	G10. Sighthounds	560	129	23.04	19.74–26.7			
	Crossbreed	10368	3830	36.94*	36.02–37.87			
	Unclassified	94	31	32.98	24.31–42.99			
	Unknown	5547	2029	36.58	35.32–37.85			
Age	<1	412	45	10.92	8.26–14.3	274.17	5	<0.001
	1–2	1154	324	28.08	25.56–30.74			
	3–4	1393	624	44.80*	42.20–47.42			
	5–6	1289	608	47.17*	44.46–49.9			
	7–8	854	344	40.28*	37.04–43.61			
	>8	958	293	30.58	27.75–33.58			
	Unknown	18176	6317	34.75	34.07–35.45			
Region	UKC	601	241	40.10*	36.26–44.07	54.06	11	<0.001
NUTS1 level	UKD	2058	706	34.31	32.29–36.38			
	UKE	1803	662	36.72	34.52–38.97			
	UKF	2831	915	32.32	30.62–34.07			
	UKG	1146	358	31.24	28.62–33.98			
	UKH	4180	1519	36.34	34.89–37.81			
	UKI	1844	721	39.10*	36.90–41.35			
	UKJ	4395	1538	34.99	33.60–36.42			
	UKK	3347	1212	36.21	34.60–37.85			
	UKL	318	101	31.76	26.89–37.07			
	UKM	1653	572	34.60	32.35–36.93			
	UKN	60	10	16.67	9.31–28.03			

* Significant differences observed (P <0.05).

time, are more likely to have gained experience in managing CanL and become more aware of the risks it poses to dogs in the UK. In fact, the rise in the proportion of positive samples could be directly related to the increasing number of samples tested each year from dogs suspected of having CanL.

The quantitative serological techniques in this study (ELISA and IFAT) are the most frequently used for testing CanL in dogs in endemic areas. In this study, the serological results showed a high proportion of canine serum samples testing positive for *L. infantum* (39.7 %). Interestingly, this is broadly consistent with previous studies conducted in sick dog populations from Mediterranean countries which reported *L. infantum* seroprevalences between 26 % and 41 % (Chaabouni et al., 2018; Gálvez et al., 2020; Miró et al., 2012; Sousa et al., 2011). These serological assays showed that 38.7 % and 40.7 % of positive samples showed high anti-*Leishmania* antibody titres by IFAT and ELISA techniques, respectively. Such high levels are more likely to indicate a clinical diagnosis of CanL. In contrast, the presence of low antibody levels may only indicate transitory exposure including vaccination with Canileish® (Solano-Gallego et al., 2017). In a clinical setting, a positive serological result should be interpreted jointly with the clinical history of the dog. Since we do not have access to the clinical history of the animals in the laboratory dataset, results here should be interpreted with caution, especially in the case of low antibody levels.

On the other hand, the overall proportion of canine positive samples by PCR was lower than the proportion of seropositive samples. Typically, in cross-sectional studies carried out in CanL endemic areas, the detection of infected dogs by PCR is usually higher than the seropositive dogs (Baneth et al., 2008), because most of the seropositive dogs, whether sick or clinically healthy, would be PCR-positive. In addition, there would be a high number of clinically healthy infected dogs, testing PCR-positive but seronegative. In a cross-sectional study in Mallorca Island (Spain), the prevalence of *Leishmania* infection by PCR was 67 % while the seroprevalence by ELISA was only 26 % (Solano-Gallego et al., 2001). The lower proportion of positive samples found by PCR in this study could be due to the distribution of CanL among the canine population in a non-endemic area like the UK, where the majority of dogs with clinical leishmaniosis have been treated, and reinfection is not probable since the sandfly vector is absent. Furthermore, according to previous studies, the frequency of parasite detection by PCR is different depending on the tissue sampled; the sensitivity of PCR is higher when DNA is extracted from target organs such as bone marrow, lymph nodes or spleen rather than from peripheral blood (Maia et al., 2009; Paltrinieri et al., 2016; Reithinger et al., 2000). It is possible that in non-endemic areas veterinary surgeons may have less experience of dealing with CanL, and where the disease may be just one of several differential diagnoses, the proportion of tested animals for which non-invasive samples such as whole blood are submitted may be higher, and allow for convenient, simultaneous analyses (e.g., biochemical profile, complete blood counts, total serum proteins and antibody detection). In this study, at least 27.8 % (103/371) of PCR-positive dog

Table 2
Multivariable logistic regression model identifying predictors of CanL in 368 case dogs from veterinary practices throughout the UK. Significant variables are shown in bold ($P < 0.05$).

