SCIENTIFIC OPINION

Scientific Opinion on canine leishmaniosis[1]

EFSA Panel Animal Health and Welfare[2,3]

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ABSTRACT

This Scientific Opinion presents a characterisation of canine leishmaniosis (CanL) in Europe and its potential for spreading. The efficacy of available preventative measures to protect dogs against CanL was assessed, with the objective of mitigating the probability of introduction of CanL into free areas in the European Union through movement of infected dogs. Several systematic reviews (SRs) of literature were carried out to evaluate the efficacy of vaccines, topically applied insecticides and prophylactic medication. Additionally, SRs on the sensitivity of diagnostic tests and treatment efficacy were carried out to evaluate the possibility of testing and excluding or treating infected dogs to mitigate the risk of introduction into free areas. The probability of introduction and establishment of CanL in a non-endemic region with competent sandflies was estimated, using a stochastic simulation model. The probability of establishment defined as the local transmission of *L. infantum* from vector to host and vice versa, was very high. The most effective mitigation measure to reduce the probability of introduction and establishment of CanL was topically applied insecticide. Vaccination had only limited effect on the probability of establishment in a non-endemic region. Testing dogs before their introduction into a non-endemic area is of limited value if applied too soon after exposure to infected sandflies, because it takes several months to obtain a positive result after exposure. Test and treatment in the endemic area, prior to movement into a non-endemic area, does not appear to be an efficient and realistic option to mitigate the probability of introduction of CanL, as no treatment against CanL can guarantee to prevent future transmission. It was concluded that the main limitation to CanL spread is represented by the vectors. This reinforces the need for knowledge on the vectorial competence, distribution and abundance of potential vectors of CanL in the EU.

KEY WORDS

canine leishmaniosis, preventive measures, introduction, establishment

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* The reference to Mattin et al. (2014) on pp.32 and 35 has been replaced by reference to Espejo et al. (2014) which is focused more specifically on the modelling aspects. This does not affect the outcomes of the opinion. The original version is available on request as is a version showing all the changes made.

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SUMMARY

Following a request from the European Commission (EC) the European Food Safety Authority (EFSA) Animal Health and Welfare (AHAW) Panel was asked to deliver a scientific opinion on canine leishmaniosis (CanL).

The first term of reference of the mandate requested a brief characterisation of canine leishmaniosis in Europe. This was based mainly on extensive literature searches and questionnaires sent to veterinary practitioners.

CanL is **endemic** in the European countries or regions surrounding the Mediterranean, where the disease distribution matches that of the phlebotomine vectors. The prevalence of infection in dogs in endemic areas is much higher than the fraction that shows clinical illness or seroconversion. On average, around 10 % of dogs in endemic countries are seropositive for *Leishmania infantum*, with wide variations between territories. Polymerase chain reaction (PCR) studies conducted in endemic areas have given much higher prevalences than serology, with up to 80 % of the dog population testing positive, indicating that only a small proportion of *Leishmania*-infected dogs mount a detectable humoral immune response.

Northern European countries, where competent vectors have not been found, experience **imported cases** in dogs with a history of travelling from endemic areas, and CanL foci in households or in kennels have been described. These foci can last for several years because of non-vectorial transmission (e.g. through direct contact, venereal transmission, vertical transmission or via blood transfusion). None of these transmission routes appears to sustain infection in a large population (i.e. larger than that of a household or a kennel). Data on sandfly distribution are limited because of the absence of systematic sampling programmes and expertise. In central European countries, knowledge of the presence of competent vectors and the presence of endemic CanL is limited.

Available field data suggest that sandflies are spreading northwards in Europe and their densities are increasing in some newly colonised areas. Extension of the distribution areas is attributed to climate changes, which can trigger shifts in sandfly distributions, or local changes in sandfly population densities, resulting in a patchy distribution of vectors.

The **impact** of infection with *L. infantum* on the health and welfare of dogs depends on its severity, which ranges from subclinical to very severe, including euthanasia. All seropositive *L. infantum*-infected dogs, whether they express clinical disease or not, are potential sources of infection for vectors and may transmit the parasite. The potential role of wild mammals as reservoirs has not been fully investigated. Black rats, wild rabbits and hares may contribute to maintaining *L. infantum* circulation in some areas of southern Europe.

The incidence in humans in endemic areas is lower that observed in dog populations. Most infections in humans with *L. infantum* are asymptomatic. Risk factors for clinical disease include young age, human immunodeficiency virus (HIV) infection and other immunosuppressive states.

For the second term of reference of this mandate, the efficacy of available preventative measures to protect dogs against *L. infantum* infection, with the objective of mitigating the probability of introduction of the infection into free areas in the European Union (EU) through movements of infected dogs, was assessed. Therefore, systematic reviews (SRs) of literature were carried out to identify studies on the efficacy of preventative measures, such as vaccines, topically applied insecticides and prophylactic medication to prevent dogs becoming infected. Further SRs on the sensitivity of diagnostic tests and the treatment efficacy, to evaluate the possibility of identifying infected dogs moving to free areas, or of treating them to provide permanent cure of infection, were carried out.

Currently there is no authorised **vaccine** in the EU that is able to confer full protection against infection or disease.

However, some vaccines, such as CaniLeish®, the only vaccine authorised in the EU, do provide partial protection against active *L. infantum* infection and clinical disease in dogs.

The efficacy of **topically applied insecticides** has been demonstrated under experimental conditions and in controlled field studies providing mass treatment effect. It is uncertain whether the same efficacy of insecticides can be obtained in individual dogs when application is their owners' responsibility.

Limited data are available on the efficacy of **prophylactic medication** with domperidone in endemic areas. Furthermore, data on treatments of immunologically naive dogs and its potential long-term toxicity are lacking, and this area needs further investigation.

The common diagnostic tests use quantitative serology and PCR to detect sick and subclinically infected dogs. In most observational studies, no reference test is available which would detect all the truly infected animals, thus hampering calculation of sensitivity estimates.

Drug therapy for CanL appears to mainly slow down the progression of infection, decrease infectiousness and improve clinical manifestations by reducing parasite loads in infected tissues. Based on the studies examined in the SRs, there is currently no drug or treatment regime that demonstrated 100 % efficacy in the elimination of the parasites.

For the third term of reference of this mandate, the probability that the infection would become established in free areas of the EU if *L. infantum* were introduced by infected dogs was assessed.

Owing to the limited available knowledge on factors such as vector competence and abundance, dog distribution and movements, the average probability of introduction and establishment of CanL in a theoretical dog network was estimated, using a mathematical stochastic model. For investigation purposes, the model was implemented with the assumption that competent vectors would be present at the moment of introduction and vectorial capacity was used to generalise the potential of the sandfly population to transmit the disease to dogs under the assumption of independence from the prevalence of infection in sandflies.

The model assessed the average probability of disease establishment, defined as the local transmission of *L. infantum* from vector to host and vice versa, leading to the temporal presence of at least one indigenous infectious host and at least one indigenous infectious vector as very high in areas in which competent vectors are present. Even in areas where sandfly populations are likely to have a lower vectorial capacity than in endemic areas, the average probability of establishment following introduction of an infected dog, remains high, according to the model. Examples of such areas could be foci with low vector densities, or the fringe areas between the endemic and the free areas. Thus, the average probability of establishment in a non-endemic region with competent sandflies is very high, according to the model. However, the long-term prevalence in a region in which CanL has been introduced and has established may vary from extremely low to high, depending mainly on the vectorial capacity.

Owing to the wide distribution of susceptible dogs and the high host–vector contact rates, it was concluded that the main limitation to CanL spread is represented by the vectors. This reinforces the need for knowledge on the vectorial competence of some sandfly species and of the distribution and abundance of known vectors.

Results from the model indicated that the probability of introduction and establishment can be reduced by mitigation measures, separately or in combination. The most effective mitigation measure to reduce the probability of introduction and establishment of CanL in a free area with sandflies was found to be

topically applied insecticide. Vaccination of dogs prior to travelling to endemic areas had only a limited effect on the probability of establishment in a non-endemic region, and this effect seems to be relevant only when the vectorial capacity and the number of imported dogs are low.

The use of topical insecticide and vaccination in travelling dogs had a synergistic effect in reducing the probability of establishment in a dog network and in reducing the probability of establishment in a region after their return to a non-endemic area, according to the model. Again, this effect was higher in areas where a low vectorial capacity of the vectors was assumed.

Testing dogs before their introduction into a non-endemic area is of limited value if applied too soon after exposure to infected sandflies. This is mainly because it takes several months for a test to give a positive result after exposure. Test and treatment in the endemic area, prior to movement into a non-endemic area, does not appear as an efficient and realistic option to mitigate the probability of introduction of CanL into a non-endemic area, as no treatment against *L. infantum* infection can provide a permanent parasitological cure.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Leishmaniosis is a parasitic disease of animals and humans caused by different species of protozoa of the genus Leishmania, which is transmitted by sandflies and represents a serious public health problem in many parts of the world. Among the different clinical forms of the disease, visceral leishmaniosis is the most severe being often lethal if untreated. Leishmania infantum is the most widespread etiological agent of zoonotic cutaneous and visceral leishmaniosis in human and of canine leishmaniosis in dogs in Mediterranean areas. Domestic dogs are deemed to be the principal reservoir hosts for this parasite since they efficiently replicate the protozoan parasite and are preferred hosts for vector phlebotomine sandflies.

Although canine leishmaniosis is not a notifiable disease in many countries and knowledge about the prevalence and spread of the disease is scattered, there are reports that Leishmania infantum has recently spread from Mediterranean to temperate climates in Europe (e.g. Hungary and northern Italy), apparently linked to socioeconomic and possible climate factors, but perhaps also to increased movements of infected hosts, mostly dogs. However, non-sand fly modes of transmission, such as blood transfusion, vertical or venereal transmission, or even dog to dog transmission through bites or wounds, have also been described or suspected. Successful control measures targeted on dogs as the main natural reservoir species should decrease the occurrence of this disease. Current preventive measures are based on long-acting insecticide applications in combination with vaccination against Leishmania infantum.

Scientific evidence from EFSA would be required in order to support the Commission in determining if canine leishmaniosis complies with the characteristics of a disease for which the Commission might adopt preventive health measures for its control pursuant to Regulation (EC) No 998/2013 of the European Parliament and of the Council. The Commission therefore requests EFSA to assess the available scientific information regarding canine leishmaniosis and to evaluate the relevance of measures aiming at mitigating the probability of introducing the disease into free areas through the movement of dogs.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

1. To collect the necessary data to characterise canine leishmaniosis in Europe and in particular:

- the inherent aspects of the epidemiology of the disease, i.e. the affected species, the life cycle, the modes of transmission and potential persistence of the parasite, the distribution of the disease (free and endemic areas);

- the impact of Leishmania infantum infections on animal health and welfare, human health, as well as its environmental impact in the regions of the EU where the disease is endemic.

2. To assess the efficacy of available preventive measures to protect dogs against Leishmania infantum infection, with the objective of mitigating the probability of introduction of the infection into free areas in the EU through movements of infected dogs.

3. To assess the probability that the infection would become established in free areas of the EU if Leishmania infantum were introduced by infected dogs.

ASSESSMENT

1. Introduction

Canine leishmaniosis (CanL) is a zoonotic disease caused in Europe by the protozoan parasite *Leishmania infantum*. The domestic dog is considered the main reservoir of human infection, and phlebotomine sandflies are the biological vectors of the parasite. In infected dogs *L. infantum* affects both viscera and skin, whilst in humans the parasite causes either visceral leishmaniosis, the most severe form of leishmaniosis, or the more benign, cutaneous leishmaniosis.

The structure of the current European Food Safety Authority (EFSA) opinion is based on the terms of reference (TORs) of the mandate. Section 2 addresses TOR1, namely the characteristics of CanL in Europe, including its epidemiological features and its impact where it is endemic. Section 3 describes the available preventative measures, thus addressing part of TOR2. Section 4 describes "test and exclusion" and "test and treatment" as options for mitigating the probability of introduction of *L. infantum* into free areas. In Section 5, the probability that the infection would become established in free areas, should it be introduced through movement of infected dogs, is assessed, thus addressing TOR3. Section 6 describes the application of the model developed in Section 5 to assess the probability reduction provided by mitigation measures, thus addressing the second part of TOR2 (efficacy of mitigation measures)

The current EFSA opinion relies partly on the preparatory work carried out by a consortium, which was published in the technical report on CanL (Mattin et al., 2014). This consisted of a systematic review on the characteristics of CanL and on preventative measures, and the development of a stochastic model for assessing the probability of introduction and establishment. The systematic review was updated for the purpose of this opinion and additional systematic reviews were conducted on diagnostics and treatment of CanL. The model was re-parameterised based on additional systematic review and expert knowledge.

2. Characterisation of CanL in Europe (TOR 1)

The question about the epidemiological characteristics of CanL (TOR1) was addressed by an extensive literature review, including information collected through the SR described in the technical report (Mattin et al., 2014). A summary of the available information on the geographic distribution of the parasite and the competent vectors in the EU, the affected species and life cycle, modes of transmission and a summary of the evidence of possible persistent infections in dogs, wildlife or sandflies or persistence of the parasite in the environment is provided in this section, as well as a brief evaluation of the impact of the disease on animal health and welfare and human health. After discussion with the requestor of the scientific opinion (European Commission (EC)), it was decided to limit the environmental impact assessment to a summary of current evidence on infection rates in wildlife and their infectiousness for vectors.

2.1. Geographical distribution of CanL in Europe

In Europe, CanL is known to be endemic in the Mediterranean countries, namely Albania, Croatia, southern France, Greece, Cyprus, Italy, Malta, Portugal, Spain and Turkey (WHO, 2010). It is also thought to be endemic in Romania, Bosnia, Macedonia, Montenegro and Bulgaria (Diza, 2008; Tsatchev et al., 2010; Alvar et al., 2012; Andric et al., 2013; Mircean et al., 2014).

There has been some recent evidence of leishmaniosis spreading north of endemic areas in Italy, to the foothills of the Alps (Maroli et al., 2008; Baldelli et al., 2011). Autochthonous cases have also been diagnosed in previously unaffected areas in northern Spain (Amusategui et al., 2004; Miró et al., 2012) and the French Pyrénées (Chamaille et al., 2010; Bourdeau et al., 2011, 2014a, b). Extension of the disease distribution areas is attributed to changes in vector distributions, which may result from climate changes, especially higher temperatures that are favourable to the vectors (Maroli et al., 2008; Ready, 2010). In addition, climate variations may trigger local and temporal increases in sandfly

population densities, which result in patchy distributions of vectors, especially at the fringe of endemic areas (Morosetti et al., 2009).

CanL is also sporadically diagnosed in countries/regions, north of the Mediterranean areas, where no competent vectors are reported. Such cases have been, so far, considered as "imported" and are related to increased travel and movement of pets and humans from endemic areas (Teske et al., 2002; El Hajj et al., 2004; Leishrisk, 2007; Dujardin et al., 2008; Menn et al., 2010). In Germany (Naucke and Schmitt, 2004), Holland (Tseke et al., 2002), Hungary (Tánczos et al., 2012), Serbia and the UK (Shaw et al., 2009; Savic et al., 2013), CanL cases are regularly reported in dogs with a history of travel to endemic areas. However, there are also some concerns that autochthonous leishmaniosis might occur in these countries, as a result of the spread of the vectors or non-vectorial transmission.

2.2. Prevalence of CanL in Europe

2.2.1. Endemic areas

An important epidemiological feature of CanL in endemic areas is a high prevalence of infection together with a low prevalence of clinical disease (Baneth et al., 2008).

2.2.1.1. Prevalence of infection

Prevalence estimates are highly variable; they depend on the true prevalence and are influenced by the diagnostic tests that are used (Morales-Yuste et al., 2012). Prevalence estimates are based either on parasite detection by polymerase chain reaction (PCR) or on immunological tests indicating exposure (serology and to a lesser extent, cellular immunity assays)

Seroprevalence in endemic countries is around 10 %, with wide variations within countries (Table 1). However, serological tests underestimate prevalence, since only a proportion of the *Leishmania*-infected dogs mount a detectable humoral immune response (see Table 2).

PCR studies conducted in endemic areas have given much higher figures, with up to 63–80 % of the dog population being PCR positive (Berrahal et al., 1996; Solano-Gallego et al., 2001; Leontides et al., 2002). These figures are considered closer to the true prevalences of infection; however, the high percentage cannot be ascertained, mainly because of the heterogeneity of the PCR methods, which precludes an overall evaluation of test performance (see Section 3). Table 2 illustrates the differences in prevalence estimates obtained in small-scale cross-sectional studies using both PCR and serology carried out over different periods.