Data origin		Practice data (368 CanL cases, 1838 controls)					
Category		**No. CanL cases** (%)	**No. Controls** (%)	B	SE	OR (95 % CI)	*P*-value
Intercept				−4.61	0.62	0.01 (0.003–0.03)	**< 0.001**
Neuter status	Entire	94 (25.54)	831 (45.21)				
	Neutered	274 (74.46)	1007 (54.79)	0.55	0.16	1.73 (1.28–2.35)	**< 0.001**
Age	≤2 (reference category)	73 (19.84)	774 (42.11)				
	3–6	197 (53.53)	448 (24.37)	1.55	0.18	4.71 (3.36–6.67)	**< 0.001**
	≥7	98 (26.99)	616 (33.51)	0.60	0.19	1.83 (1.27–2.63)	**0.001**
Region NUTS1 level	South East (UKJ) (reference category)	127 (34.51)	312 (16.97)				
	North East (UKC)	17 (4.62)	177 (9.63)	−1.01	0.32	0.36 (0.19–0.67)	**0.001**
	North West (UKD)	34 (9.24)	282 (15.34)	−0.85	0.25	0.43 (0.26–0.69)	**< 0.001**
	Yorkshire and The Humber (UKE)	12 (3.26)	170 (9.25)	−1.29	0.36	0.28 (0.13–0.54)	**< 0.001**
	East Midlands (UKF)	37 (10.05)	158 (8.60)	−0.55	0.26	0.58 (0.35–0.95)	**0.031**
	West Midlands (UKG)	14 (3.80)	73 (3.97)	−0.40	0.36	0.67 (0.32–1.34)	0.273
	East of England (UKH)	57 (15.49)	240 (13.06)	−0.29	0.21	0.75 (0.49–1.14)	0.182
	London (UKI)	11 (2.99)	31 (1.69)	−0.04	0.43	0.96 (0.40–2.20)	0.932
	South West (UKK)	45 (12.23)	228 (12.40)	−0.55	0.23	0.57 (0.36–0.90)	**0.015**
	Wales (UKL)	6 (1.63)	59 (3.21)	−0.50	0.49	0.61 (0.21–1.50)	0.311
	Scotland (UKM)	8 (2.17)	95 (5.17)	−1.46	0.44	0.23 (0.09–0.52)	**< 0.001**
Year of consult	2014 (reference category)	4 (1.09)	120 (6.53)				
	2015	15 (4.08)	203 (11.04)	0.74	0.62	2.09 (0.67–8.12)	0.236
	2016	30 (8.15)	313 (17.03)	0.77	0.59	2.17 (0.75–8.003)	0.189
	2017	30 (8.15)	192 (10.45)	1.2	0.59	3.32 (1.15–12.33)	**0.042**
	2018	46 (12.50)	262 (14.25)	1.34	0.58	3.82 (1.36–13.96)	**0.021**
	2019	69 (18.75)	257 (13.98)	1.82	0.58	6.18 (2.23–22.32)	**0.001**
	2020	44 (11.96)	158 (8.60)	1.9	0.59	6.69 (2.35–24.59)	**0.001**
	2021	82 (22.28)	181 (9.85)	2.49	0.58	12.10 (4.37–43.79)	**< 0.001**
	2022	48 (13.04)	152 (8.27)	2.13	0.59	8.41 (2.96–30.85)	**< 0.001**
Breed FCI	G8. Retrievers (reference category)	30 (8.15)	381 (20.73)				
	G1. Sheepdogs	3 (0.82)	86 (4.68)	−0.8	0.63	0.45 (0.10–1.34)	0.203
	G2. Schnauzer	22 (5.98)	129 (7.02)	0.68	0.32	1.97 (1.04–3.71)	**0.035**
	G3. Terriers	9 (2.45)	334 (18.17)	−1.09	0.40	0.34 (0.15–0.71)	**0.006**
	G4. Dachshunds	2 (0.54)	26 (1.41)	−0.13	0.79	0.88 (0.13–3.41)	0.868
	G5. Spitz	3 (0.82)	32 (1.74)	0.11	0.68	1.11 (0.24–3.73)	0.876
	G6. Scent hounds	7 (1.90)	31 (1.69)	0.72	0.50	2.05 (0.73–5.20)	0.147
	G7. Pointing dogs	41 (11.14)	34 (1.85)	2.63	0.35	13.86 (7.10–27.60)	**< 0.001**
	G9. Toy Dogs	4 (1.09)	239 (13.00)	−1.67	0.55	0.19 (0.05–0.50)	**0.002**
	G10. Sighthounds	3 (0.82)	27 (1.47)	0.49	0.68	1.64 (0.35–5.51)	0.467
	Crossbreed	244 (66.30)	519 (28.23)	1.56	0.22	4.75 (3.13–7.42)	**< 0.001**

SE: Standard Error; OR: Odds Ratio; CI: 95 % Confidence Interval.

samples were from blood. Blood is not the most sensitive sample, frequently testing negative in clinically healthy infected dogs (Crawford et al., 2013; Reithinger et al., 2000). This could suggest those testing positive by PCR in our study largely comprised sick dogs showing high levels of parasitaemia at the time of diagnosis. Unfortunately, such clinical detail is not currently linkable to these laboratory datasets.