Table 1: Seroprevalence of CanL in different periods in western European countries, according to 947 surveys including a total of 504 369 dogs, from 1971 to 2006 (modified from Franco et al., 2011)

Country	No of surveys	No of dogs tested	1971–1980 Prevalence [a] (range) [b]	1981–1990 Prevalence (range)	1991–2000 Prevalence (range)	2001–2006 Prevalence (range)	Total median (range)
France	169	39 259	4.4 % (0–40.0 %)	13.2 % (0.7–43.3 %)	9.1 % (0–27.6 %)	11.1 % (4.1–17.7 %)	8 % (0–43.3 %)
Italy	377	423 831	11.1 % (0–100 %)	19.1 % (2.5–66.7 %)	21.1 % (0–100 %)	10.7 % (0–100 %)	17.7 % (0–100 %)
Portugal	188	15 896	0 % (0–3.6 %)	10.4 % (0–39.8 %)	6.3 % (0–81.1 %)	17.6 % (0–21.4 %)	7.3 % (0–81.1 %)
Spain	213	25 383	– (–)	6.9 % (0–45.5 %)	5.2 % (0–100 %)	16.9 % (3.8–66.1 %)	5.9 % (0–100 %)
All countries	947	504 369	6.3 % (0–100%)	10.2 % (0–66.7 %)	11.7 % (0–100 %)	11.1 % (0–100 %)	10.0 % (0–100 %)

(a): Median seroprevalence from the different studies.
(b): Minimum and maximum seroprevalence values.

Table 2: Examples of prevalence of disease, seroprevalence and prevalence of *L. infantum* infection, based on both serology and molecular techniques, in several locations of various countries where CanL is endemic (modified from Solano-Gallego et al., 2009)

Country (region)	No of dogs	Prevalence of clinical disease	Seroprevalence	Prevalence of infection (PCR) [(a)]	Reference
France (south)	30	0 %	3 % (IFAT); 67 % (WB)	80 %	Berrahal et al. (1996)
	253	26 %	30 %	83 %	Lachaud et al. (2002)
Greece (centre)	73	0 %	12.3 % (IFAT)	63 %	Leontides et al. (2002)
Spain (Mallorca island)	100	13 %	26 % (ELISA)	67 %	Solano-Gallego et al. (2001)
Italy (south)	43	0 %	7 % (IFAT)	23 %	Oliva et al. (2006)
Cyprus	900	1.3 %	14.9 % (ELISA); 11.8 % (IFAT)	25 %	Mazeris et al. (2010)

(a): Different PCR methods were used.
ELISA, enzyme-linked immunosorbent assay; IFAT, immunofluorescence antibody test; WB, western blot.

2.2.1.2. Prevalence of clinical disease

As illustrated in Table 2, prevalence of clinical disease is consistently lower than prevalence of infection.

A questionnaire prepared by Mattin et al. (2014), which was sent to practices in France, Spain, Portugal and Italy, indicated an annual percentage of practice-attending dogs with a confirmed veterinary diagnosis of CanL ranging from 0.71 % (France) to 7.8 % (Greece), with 3–4 % in Spain, Italy and Portugal. Data from shelters show similar levels of disease prevalence in non-owned dogs as in those attending veterinary practices (Mattin et al., 2014).

Other data from Greece (Saridomichelakis, 2009) demonstrate prevalence estimates ranging from 0.98 % to 7.46 % across the country (average 3.3 %). The prevalence rates of clinical CanL in France, and to a lesser extent in Spain, have an even more heterogeneous spatial distribution. While very few cases are reported in central and northern France (clinical prevalence close to 0 %), the prevalence of the disease in the endemic areas of southern France may be as high as 1 %, and 2.7 % in Corsica. On average, the national disease prevalence (number of sick dogs treated per year) has been estimated as 0.4 % according to two surveys (Bourdeau et al., 2011).

2.2.2. Non-endemic areas

CanL occurrence in non-endemic countries is probably underestimated because of difficulties associated with diagnosis and under-reporting. Under-reporting is to be expected, considering that CanL is not a notifiable disease in most EU countries. In the UK, at least 257 dogs with confirmed leishmaniosis were presented between April 2005 and December 2007 (Shaw et al., 2009). In Sweden, according to the annual report of notifiable diseases of animals of the Swedish Board of Agriculture, 24 and 23 cases of affected dogs were reported in 2011 and 2012, respectively. In Germany, antibodies against *L. infantum* were detected in 569 out of 4 681 serum samples collected from dogs imported from endemic regions (4 226) or dogs having travelled to those areas (87) (Menn et al., 2010). Suspected autochthonous cases of CanL in non-endemic countries were recorded in Germany (Naucke and Schmitt, 2004), Hungary (Tánczos et al., 2012) and Romania (Mircean et al., 2014) as well as in Spain outside the endemic areas (Amusategui et al., 2004).

2.3. Geographic distribution of phlebotomine vectors in Europe

There are many sandflies species, but only a minority are competent vectors for *L. infantum* (Killick-Kendrick, 1999). Sandflies are mainly present in the southern Mediterranean parts of Europe;

however, some species with the potential for acting as competent vectors have been recorded at higher latitudes.

In Mediterranean countries of Europe, where CanL is endemic, there are at least nine species of phlebotomine sandflies possibly involved in the transmission of CanL (VBORNET (2012)).

The most widespread vector of *L. infantum* in the western Mediterranean basin is *Phlebotomus perniciosus* (Killick-Kendrick, 1999), a proven vector in France, Italy, Malta, Portugal and Spain (Maroli et al., 2013).

Other proven vectors of *L. infantum* in European countries are *P. ariasi*, *P. neglectus*, *P. perfiliewi* and *P. tobbi* (Killick-Kendrick, 1999; Ready, 2010).

The species *P. langeroni*, a proven vector in Egypt, was reported in Spain (Martinez-Ortega, 1996).

P. alexandri (Azizi et al., 2006; Colacicco-Mayhugh et al., 2010) and *P. syriacus* (Maroli et al., 2013), suspected vectors of *L. infantum* in some Middle East countries (Iran, Iraq, Oman), are also found in eastern Mediterranean countries (Greece, Israel, Lebanon, Palestine, Syria, Turkey) (Maroli et al., 2013). *P. mascittii* is widely distributed in temperate western Europe (see maps in Appendix A). A role for *P. mascittii* as a vector of leishmaniosis has been suspected but could not be proven by detection of *Leishmania* parasites in specimens belonging to this species.

2.3.1. In other EU countries

Phlebotomine sandflies have not been found, so far, in the Baltic countries, the Czech Republic, the Netherlands, Poland, the Nordic countries, Slovakia, Ireland or the UK.

The species *P. perniciosus*, *P. mascittii* and *P. ariasi* have been identified in France outside the Mediterranean regions, while *P. mascittii* and *P. perniciosus* have been signalled in south-western Germany, in Baden-Württemberg and the Rhineland-Palatinate (Naucke and Schmitt, 2004).

Specimens of *P. mascittii* were also sampled in Belgium (Depaquit et al., 2005). More recently, *P. mascittii* has been recorded in Austria (Naucke et al., 2011; Poeppl et al., 2013), while *P. neglectus* and *P. perfiliewi* were found in Hungary (Farkas et al., 2011).

Overall, it is difficult to give a precise picture of sandfly species distributions and densities, since data are scarce, especially in the northern parts of their distribution areas, because of limited or absence of trapping and monitoring. It should also be noted that a negative result from trapping does not demonstrate the absence of a given sandfly species within an area. Maps of the reported presence in the EU of *P. perniciosus*, *P. ariasi*, *P. neglectus*, *P. perfiliewi*, *P. tobbi*, *P. alexandri*, *P. syriacus and P. mascittii* are provided in Appendix A.

2.4. Affected species

For the purpose of this mandate, the "affected species" are those mammalian species that can be found infected by *L. infantum* (host species), whether or not they are clinically affected. Some of the affected species may serve as reservoirs of the disease.

Dogs are the main domestic host species infected by *L. infantum* in endemic areas, and it is widely accepted that they represent the main domestic reservoir host (Alvar et al., 2004; Baneth et al., 2008; Quinnell and Courtenay, 2009). Most infected dogs, however, do not develop the disease until a long time has passed after infection, and sometimes never do so (subclinical infections) (Baneth et al., 2008; Miro et al., 2008; Moreno and Alvar, 2002; Solano-Gallego et al., 2009). Clinical cases present a wide spectrum of clinical signs and clinicopathological abnormalities, generally involving both skin and internal organs (see Section 2.8). Disease severity may range from mild and self-limiting to very severe, and can be fatal (Solano-Gallego et al., 2009). There appear to be variations in dog genetic

susceptibility to disease, with some breeds being more susceptible, such as the boxer (Sanchez-Robert et al., 2008; Quilez et al., 2012), while other breeds, such as the Ibizan hound, exhibit mainly subclinical infections (Solano-Gallego et al., 2001).

Domestic cats can be infected by *L. infantum*. Clinical manifestations are rare and, when present, have many similarities with CanL, including a combination of visceral and cutaneous signs (Hervas et al., 1999; Marcos et al., 2009; Navarro et al., 2010; Ozon et al., 1998; Poli et al., 2002). Transmission of *Leishmania* from cats to sandflies has been demonstrated (Maroli et al., 2007).

Infection with or without clinical disease has also been reported in non-domestic canines, such as foxes, wolves and jackals (Beck et al., 2008; Fallah and Khanmohammadi, 2011; Luppi et al., 2008; Tenorio Mda et al., 2011), and in lions (Dahroug et al., 2011; Libert et al., 2012). *Leishmania* has also been detected in other wild carnivores, such as Egyptian mongooses and genets and Iberian lynx (Sobrino et al., 2008). Hares can be infected (Ruiz Fons et al., 2013), as can some species of rodents, such as black rats (*Rattus rattus*) (Gradoni et al., 1983; Zanet et al., 2014). The epidemiological role that these wild species may play as reservoirs of *L. infantum* is discussed in Section 2.6.2.

Horses have been reported to have only self-limiting cutaneous forms of leishmaniosis (Koehler et al., 2002; Rolao et al., 2005; Solano-Gallego et al., 2003).

Finally, leishmaniosis is a zoonosis, which remains a public health concern in southern Europe (Quinnell and Courtenay, 2009; Ready, 2010). As with CanL, the number of subclinically infected human individuals in endemic areas exceeds, by far, the number of clinical cases (see Section 2.8).

2.5. Life cycle

Sandflies acquire the parasite via ingestion of amastigote-infected mononuclear cells during a blood meal. In the sandflies, amastigotes transform into procyclic promastigotes and undergo a differentiation process in the midgut, leading to infectious metacyclic promastigotes within four to five days. After another three to five days, these invade the pharynx and the proboscis, through which they can be inoculated into a new mammalian host (Figure 1). In summary, sandflies infected with *L. infantum* are infectious to new mammalian hosts at least one week after taking an infective blood meal (Pozio et al., 1985; Maia et al., 2011).

When a female phlebotomine sandfly inoculates metacyclic promastigotes into the skin of a dog during a blood meal, these are rapidly phagocytosed by macrophages in the upper dermis. They differentiate into amastigote forms within hours, multiply intracellularly and disseminate to various organs via macrophage or dendritic cell circulation (Saint André Marchal et al., 1997). Intracellular amastigotes, the infective form for sandflies, can be taken up by a new sandfly during blood feeding several months after the initial infective bite (Saridomichelakis, 2009); Courtenay et al., 2014); however, the time between infection and infectiousness is variable.

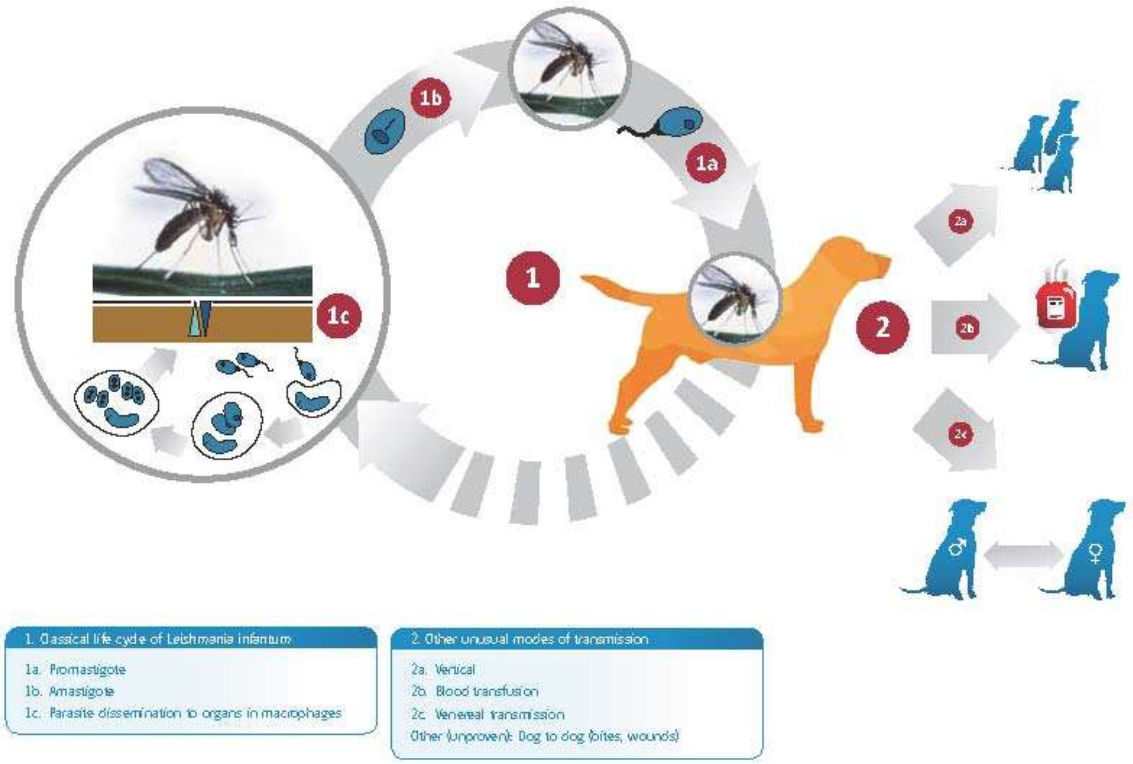


Figure 1: Life cycle and transmission of *L. infantum*

2.6. Modalities of transmission between mammals

2.6.1. Vectorial transmission

The main route of transmission of the parasite to mammals is via the bite of the female phlebotomine sandfly (Killick-Kendrick, 1999). Sandflies are the only demonstrated biological vectors of leishmaniosis, and there is no evidence that non-sandfly vectors are important in the spread of CanL in dog populations.

A possible role as *Leishmania* vectors was proposed for fleas (Coutinho and Linardi, 2007) and ticks, particularly for the tick *Rhipicephalus sanguineus*, in which DNA of *L. infantum* parasites has been detected in larvae from infected females (Dantas-Torres et al., 2010). However, these findings have not been confirmed under natural conditions. Thus, it appears that, even if non-sandfly arthropods play a role in *Leishmania* transmission, this is likely to be a secondary role, with little impact, if any, in the epidemiology of CanL.

2.6.2. Non-vectorial transmission

Direct transmission by dog-to-dog contact has been suspected in areas with no competent sandflies. CanL transmission has occurred between infected dogs imported from endemic areas and naive dogs that were part of the same household (Slappendel, 1988). It has also been proposed that the spread of *L. infantum* in foxhounds in the USA was the result of dog-to-dog transmission (Gaskin et al., 2002; Duprey et al., 2006).

Case reports of natural *Leishmania* infection in dogs transmitted via venereal (Silva et al., 2009; Naucke and Lorentz, 2012) or vertical routes (Boggiatto et al., 2011) provide further evidence of

alternative transmission routes. Pups born from infected dams were found infected, with evidence for infection having occurred *in utero* (Boggiatto et al., 2011; Kasbari et al., 2012).

Finally, successful transfer of *L. infantum* infection has been achieved by blood transfusion (Owens et al., 2001).

None of these transmission routes (direct contact, venereal, vertical, via blood transfusion) appears to sustain infection in a large population (i.e. larger than that of a household or a kennel). This is substantiated by the absence of spread in areas without competent vectors, while introduction of CanL into free areas is frequent. However, the occurrence and persistence of limited CanL foci (e.g. in households or in kennels) is a threat for further spread of the disease in non-endemic areas, should competent vectors be introduced.

There is no evidence that non-vectorial transmission can pose a risk to humans, as dog-to-human transmission by direct contact has not been reported. Non-vectorial transmission (vertical, venereal or via blood transfusion) between humans, however, has been reported occasionally (see Section 2.9).

2.7. Potential persistence of *L. infantum*

2.7.1. Persistent infections in dogs

2.7.1.1. Role of the dog immune response in the maintenance of the parasite

Several outcomes of infection are observed in dogs, ranging from subclinical to clinical, and in the clinical forms, from mild to severe and fatal disease (see Section 2.8). The following profiles of immune responses are observed at the two extremes of clinical spectrum:

(1) "Resistant" dogs develop a protective CD4+ T-cell-mediated immune response characterised by production of type 1 T helper (Th1) cytokines, such as interferon-γ, interleukin 2 (IL-2) and tumour necrosis factor alpha (TNFα), which contributes to the control of infection. TNFα has been shown to induce apoptosis of amastigotes in macrophages via nitric oxide synthesis (Holzmuller et al., 2006),

(2) Sick dogs develop limited cell-mediated responses with a mixed Th1 and Th2 cytokine pattern and a marked humoral immune response with no protective effect. As a consequence of ineffective cellular immune responses, high parasite burdens can be maintained and persist for months or even years in macrophages in several tissues of the infected individuals, such as the skin (Baneth et al., 2008).