Since SAVSNET does not collect data from all veterinary practices or diagnostic laboratories, the total UK CanL confirmed cases in dogs are likely to be higher. Because each sample tested could not be linked to the individual dog of origin, it is possible that samples from the same animal at different times may be included in the analysis. This may have resulted in an overestimated seroprevalence in our study population, especially in the case of chronically infected sick dogs where response to treatment may be monitored by measuring serological levels over time. However, this effect may to some extent be counteracted by the large sample size included in the study. Another limitation of this study is that the clinical status of the seropositive and PCR-positive dogs was unknown.

In the present study, epidemiological variables from two independent datasets collected from SAVSNET database were analysed. Factors in the veterinary practice data associated with CanL in the UK canine population included the dog's age, neuter status, breed, geographical region of residence and year of the consultation. The laboratory data results were consistent with these findings, as significant differences were also identified in the same categories.

Adult dogs (3–6 years) were significantly more likely to have CanL than those less than 3 years of age, consistent with our laboratory findings and previous studies in endemic areas such as Portugal, Spain and Italy (Amela et al., 1995; Cardoso et al., 2004; Cortes et al., 2012; Maresca et al., 2009; Martín-Sánchez et al., 2009). Generally, in Mediterranean areas, the increased risk in older animals may be attributed to repeat exposure to *Leishmania*-infected sandflies. In non-endemic areas like the UK, it is perhaps more likely to be associated with the age at which animals travel and the frequency the owner travels with their pets, either for work or vacation, creating an accrual of risk over multiple trips. A possible suggestion for the majority of dogs being in the age range 3–6 years, could be that owners travel less frequently with older dog and puppies.

In other studies, from Spain, seroprevalence showed a bimodal distribution with a peak in young animals (1 – 2 years) and those older than seven years (Amela et al., 1995; Gálvez et al., 2010), attributed to very young and old animals being at greater risk of leishmaniosis because of their immune system status. Interestingly, we did not see a peak in the older UK dog SAVSNET population in either dataset (≥7 years). The lower frequency of older dogs infected with *Leishmania* living in the UK may be due to most imported infected dogs arriving at a young age. After therapy is administrated, reinfection is not probable which can improve the prognosis of the disease.

The results from both the laboratory and veterinary practice data showed a significantly increased risk in the group of Pointing dog breeds, which includes continental pointing dogs, pointers, and setters (FCI breed Group 7). In addition, crossbreed dogs had a higher risk of