2.7.1.2. Infectiousness of infected dogs to sandflies

Dogs with clinical or with subclinical infection may be infectious to sandflies (Baneth et al., 2008). Most studies have shown a strong association between clinical signs, high antibody levels and infectiousness (Courtenay et al., 2002; Quinnell and Courtenay, 2009); thus, clinically affected, seropositive dogs generally have higher parasite skin loads and are more likely to be infectious for sandflies than subclinically infected dogs (Courtenay et al., 2014). However, in a recent study using xenodiagnosis (i.e. feeding laboratory-reared female sandflies on infected hosts), a subclinical seronegative dog was found to be infectious for sandflies, although at a very low level (Bongiorno et al., 2013).

In conclusion, it should be considered that all seropositive dogs, whether or not they express clinical disease, are potential sources of infection for vectors and may transmit the disease. Seronegative infected dogs (generally subclinical) are unlikely to be infectious; however, during the course of infection, which may take months or years, they may develop active infection, seroconvert and become infectious.

2.7.2. Persistence of *Leishmania* in wildlife—the role of wildlife in CanL epidemiology

Several wildlife species can be infected by *L. infantum*; some may be dead-end hosts, unable to transfer the infection back to vectors and thereby to dogs or humans, e.g. the New World crab-eating foxes (*Cerdocyon thous*), while others have a suspected or proven role as reservoir hosts, either primary or secondary (Quinnell and Courtenay, 2009). An undisputable role of reservoir can be proven only by xenodiagnosis using competent vectors; however, these studies are lacking for most species.

The Iberian hare (*Lepus granatensis*) is abundant in some areas of the Iberian Peninsula, and high rates of *Leishmania* infection (30–50 %) have recently been reported in this species (Arce et al., 2013; Ruiz-Fons et al., 2013; Moreno et al., 2014). Investigations in Fuenlabrada, Madrid, concluded that hares were a likely reservoir host of *L. infantum* and that infection in this species contributed to a recent outbreak of human leishmaniosis (Molina et al., 2012; Aguado et al., 2013). Molina et al. (2012) demonstrated that apparently healthy, naturally infected hares were infectious to *P. perniciosus*, a competent vector for *L. infantum* (Molina et al., 2012). Moreover, blood meal analysis of 10 sandflies captured in the area showed a feeding preference for hares (n = 6), followed by humans (n = 3) and cats (n = 1) (Jimenez et al., 2013).

Díaz-Sáez et al. (2014) suggested that wild rabbits (*Oryctolagus cuniculus*) meet most of the conditions for being considered a reservoir host, with 20.7 % of the 150 rabbits tested found infected over a period of three years in the south-east of Spain. Furthermore, a xenodiagnostic study carried out by Jimenez et al. (2014) demonstrated that *L. infantum* was transmitted to *P. perniciosus* from wild rabbits and analysis of blood meals of *P. perniciosus* gives strong evidence that rabbits are contributing to the maintenance of the sandfly population in the study area.

The black rat (*R. rattus*) is a widespread and abundant rodent species in Europe. High rates of *Leishmania* infection (15–45%) have been found in this species in Italy using PCR (Di Bella et al., 2003). Infectiousness of black rats to the proven competent vector *P. perniciosus*, has been demonstrated under laboratory conditions (Gradoni et al., 1983; Pozio et al., 1985). Recently, on the island of Montecristo (Italy), where no dogs or other carnivores are present, up to 15.5 % of the local black rats tested were found to be infected with *L. infantum.* The only sandfly collected on the island was *P. mascittii*, whose vectorial competence has not been ascertained; thus, other transmission routes remain to be considered (Zanet et al., 2014).

High rates of *Leishmania* infection (10–40 %) have also been found in wild carnivores (Sobrino, et al., 2008; Millan et al., 2011) in areas of Europe where the disease is common in dogs. However, longitudinal studies evaluating persistence of infection and xenodiagnostic studies are lacking in wild carnivores (Millan et al., 2014). In addition, a high rate of infection in a given species does not provide proof that this species acts as a reservoir (Quinnell and Courtenay, 2009).

In conclusion, while the role as reservoir of wild carnivores has not been fully demonstrated, black rats, wild rabbits and hares might contribute to maintaining *L. infantum* circulation in some areas of southern Europe (Millan et al., 2014).

2.7.3. Vector biology and persistence of *Leishmania* in the vectors

2.7.3.1. Vector biology

Sandfly larvae feed on organic materials in soil or burrows of animals. A high humidity is required for their breeding sites, but they do not live in water. Their breeding sites are very difficult to find and therefore larvicidal treatments are not applicable. The duration of the preimaginal stages vary (from weeks to months) depending on environmental conditions (particularly temperature) (Busvine, 1980; Killick-Kendrick, 1999). The duration of *P. perniciosus* development, from a blood meal to the next generation of adults, was found to be 42 days under laboratory conditions (Romi et al., 1994). Under

natural conditions, the development time could be longer considering the diapausing capacity of the larval stage, which represents the overwintering stage in Palaearctic species.

Both adult males and females feed on natural sources of sugar, such as nectar, plant sap and honeydew of aphids or coccids; only females take blood meals, which are needed for egg maturation (except for *P. mascittii*, which occasionally may lay eggs without taking a blood meal). The various sandfly species differ in the number of blood meals taken to maturate one batch of eggs (Killick-Kendrick, 1999). The Mediterranean species acting as vectors of *L. infantum* normally take one blood meal for each gonotrophic cycle (Killick-Kendrick, 1999). Maturation of eggs after female engorgement takes at least six days, (Killick-Kendrick, 1999). The adult lifespan is around two to three weeks (Lewis, 1971). In a mark–release–recapture trial, a female *P. ariasi* was captured 28 days after feeding on a dog (Killick-Kendrick and Rioux, 2002).

The activity of adult sandflies is nocturnal or crepuscular. They rest in protected sites during the day. Sandflies do not disperse far from the breeding sites, with reported travelling distances ranging from hundreds of metres to one kilometre; a distance of more than 2 km was recorded for only *P. ariasi* (Killick-Kendrick, 1999).

Mediterranean sandflies seem to feed on any available mammals and birds (Rossi et al., 2008), but not on reptiles (Killick-Kendrick, 1999). They have a focal distribution in the geographical areas where they are present. This "patchy" distribution is linked with the availability of breeding sites and hosts. Furthermore, the abundance of sandflies in these foci fluctuates during the active season, which hampers their sampling.

2.7.3.2. Persistence of *Leishmania* in their vectors

Once infected, a sandfly remains infected for life, that is, on average, for two to three weeks. Vertical transmission of *Leishmania* has not been reported in sandflies.

2.7.4. Persistence of *Leishmania* parasites outside the vectors and the mammalian hosts

Leishmania tropica and *L. donovani* can persist in a viable form in blood products stored under blood bank conditions (refrigerated at 1–6 °C) for at least 25 days after human blood donation (Grogl et al., 1993). This information is lacking for *L. infantum* after human or canine blood donation.

2.8. Impact of the disease and disease prevention and control measures on animal health and welfare

2.8.1. Clinical illness

Clinical signs have been observed as early as six months of age (Tarantino et al., 2001; Lombardo et al., 2014), but often it takes more than a year for a susceptible dog to develop the first clinical signs or clinicopathological abnormalities following natural exposure to sandflies bites in an endemic setting (Foglia-Manzillo et al., 2013).

When clinically expressed, *L. infantum* infection in dogs can manifest as a chronic self-limiting disease, or a severe non-self-limiting illness (Baneth et al., 2008; Solano-Gallego et al., 2009). Thus, a wide spectrum of clinical disease presentations and degrees of severity are found in dogs and four clinical stages of disease have been proposed by the LeishVet group (Solano-Gallego et al., 2009, 2011), with different treatments and prognosis for each stage. Sick dogs may go through different clinical stages during their lifetime, and the duration of each stage is variable in different individuals. Immunocompromising conditions, such as neoplasia, immunosuppressive drugs and old age, may trigger the transition to more severe clinical stages (Solano-Gallego et al., 2009).

Skin lesions are the most obvious clinical manifestation of CanL (Solano-Gallego et al., 2011) and include alopecia and exfoliative dermatitis, ulcerative dermatitis, nodular, pustular and papular

dermatitis (Ferrer et al., 1988a) and onychogryphosis (Ciaramella et al., 1997; Ordeix et al., 2005). Lymphadenomegaly, splenomegaly, lethargy and weight loss are also common clinical findings (Slappendel, 1988; Ciaramella et al., 1997).

Glomerulonephritis and proteinuria are frequently reported in CanL (Costa et al., 2003; Zatelli et al., 2003). Estimates of the proportion of sick dogs with renal alterations range from 10 to 30 % of the total number of sick dogs (Baneth and Solano-Gallego, 2012). Although azotaemia is a relatively uncommon finding, severe renal failure can develop and is a major cause of mortality in affected dogs (Ciaramella et al., 1997; Solano-Gallego et al., 2011).