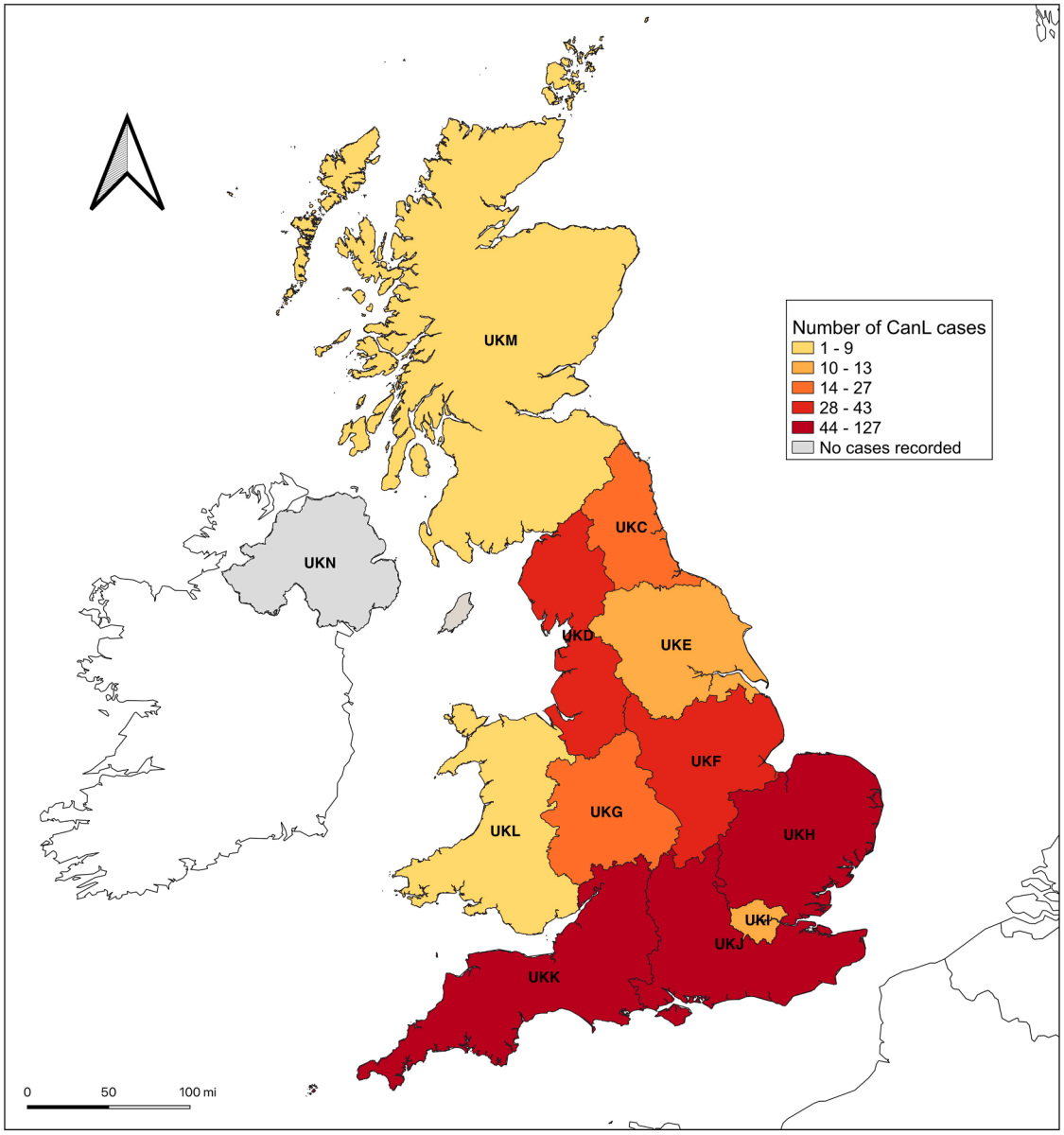

Fig. 6. Geographical distribution of the recorded cases of CanL within each UK NUTS1 region based on the veterinary dataset (n=368).

being infected by *L. infantum*. There are some breeds more susceptible to developing CanL (e.g., Boxer, Cocker Spaniel, Rottweiler, German Shepherd; França-Silva et al., 2003), whilst others are more resistant (Ibizan hound, an ancient Mediterranean dog breed) (Solano-Gallego et al., 2000). On the other hand, the use and management of certain types of breeds, such as hunting dogs, could favour infection by *Leishmania*. Several authors have reported that outdoor living (spending exclusively/majority of the time outdoors) and hunting were more important than dog breeds in the transmission of *L. infantum* infection in endemic areas directly related to the exposure risk to sandflies biting (Cortes et al., 2012; Gálvez et al., 2010; Rombolà et al., 2021). However, the higher risk found in crossbreed dog contrast with other studies, which have reported that crossbreeds had a protective effect (Cortes et al., 2012; Rombolà et al., 2021) and may suggest important differences in the study populations.

In the present study, the UK region with the majority of recorded CanL cases (34.41 %) was the Southeast of England. In addition, the East and South of England were the regions where a higher number of positive dog samples of *L. infantum* were recorded. Our results are consistent with the findings of a previous UK study using EHRs (Silvestrini et al., 2016), where most dogs with CanL were reported to live in south and central England. The presence of more *L. infantum* infected dogs, either clinically healthy or sick, could be explained if owners from these areas were more likely to travel with their pets to endemic areas; indeed, these areas are closer to important ports which are well connected to continental Europe. Furthermore, uneven UK risk could also suggest a greater number of rescue dogs from European countries residing in these high-risk areas. Indeed, in the 51.3 % of the dogs with CanL that had travel history recorded, many of them were rescue dogs from Southern European countries with Spain and Greece being the most popular origin countries. In 2016, Silvestrini et al., reported that all dogs in their study population of referral patients had a history of travel to or from an endemic country. Further research is needed to provide more information about the circulation of both the imported and rescue dogs in the UK and their health status.