A range of ocular and periocular manifestations (Ciaramella et al., 1997; Peña et al., 2000), lameness due to polyarthritis (Santos et al., 2006) and/or bone lesions (Agut et al., 2003) and epistaxis have also been reported (Petanides et al., 2008).

During disease progression, lymph node enlargement and weight loss are the earliest and most frequently observed signs, followed by cutaneous abnormalities and then ocular signs (Foglia Manzillo et al., 2013).

2.8.2. Mortality

There is relatively few data on mortality rates and survival time in dogs with clinical CanL. In a survey conducted in 994 veterinary clinics in France in 2004, 22.5 % of infected and treated dogs survived less than a year, 13.5 % survived one to two years, 35.7 % survived two to five years and 30.3 % survived for more than five years (Bourdeau et al., 2007). The most common cause of death in CanL is severe chronic renal disease, which causes azotaemia and proteinuria (Solano-Gallego, et al., 2009). A short survival time has been associated with proteinuria and hypoalbuminaemia (Geisweid et al., 2012). The survival time may be influenced mainly by the initial clinical status at the time of diagnosis and the quality of management, including drug therapy and follow-up. A multinational survey was conducted in six countries. In five countries (Spain, Italy, France, Portugal and Slovenia), the survival time was more than five years in 30.3–45.1 % of sick dogs, two to five years in 35 % of sick dogs, one to two years in 8–28.8 % of sick dogs, three to six months in 1–34.7 % of sick dogs and less than three months in 0–9.6 % of sick dogs. A marked difference was identified in a sixth country (Greece), with much shorter survival times (only 7.1 % of sick dogs survived more than five years) (Bourdeau et al., 2014a).

The decision to euthanise a sick dog depends on the severity of the disease. Veterinary clinicians in Portugal, when questioned about the frequency of elective euthanasia as opposed to treatment for CanL, provided the following responses: never (14 % of respondents), occasionally (58 %), frequently (23 %) or always (5 %) (Oliveira et al., 2010). In the same survey, 43.1 % of the vets reported that less than 10 % of treated dogs were euthanised, with 34.3 % reporting that between 10 and 25 % of such dogs were euthanised. In France, 161 (91.0 %) of the leishmaniosis cases identified through the PanelVet survey were treated, whereas 16 (9.0 %) were euthanised within 60 days of the last test or treatment. Altogether, these figures suggest that approximately 10–25 % of CanL-diagnosed dogs are likely to be euthanised as a result of clinical disease in France and Portugal regardless of whether or not they receive treatment (Mattin et al., 2014).

2.8.3. Welfare impact at different disease stages

The impact of infection with *L. infantum* on the welfare of dogs is variable; subclinical infections do not affect animal welfare, while in sick dogs the disease impact depends on its severity (Solano-Gallego et al., 2009). There are four stages of the disease, of increasing severity, based on the dog's serological status, clinical signs and laboratory findings (Solano-Gallego et al., 2009). Disease severity is mainly established based on the evidence of renal disease evaluated by laboratory findings. In stage I (mild disease), there is no evidence of renal disease. In stage II (moderate disease), a very mild proteinuria might be present. In stages III and IV (severe and very severe diseases), there is a moderate to marked evidence of renal disease (Figure 12 and 13 in Appendix A). The LeishVet group proposed

a staging system, with stage I (mild disease) having a minor or no impact on the dog's welfare (see Appendix B), and being more likely to result in complete recovery. Stage IV (very severe disease) generally has a high to extreme impact on the animal's welfare, although several experts thought that this stage is only moderately associated with pain. Complete clinical recovery is thought to be rare in this stage of the disease. Death and euthanasia were considered common outcomes in dogs with stage IV disease. Stages II and III of the disease had intermediate scores. It was not possible to estimate the frequency at which each clinical stage of disease occurred in the canine population (see Appendix B and Mattin et al., 2014).

2.9. Impact of *L. infantum* infection on human health

2.9.1. Incidence of visceral and cutaneous leishmaniosis in Europe

Visceral and cutaneous leishmaniosis incidence ranges were estimated by Alvar et al. (2012), by country and epidemiological region, based on reported incidence, under-reporting rates, if available, and the judgement of national and international experts. The reported and estimated incidence of visceral leishmaniosis and of cutaneous leishmaniosis in the Mediterranean region is shown in Table 3.

The authors recognised the uncertainties inherent to these data and, for that reason, presented rough ranges rather than single estimates for each outcome. Conservative assumptions for the under-reporting rates have been used deliberately and the resultant multipliers (true leishmaniosis incidence rates) may therefore be substantially higher (Alvar et al., 2012). Under-reporting was considered uncommon in all European countries (1.2- to 1.8-fold).

Table 3: Reported and estimated incidence of visceral leishmaniosis and cutaneous leishmaniosis in humans in southern European countries [(a)]

Country	Period	Average no of VL cases per year	Estimated Annual VL incidence [(b)]	Period	Average no of CL cases per year	Estimated annual CL incidence [(b)]
Albania	2004–2008	114	1400 to 2100	2004–2008	6	
Bosnia and Herzegovina	2002–2005	2	20 to 30	2008	0	
Bulgaria	2004–2008	7	8 to 120	2008	0	
Croatia	2004–2008	5	60 to 80	2004–2008	2	60 to 100
Cyprus	2008	2	20 to 40	2006–2008	1	
France	2004–2008	18	180 to 300	2004–2008	2	60 to 100
Greece	2004–2008	42	500 to 800	2004–2008	3	80 to 130
Italy	2003–2007	134	1600 to 2400	2003–2007	49	1400 to 2300
Macedonia	2005–2009	7	90 to 130	2008	0	
Monaco	No data			No data		
Montenegro	2004–2008	3	40 to 50	2008	0	
Portugal	2003–2007	15	200 to 300	2004–2008	0	
Slovenia	No data			No data		
Spain	2004–2008	117	1400 to 2190	2004–2008	0	

CL, cutaneous leishmaniosis; VL, visceral leishmaniosis.
(a): In all other listed countries, apart from Greece where *Leishmania tropica* is also endemic, cutaneous leishmaniosis is caused by Leishmania infantum (modified from Alvar et al., 2012).
(b): Annual incidence estimated per 100 000 habitants

2.9.2. Symptoms in humans

Visceral leishmaniosis is the most severe clinical manifestation of *L. infantum* infection in humans, and is frequently fatal if left untreated (World Health Organization, 2010; Desjeux, 2004; Ready,

2010). Common clinical signs and clinicopathological abnormalities include hepatosplenomegaly, pyrexia, anaemia, leucopaenia and thrombocytopaenia (Maltezou et al., 2000; Lita et al., 2002; Fernandez-Guerrero et al., 2004; Papadopoulou et al., 2005). The cutaneous form of the disease is more benign and can manifest as single (Murray et al., 2005) or multiple lesions (Bongiorno et al., 2009). Humans can be infected with *L. infantum* via the bite of an infected sandfly (Killick-Kendrick, 1999). However, non-vector routes have also been reported, including vertical transmission (Meinecke et al., 1999; Boehme et al., 2006), venereal transmission (Symmers, 1960), transmission through organ transplantation (Antinori et al., 2008), blood transfusions (Cummins et al., 1995; Dey and Singh, 2006) and through sharing contaminated syringes (Cruz et al., 2002; Morillas-Marquez et al., 2002).

A review by Michel et al. (2011) concluded that most humans infected with *L. infantum* have asymptomatic infections. Risk factors for clinical disease include young age (Maltezou et al., 2000), human immunodeficiency virus (HIV) infection (Desjeux and Alvar, 2003) and other immunosuppressive states (Antinori et al., 2008). The prevalence of HIV among hospital patients with a primary diagnosis of leishmaniosis in Spain was 21.33 %, and co-infection with HIV was recorded in 7.0 % of hospital admissions with a primary diagnosis of leishmaniosis in Italy. When compared with the prevalence of HIV among the general population aged 15–49 (0.4 % for both countries (WHO, 2013)), there appears to be an association between being diagnosed with leishmaniosis and HIV. A bimodal age distribution was observed in hospital data from Spain and Italy, with peaks in infancy and middle age.

Cutaneous leishmaniosis commonly presents as single or multiple erythematous plaques or papules (Aguado et al., 2013). The cure rate of leishmaniosis with treatment is reported to exceed 95 % in France, Italy, Portugal and Greece. Spain reported a fatality rate for visceral leishmaniosis of 5 % (Alvar et al., 2012). Fatality rates of 4.1 % and 2.7 % among hospitalised leishmaniosis cases in Portugal and Spain, respectively, were calculated from data described in Section 3.5.3.2 of the External Report delivered to EFSA (Mattin et al., 2014). All of the fatal leishmaniosis cases in Spain were diagnosed with comorbidities, including two patients with concurrent HIV.

3. Preventative interventions

The probability of introducing *L. infantum* into free areas could potentially be reduced by advising dog owners from non-endemic areas not to travel to endemic areas with their dogs. This section describes the available preventative measures to protect susceptible dogs against *Leishmania* infection and assesses their overall efficacy through a systematic review, thus addressing the first part of TOR2. The efficacy of preventative measures in reducing the probability of introduction of CanL in free areas of the EU (second part of TOR2) is analysed through a simulation model in Section 5.

The systematic review aimed to assess the efficacy of different prophylactic measures, i.e. vaccination, prevention of vector bites using insecticides, prophylactic medication, in preventing *L. infantum* infection in individual dogs. The detailed protocol is described in an external scientific report of EFSA (Mattin et al., 2014). The systematic review examined randomised control trials (RCTs), non-randomised clinical trials (OCTs), cohort studies and case–control studies, which investigated prophylactic control measures for naturally occurring *L. infantum* infection. The primary outcome measure examined was the proportion of dogs infected with *L. infantum*, based on serology and/or parasite detection.

From the literature search, which identified 937 publications up to 31 December 2012, only 23 studies met the selection criteria and thus were included in data extraction. Among the 23 studies, there were 12 studies on vaccination, 10 studies on topically applied insecticides (5 on collars, 5 on spot-ons) and 3 studies on prophylactic medication. Pooling of outcome data from multiple studies investigating the same category of intervention (for the purpose of meta-analyses) was not possible because of the heterogeneity of the study designs and the interventions themselves (e.g. vaccine trials with different antigens). The systematic review was later updated to cover the period between 31 December 2012

and 28 February 2014, and 182 additional papers were identified. Of these, only one paper met the selection criteria, from which data were extracted.

3.1. Vaccination

3.1.1. Objectives

The ultimate goal of vaccination, which is prevention of infection, is very difficult to achieve with protozoan pathogens. More realistic objectives of CanL vaccination include (1) reduction of the infection rate in vaccinated dogs, (2) reduction of the parasite load in vaccinated dogs in case they become infected despite vaccination, thereby reducing infectiousness of those infected dogs for sandflies and (3) protection against the clinical illness in infected dogs. Both the reduction of infection rate (1) and that of infectiousness (2) may contribute to limiting transmission of *L. infantum* in dog populations, thereby reducing incidence of CanL. Moreover, if vaccination in the dog population results in a reduction of the *L. infantum* reservoir, public health benefits may be expected.

3.1.2. Protective antigens

Proteins involved in promastigote entry and/or survival into the host phagocytes are major targets for vaccine development. However, other proteins playing a role in the host–parasite relationship may also contribute to inducing protective responses, as reviewed elsewhere (Reis et al., 2009).

The leishmanial antigens which have shown protective potential so far include killed promastigotes (ALM) (Mohebali et al., 1999, 2004), excreted–secreted products of *L. infantum* purified from culture supernatants (Lemesre et al., 2007) (LiESAp, CaniLeish®), the fucose–mannose ligand from *L. donovani* (FML, Leishmune®) (Nogueira et al., 2007 Lima et al., 2010) and single or multiple recombinant antigens (e.g. MAPS–M16–LeiF). They have been associated with various types of adjuvants (muramyl dipeptide or MDP, QuilA, saponin or Bacillus Calmette–Guérin), the choice of which is important both for improving immunogenicity of leishmanial antigens and for triggering a protective, Th1-oriented response.

The only commercial vaccine currently available in Europe is CaniLeish®, which consists of excreted–secreted products obtained by *in vitro* culture of promastigotes (equivalent to LiESAp) adjuvanted with QuilA (EMA, 2011). The first immunisation consists of three subcutaneous injections at three-week intervals and is carried out on dogs more than six months of age. The manufacturer recommends the vaccination of only non-infected dogs, based on a negative rapid serological test. A booster vaccination is recommended every year, as the duration of immunity has been estimated to be at least one year based on the persistence of a delayed-type hypersensitivity (DTH) response to leishmanial antigens (Moreno et al., 2014). A survey sent out to veterinarians in 2014 revealed that the cost of the first immunisation varies in EU countries from €140 to €200 per dog (Mattin et al., 2014), which may limit vaccine coverage in the dog population.

3.1.3. Protective immunity

It is assumed that protective immunity conferred by vaccination against leishmaniosis relies on induction of strong *Leishmania*-specific cellular responses, of a mixed Th1/Th2 phenotype with a predominant Th1-type (Carrillo and Moreno, 2009). Thus, biomarkers that are considered as possible correlates of protection include markers of cell-mediated immune response (peripheral blood mononuclear cell (PBMC) proliferation in response to *Leishmania* antigens, DTH, macrophage leishmanicidal activity) and Th1 cytokines, with interferon gamma (IFNγ) considered as a major hallmark of protective immunity. Conversely, the development of a prominent *Leishmania*-specific humoral immune response generally reflects active parasite multiplication and does not correlate with protection (Baneth et al., 2008).

3.1.4. Assessing vaccine efficacy

Experimental studies have indicated that achievement of the above immunological profile via immunisation may be a prerequisite for protective immunity; however, it is not always predictive of protection against infection and/or disease (EMA, 2011). Therefore, "immunisation and challenge" strategies are necessary for a full assessment of vaccine efficacy.

3.1.4.1. Immunisation studies

Immunisation trials have been conducted either under experimental conditions (including artificial challenge) or/and in field trials involving natural exposure to sandfly bites.

Experimental challenge

Experimental challenge (e.g. intravenous injection of *Leishmania* promastigotes) does not consistently reproduce infection and clinical disease in dogs, which limits its applicability for vaccine testing. In a study involving vaccination of 10 dogs with CaniLeish®, the vaccine failed to consistently prevent infection, but 7/10 dogs had no or only transient infection and 3/10 developed active, persistent infection (i.e. showing biomarkers prognostic for progression towards disease) whereas the opposite (3/10 and 7/10, respectively) was observed in the non-vaccinated, inoculated controls. Average parasite loads, as determined by quantitative polymerase chain reaction (qPCR), were significantly lower in the vaccinated dogs throughout the challenge period (Martin et al., 2014).

Natural challenge

The systematic review examined 12 studies on the effect of vaccination, in combination with natural challenge up to 31 October 2014 (Wylie et al., 2014).

The diagnostic methods used to assess vaccine efficacy, i.e. the proportion of *L. infantum* infected dogs in control versus vaccinated groups, were parasite detection and/or serology.

Of the 12 studies examined by the systematic review 6 showed a significant protective effect of vaccination. These were two studies with LiESAp or CaniLeish® (Oliva et al., 2012), two studies with Leishmune® (Nogueira et al., 2005; Lima et al., 2010) and two studies with ALM (Mohebali et al., 1999, 2004).

One of the studies examined was a double-blind field trial with the LiESAp–MDP vaccine (equivalent to CaniLeish® except for the adjuvant) involving 340 initially seronegative, healthy dogs in endemic areas of southern France, which were followed for two years following vaccination (Lemesre et al., 2007). At the end of the observation, based on serology, parasite culture from bone marrow aspirates and PCR, only 1 out of 165 vaccinated dogs was infected (infection rate 0.6 %) whereas 12 of the 175 controls were infected (infection rate 6.9 %).

CaniLeish® was subsequently tested in young beagle dogs vaccinated in the laboratory and thereafter exposed to high natural challenge for two years in Spain (Barcelona) and Italy (Napoli). In each site, 22 vaccinated dogs and 23 controls were exposed to natural infection.

Fifty-nine per cent of the vaccinated and 72 % of the controls tested positive by PCR at least once during the period (a non-significant difference between groups), whereas significantly fewer dogs expressed clinical disease in the vaccinated group. Overall, the vaccine appears to reduce the proportion of dogs developing active infection (12 % in vaccinated vs. 33 % in controls) or clinical disease (7 % vs. 23%) (Oliva et al., 2014).

Studies involving xenodiagnosis techniques have revealed that vaccinated dogs that develop active infection and disease despite vaccination may be infectious to sand flies, although at lower rates (fewer sandflies were infected after feeding on vaccinated dogs than on the controls) and lower infection burdens in gut sandflies (Bongiorno et al., 2013). These results suggest an effect of

vaccination on transmission to sandflies. The Leishmune® vaccine, which is commercialised in Brazil, has also been reported to reduce transmission of *L. infantum* from dogs to sandfly vectors in South America (Saraiva et al., 2006; Santos et al., 2007).

3.1.4.2. Epidemiological studies

CaniLeish®, the only vaccine commercialised in Europe, has only recently become available. Full validation will require long-term monitoring of dogs after vaccination and natural exposure to *L. infantum* infection. Thus, several years of vaccine use in veterinary practice will be necessary before full validation, with regard to reduction of the incidence of disease in endemic areas and individual protection of dogs vaccinated prior to potential exposure.