In the veterinary practice data, we observed a significantly higher probability of CanL in dogs from 2017 to 2022 compared to earlier years. This could be explained by a combined effect of both new cases being imported, and the cumulative effect of the disease, whereby even if dogs with CanL recover clinically after treatment, complete

elimination of the parasite is rarely achieved, and they remain infected and may relapse (Kaszak et al., 2015). It is important to note that the CanL incubation period is long (at least three months), and the clinical manifestation of disease could be delayed up to several years post-infection (Miró et al., 2008). Therefore, it is very difficult to determinate when a positive dog was infected.

Data from the clinical narratives showed that veterinary surgeons from the UK are already having to deal with the diagnosis of CanL and the management of both sick and healthy dogs infected by *L. infantum*. Cutaneous lesions were the most commonly recorded clinical signs (46.7 %), followed by lymphadenomegaly (16.1 %), ocular disorders (14.5 %), joint diseases (12.3 %), and abnormalities in the renal function (11.3 %). This is in broad agreement with Silvestrini et al., 2016, where cutaneous disease, lethargy, loss of appetite, and lameness were common reasons for presentation of dogs with CanL to a UK referral hospital. Regarding treatment, most dogs in our vet-visiting dataset had been treated with allopurinol alone, consistent with previous studies in the UK and other non-endemic areas such as Switzerland and Germany (Helm et al., 2013; Silvestrini et al., 2016). In contrast, the combination of meglumine antimoniate or miltefosine with allopurinol were rarely used (2.5 % and 14.3 %, respectively); such combinations are currently the first lines of treatment in endemic areas (Montoya et al., 2020). The low use of these drugs in the UK likely reflects a need for specific treatment certificates.

5. Conclusion

This is a novel study using large-scale EHR data from both commercial veterinary diagnostic laboratories and veterinary practices from the UK to provide new insights into recent trends in *Leishmania* infection in dogs in the UK. The results obtained showed an increase in both the proportion of dog samples testing positive for *L. infantum* infection as well as the number of dogs with canine leishmaniosis in the UK during the study period. In addition, risk factors and countries likely associated with imported cases were identified. This study highlights the spread of CanL in the UK via travelling dogs and raises awareness of the importance of testing clinically healthy dogs travelling to or from endemic areas. Maintaining up-to-date knowledge on CanL in emerging areas is essential to prevent and combat this zoonotic disease.

Funding sources

SAVSNET is currently funded by Dogs Trust as part of SAVSNET-Agile. We are grateful for the support and major funding from BBSRC and previously BSAVA. Hayley Jones and Alan Radford are funded by PetPlan Charitable Trust on a project to explore vaccine preventable diseases.

CRediT authorship contribution statement

Alan David Radford: Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Peter-John Noble:** Software, Resources. **Hayley Jones:** Methodology, Formal analysis. **Gina Pinchbeck:** Writing – review & editing, Resources, Data curation. **Guadalupe Miró:** Writing – review & editing, Supervision, Conceptualization. **Fernando Sánchez-Vizcaíno:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Rocio Checa:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Veterinary practices and diagnostic laboratories that provide health data and without whose support these reports would not be possible. Thanks to the Ministry of Universities, Government of Spain to funding the “Margarita Salas” grant for the training of early-stage researchers.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110350.

References

Alcover, M.M., Basurco, A., Fernandez, A., Riera, C., Fisa, R., Gonzalez, A., Verde, M., Garrido, A.M., Ruíz, H., Yzuel, A., Villanueva-Saz, S., 2021. A cross-sectional study of *Leishmania infantum* infection in stray cats in the city of Zaragoza (Spain) using serology and PCR. Parasit. Vectors 14, 178. https://doi.org/10.1186/s13071-021-04682-w.

Amela, C., Mendez, I., Torcal, J.M., Medina, G., Pachón, I., Cañavate, C., Alvar, J., 1995. Epidemiology of canine leishmaniasis in the Madrid region, Spain. Eur. J. Epidemiol. 11, 157–161. https://doi.org/10.1007/BF01719481.

Baneth, G., Koutinas, A.F., Solano-Gallego, L., Bourdeau, P., Ferrer, L., 2008. Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. Trends Parasitol. 24, 324–330. https://doi.org/10.1016/j.pt.2008.04.001.

Brant, B.J., Singleton, D.A., Noble, P.J.M., Radford, A.D., 2021. Seasonality and risk factors for grass seed foreign bodies in dogs. Prev. Vet. Med 197, 105499. https://doi.org/10.1016/j.prevetmed.2021.105499.

Cardoso, L., Schallig, H.D.F.H., Neto, F., Kroon, N., Rodrigues, M., 2004. Serological survey of *Leishmania* infection in dogs from the municipality of Peso da Régua (Alto Douro, Portugal) using the direct agglutination test (DAT) and fast agglutination screening test (FAST). Acta Trop. 91, 95–100. https://doi.org/10.1016/j.actatropica.2004.03.004.

Chaabouni, A., Boubaker Elandoulsi, R., Mhadhbi, M., Gharbi, M., Sassi, A., 2018. Comparative analysis of the *Leishmania infantum*-specific antibody repertoires and the autoantibody repertoires between asymptomatic and symptomatic dogs. Vet. Parasitol. 261, 9–17. https://doi.org/10.1016/j.vetpar.2018.07.011.

Cortes, S., Vaz, Y., Neves, R., Maia, C., Cardoso, L., Campino, L., 2012. Risk factors for canine leishmaniasis in an endemic Mediterranean region. Vet. Parasitol. 189, 189–196. https://doi.org/10.1016/j.vetpar.2012.04.028.

Crawford, K., Walton, J., Lewis, D., Tasker, S., Warman, S.M., 2013. Infectious agent screening in canine blood donors in the United Kingdom. J. Small Anim. Pr. 54, 414–417. https://doi.org/10.1111/jsap.12109.

Del Río, L., Chitimia, L., Cubas, A., Victoriano, I., De la Rúa, P., Gerrikagoitia, X., Barral, M., Muñoz-García, C.I., Goyena, E., García-Martínez, D., Fisa, R., Riera, C., Murcia, L., Segovia, M., Berriatua, E., 2014. Evidence for widespread *Leishmania infantum* infection among wild carnivores in *L. infantum* periendemic northern Spain. Prev. Vet. Med 113, 430–435. https://doi.org/10.1016/j.prevetmed.2013.12.001.

Duprey, Z.H., Steurer, F.J., Rooney, J.A., Kirchhoff, L.V., Jackson, J.E., Rowton, E.D., Schantz, P.M., 2006. Canine visceral leishmaniasis, United States and Canada, 2000-2003. Emerg. Infect. Dis. 12, 440–446. https://doi.org/10.3201/eid1203.050811.

Ferreira-Silva, M.M., Teixeira, L. a S., Tibúrcio, M.S., Pereira, G.A., Rodrigues, V., Palis, M., Afonso, P., Alves, M., Feitosa, J.M., Urias, E., Santos, E.M., Carvalho, S.F. G., Moraes-Souza, H., 2018. Socio-epidemiological characterisation of blood donors with asymptomatic *Leishmania infantum* infection from three Brazilian endemic regions and analysis of the transfusional transmission risk of visceral leishmaniasis. Transfus. Med 28, 433–439. https://doi.org/10.1111/tme.12553.

França-Silva, J.C., da Costa, R.T., Siqueira, A.M., Machado-Coelho, G.L.L., da Costa, C.A., Mayrink, W., Vieira, E.P., Costa, J.S., Genaro, O., Nascimento, E., 2003. Epidemiology of canine visceral leishmaniosis in the endemic area of Montes Claros Municipality, Minas Gerais State, Brazil. Vet. Parasitol. 111, 161–173. https://doi.org/10.1016/s0304-4017(02)00351-5.

Gálvez, R., Miró, G., Descalzo, M.A., Nieto, J., Dado, D., Martín, O., Cubero, E., Molina, R., 2010. Emerging trends in the seroprevalence of canine leishmaniasis in the Madrid region (central Spain). Vet. Parasitol. 169, 327–334. https://doi.org/10.1016/j.vetpar.2009.11.025.

Gálvez, R., Montoya, A., Cruz, I., Fernández, C., Martín, O., Checa, R., Chicharro, C., Migueláñez, S., Marino, V., Miró, G., 2020. Latest trends in *Leishmania infantum* infection in dogs in Spain, Part I: mapped seroprevalence and sand fly distributions. Parasit. Vectors 13, 204. https://doi.org/10.1186/s13071-020-04081-7.

Helm, M., Müller, W., Schaarschmidt, D., Grimm, F., Deplazes, P., 2013. Allopurinol therapy in imported dogs with leishmaniasis treated outside the endemic area. Schweiz Arch. Tierheilkd. 155, 559–567. https://doi.org/10.1024/0036-7281/a000512.

Kaszak, I., Planellas, M., Dworecka-Kaszak, B., 2015. Canine leishmaniosis - an emerging disease. Ann. Parasitol. 61, 69–76.

Killick-Kendrick, R., 1990. Phlebotomine vectors of the leishmaniases: a review. Med Vet. Entomol. 4, 1–24.

LeishVet, 2024. URL ⟨https://www.leishvet.org/⟩ (accessed 7.1.24).

Leonel, J.A.F., Tannihão, B., Arantes, J.A., Vioti, G., Benassi, J.C., Brandi, R.A., Ferreira, H.L., Keid, L.B., Soares, R.M., Oliveira, T.M.F., de, S., 2021. Detection of *Leishmania infantum* DNA in blood samples of horses (*Equus caballus*) and donkeys

(*Equus asinus*) by PCR. Rev. Inst. Med Trop. Sao Paulo 63, e12. https://doi.org/10.1590/S1678-9946202163012.
Maia, C., Cardoso, L., 2015. Spread of *Leishmania infantum* in Europe with dog travelling. Vet. Parasitol. 213, 2–11. https://doi.org/10.1016/j.vetpar.2015.05.003.
Maia, C., Ramada, J., Cristóvão, J.M., Gonçalves, L., Campino, L., 2009. Diagnosis of canine leishmaniasis: conventional and molecular techniques using different tissues. Vet. J. 179, 142–144. https://doi.org/10.1016/j.tvjl.2007.08.009.
Maresca, C., Scoccia, E., Barizzone, F., Catalano, A., Mancini, S., Pagliacci, T., Porrini, M., Principato, M., Venditti, G., Grelloni, V., 2009. A survey on canine leishmaniasis and phlebotomine sand flies in central Italy. Res. Vet. Sci. 87, 36–38. https://doi.org/10.1016/j.rvsc.2008.11.009.
Martín-Sánchez, J., Morales-Yuste, M., Acedo-Sánchez, C., Barón, S., Díaz, V., Morillas-Márquez, F., 2009. Canine leishmaniasis in southeastern Spain. Emerg. Infect. Dis. 15, 795–798. https://doi.org/10.3201/eid1505.080969.
McKenna, M., Attipa, C., Tasker, S., Augusto, M., 2019. Leishmaniosis in a dog with no travel history outside of the UK, 441–441 Vet. Rec. 184. https://doi.org/10.1136/vr.105157.
Miró, G., Cardoso, L., Pennisi, M.G., Oliva, G., Baneth, G., 2008. Canine leishmaniosis–new concepts and insights on an expanding zoonosis: part two. Trends Parasitol. 24, 371–377. https://doi.org/10.1016/j.pt.2008.05.003.
Miró, G., Checa, R., Montoya, A., Hernández, L., Dado, D., Gálvez, R., 2012. Current situation of *Leishmania infantum* infection in shelter dogs in northern Spain. Parasit. Vectors 5, 60. https://doi.org/10.1186/1756-3305-5-60.
Miró, G., Troyano, A., Montoya, A., Fariñas, F., Fermín, M.L., Flores, L., Rojo, C., Checa, R., Gálvez, R., Marino, V., Fragío, C., Martínez-Nevado, E., 2018. First report of *Leishmania infantum* infection in the endangered orangutan (*Pongo pygmaeus pygmaeus*) in Madrid, Spain. Parasit. Vectors 11, 185. https://doi.org/10.1186/s13071-018-2772-1.
Montoya, A., Gálvez, R., Checa, R., Sarquis, J., Plaza, A., Barrera, J.P., Marino, V., Miró, G., 2020. Latest trends in *L. infantum* infection in dogs in Spain, Part II: current clinical management and control according to a national survey of veterinary practitioners. Parasit. Vectors 13, 205. https://doi.org/10.1186/s13071-020-04080-8.
Naucke, T.J., Amelung, S., Lorentz, S., 2016. First report of transmission of canine leishmaniosis through bite wounds from a naturally infected dog in Germany. Parasit. Vectors 9, 256. https://doi.org/10.1186/s13071-016-1551-0.
Naucke, T.J., Lorentz, S., 2012. First report of venereal and vertical transmission of canine leishmaniosis from naturally infected dogs in Germany. Parasit. Vectors 5, 67. https://doi.org/10.1186/1756-3305-5-67.
Norman, C., Stavisky, J., Westgarth, C., 2020. Importing rescue dogs into the UK: reasons, methods and welfare considerations. Vet. Rec. 186, 248. https://doi.org/10.1136/vr.105380.
Paltrinieri, S., Gradoni, L., Roura, X., Zatelli, A., Zini, E., 2016. Laboratory tests for diagnosing and monitoring canine leishmaniasis. Vet. Clin. Pathol. 45, 552–578. https://doi.org/10.1111/vcp.12413.
Reithinger, R., Lambson, B.E., Barker, D.C., Davies, C.R., 2000. Use of PCR to detect *Leishmania* (*Viannia*) spp. in dog blood and bone marrow. J. Clin. Microbiol 38, 748–751. https://doi.org/10.1128/JCM.38.2.748-751.2000.
Rombolà, P., Barlozzari, G., Carvelli, A., Scarpulla, M., Iacoponi, F., Macrì, G., 2021. Seroprevalence and risk factors associated with exposure to *Leishmania infantum* in dogs, in an endemic Mediterranean region. PLoS One 16, e0244923. https://doi.org/10.1371/journal.pone.0244923.
Shaw, S.E., Langton, D.A., Hillman, T.J., 2009. Canine leishmaniosis in the United Kingdom: a zoonotic disease waiting for a vector? Vet. Parasitol. 163, 281–285. https://doi.org/10.1016/j.vetpar.2009.03.025.
Silva, F.L., Oliveira, R.G., Silva, T.M.A., Xavier, M.N., Nascimento, E.F., Santos, R.L., 2009. Venereal transmission of canine visceral leishmaniasis. Vet. Parasitol. 160, 55–59. https://doi.org/10.1016/j.vetpar.2008.10.079.
Silvestrini, P., Batchelor, D., Allenspach, K., Maunder, C., Seth, M., Mas, A., Hill, T., Serrano, G., Roura, X., Planellas, M., German, A.J., Pastor, J., 2016. Clinical leishmaniasis in dogs living in the UK. J. Small Anim. Pract. https://doi.org/10.1111/jsap.12503.
Solano-Gallego, L., Cardoso, L., Pennisi, M.G., Petersen, C., Bourdeau, P., Oliva, G., Miró, G., Ferrer, L., Baneth, G., 2017. Diagnostic Challenges in the Era of Canine *Leishmania infantum* Vaccines. Trends Parasitol. 33, 706–717. https://doi.org/10.1016/j.pt.2017.06.004.
Solano-Gallego, L., Koutinas, A., Miró, G., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., 2009. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. Vet. Parasitol. 165, 1–18. https://doi.org/10.1016/j.vetpar.2009.05.022.
Solano-Gallego, L., Llull, J., Ramos, G., Riera, C., Arboix, M., Alberola, J., Ferrer, L., 2000. The Ibizian hound presents a predominantly cellular immune response against natural *Leishmania infection*. Vet. Parasitol. 90, 37–45. https://doi.org/10.1016/S0304-4017(00)00223-5.
Solano-Gallego, L., Morell, P., Arboix, M., Alberola, J., Ferrer, L., 2001. Prevalence of *Leishmania infantum* infection in dogs living in an area of canine leishmaniasis endemicity using PCR on several tissues and serology. J. Clin. Microbiol 39, 560–563. https://doi.org/10.1128/JCM.39.2.560-563.2001.
Sousa, S., Lopes, A.P., Cardoso, L., Silvestre, R., Schallig, H., Reed, S.G., Cordeiro da Silva, A., 2011. Seroepidemiological survey of *Leishmania infantum* infection in dogs from northeastern Portugal. Acta Trop. 120, 82–87. https://doi.org/10.1016/j.actatropica.2011.06.003.
Teske, E., van Knapen, F., Beijer, E.G.M., Slappendel, R.J., 2002. Risk of Infection with *Leishmania* spp. in the Canine Population in the Netherlands. Acta Vet. Scand. 43, 1–7. https://doi.org/10.1186/1751-0147-43-195.
Trees, A.J., Ridge, A., 2016. Threat of imported diseases to UK dogs. Vet. Rec. 178, 347–348. https://doi.org/10.1136/vr.i1799.
Tulloch, J.S.P., McGinley, L., Sánchez-Vizcaíno, F., Medlock, J.M., Radford, A.D., 2017. The passive surveillance of ticks using companion animal electronic health records. Epidemiol. Infect. 145, 2020–2029. https://doi.org/10.1017/S0950268817000826.
Vida, B., Toepp, A., Schaut, R.G., Esch, K.J., Juelsgaard, R., Shimak, R.M., Petersen, C.A., 2016. Immunologic progression of canine leishmaniosis following vertical transmission in United States dogs. Vet. Immunol. Immunopathol. 169, 34–38. https://doi.org/10.1016/j.vetimm.2015.11.008.
World Organisation for Animal Health (WOAH), 2021. Leishmaniosis, in: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Twelfth edition.
Wright, I., Baker, S., 2019. Leishmaniosis in a dog with no history of travel outside the UK, 387.3-388 Vet. Rec. 184. https://doi.org/10.1136/vr.l1268.
Wright, I., Moral-Gant, J.M., 2020. Possible vertical transmission of *Leishmania* in a dog. Vet. Rec. 187, 35. https://doi.org/10.1136/vr.m2703.