For evaluating the preventative effect of vaccination on infection/infectiousness/disease, it is essential that dogs are not infected at the time when vaccination is applied; this is achieved, in principle, by carrying out serology prior to vaccination and vaccinating only seronegative dogs. However, in endemic areas, a proportion of infected dogs may remain seronegative for a long time. A recent study has also indicated that the sensitivity of rapid serological tests used for dog screening prior to vaccination with CaniLeish® is low (Solano-Gallego et al., 2014). Thus, it is likely that some dogs will be already *Leishmania*-infected at the time of vaccination, and this will be a complicating factor for assessing vaccine efficacy in the field.

3.1.5. Adverse effects of vaccines

The adverse effects of CaniLeish® do not seem to differ significantly from other saponin-adjuvanted vaccines. Inflammatory local reactions at the site of injection may occur, which resolve spontaneously in 2–15 days. General signs such hyperthermia, apathy and digestive disorders have been reported, lasting for one to six days (NOAH, 2013).

3.1.6. Conclusion on vaccination

Although protective immunity has been well characterised in CanL, no biomarker has been validated, so far, as a measure of vaccine efficacy; thus, immunisation and challenge experiments are required to test vaccine efficacy. Moreover, as the disease is difficult to reproduce under laboratory conditions, vaccine validation requires RCTs under natural challenge conditions and ultimately analyses of data from large-scale use in the field.

No CanL vaccine developed so far is able to confer full protection against infection. However, some vaccines, such as CaniLeish®, the only vaccine commercialised in EU, provide partial protection against active *L. infantum* infection and clinical disease in vaccinated dogs. Since disease severity appears to be generally associated with high parasite loads in the skin and infectiousness, vaccinating with CaniLeish® may reduce parasite load and infectiousness in those dogs which get infected despite vaccination, as suggested by a recent study using xenodiagnosis (Bongiorno et al., 2013). Thus, vaccination may effectively complement other preventative measures. However, it has not yet fully proven its long-term efficacy in reducing transmission, and therefore incidence of infection in dogs and humans, in endemic areas. Furthermore, there are limited published data on its protective potential (prevention of infection) in individual dogs from non-endemic areas newly and temporarily exposed to challenge in endemic areas (Oliva et al., 2014).

It should be stressed, however, that reduced transmission of *L. infantum* infection in an endemic area, as a result of implementing vaccination in the local dog population, does not imply that a high level of individual protection from infection and/or disease is achieved by vaccinating naive dogs prior to exposure.

Furthermore, antibodies elicited on vaccinated dogs cannot be discriminated with current serological methods, which will have implications in the context of pre-movement testing dogs from endemic areas to prevent introduction of *L. infantum* in free areas.

3.2. Topical insecticides

Insecticides used for protecting dogs by topical application are of the pyrethroid family—mainly deltamethrin, permethrin and flumethrin (some of them in combination with neonicotinoids, such as imidacloprid and dinotefuran). They can be applied to dogs as spot-on, sprays or collars (Podaliri Vulpiani et al., 2011).

Their main effect as contact repellents is to avoid sandfly bites, thereby reducing the vectorial capacity; moreover, they have a secondary insecticide effect on those sandflies that are able to feed on the treated dogs.

Insecticides for environmental vector control were not considered in this assessment as their use raise environmental concerns that do not seem proportionate with the objective of protecting individual dogs from sandflies. Moreover, the use of insecticides in the outdoor environment is not suitable for the control of sandflies, which tend to concentrate in focal areas that may be difficult to identify and whose breeding sites are mostly unknown (Alexander and Maroli, 2003). Indoor residual insecticide spraying has proved to be effective on endophilic vectors (i.e. mainly resting indoor after blood-feeding). Most studied phlebotomine vector populations in Europe, however, are mainly exophilic (WHO, 2010).

3.2.1. Collars

Six papers were reviewed reporting on studies that evaluated the effect of collars using active substances of the pyrethroid family. Four of five studies that evaluated deltamethrin collars provided evidence that deltamethrin had a protective effect (Maroli et al., 2001; Gavgani et al., 2002; Foglia Manzillo et al., 2006; Ferroglio et al., 2008). One additional study included in the systematic review showed that collars combining imidacloprid and flumethrin have a protective effect (Otranto et al., 2013) (see Table 4). Full protection is obtained one week after application because of the time needed for the insecticide to diffuse over the entire body surface.

Table 4: Effect of pyrethroid impregnated collars on the proportion of dogs infected with *L. infantum*

Description of intervention	Control group		Intervention group		RR (95 % CI)	1 – RR (95 % CI)	Reference
Active substance (dose)	Positive [(a)]	Negative [(b)]	Positive [(a)]	Negative [(b)]			
Deltamethrin collar (0.76 g)	6	32	0	42	0.07 (0.00–1.2)	0.93 (–0.2–1)	Aoun et al. (2008)
Deltamethrin collar (dose not mentioned)	30	158	3	116	0.16 (0.05–0.51)	0.84 (0.49–0.95)	Ferroglio et al. (2008)
Deltamethrin collar (0.76 g small/medium dog; 1 g to large dogs)	21	10	12	24	0.49 (0.29–0.83)	0.51 (0.17–0.71)	Foglia Manzillo et al. (2006)
Deltamethrin collar (40 mg/g)	31	435	11	343	0.47 (0.24–0.92)	0.53 (0.08–0.76)	Gavgani et al. (2002)
Deltamethrin collar (0.76 g to small dogs; 1 g to large dogs; 1.52 g to very large dogs)	24	69	4	110	0.14 (0.05–0.39)	0.86 (0.61–0.95)	Maroli et al. (2001)
Imidacloprid (10 %) and flumethrin (4.5 %) collar	21	30	0	63	0.02 (0.00–0.3)	0.98 (0.7–1)	Otranto et al. (2013)
Imidacloprid (10 %) and flumethrin (4.5 %) collar	3	83	41	104	0.09 (0.03–0.28)	0.91 (0.72–0.97)	Brianti et al. (2014)

(a): Dogs tested positive by one or more parasitological, serological and/or PCR tests.

(b): Dogs tested negative by one or more parasitological, serological and/or PCR tests.
1 – RR, efficacy; CI, confidence interval; RR, relative risk.

3.2.2. Spot-on devices

Four of five studies of spot-on preventives found that they had a significant protective effect against CanL (Table 6): one using 65 % permethrin (Ferroglio et al., 2008), another using 10 % imidacloprid and 50 % permethrin, evaluated by Otranto et al. (2010), and both the 28 ± 2-day and 14 ± 2-day treatments with 10 % imidacloprid and 50 % permethrin, evaluated by Otranto et al. (2007). Full protection is obtained about two days after application.

Table 5: Effect of pyrethroid based spot-on applications on the proportion of dogs infected with *L. infantum*

Description of intervention	Control group		Intervention group		RR (95 % CI)	1 – RR (95 % CI)	Reference
Active substance (dose)	Positive [(a)]	Negative [(b)]	Positive [(a)]	Negative [(b)]			
Permethrin (65 %)	30	158	3	117	0.16 (0.05–0.51)	0.84 (0.49–0.95)	Ferroglio et al. (2008)
Permethrin (65 %)	17	84	8	66	0.64 (0.29–1.40)	0.36 (–0.04–0.71)	Giffoni et al. (2002)
Imidacloprid (10 %) and permethrin (50 %)	20	169	2	183	0.10 (0.02–0.42)	0.9 (0.58–0.98)	Otranto et al. (2007)
Imidacloprid (10 %) and permethrin (50 %)	20	169	1	193	0.05 (0.01–0.37)	0.95 (0.63–0.99)	Otranto et al. (2007)
Imidacloprid (10 %) and permethrin (50 %)	21	35	0	71	0.02 (0.00–0.32)	0.98 (0.68–1)	Otranto et al. (2010)

(a): Dogs tested positive by one or more parasitological, serological and/or PCR tests.
(b): Dogs tested negative by parasitological, serological and/or PCR tests.

3.2.3. Sprays

Spray pump formulations containing permethrin have also been shown to be active experimentally in preventing sandfly bites for periods of two to three weeks, thus potentially mitigating the vectorial transmission of *Leishmania*. To date, however, no study has been conducted to verify their preventative effect on *Leishmania* transmission although this indication has been authorised in some countries. Sprays are protective immediately after application (Mercier et al., 2006).

3.2.4. Possible adverse effects of topically applied insecticides

The product datasheets report that skin sensitivity, lethargy, behaviour changes, gastro-intestinal and neurological signs rarely occur following application of imidacloprid and permethrin (NOAH, 2013) or deltamethrin (NOAH, 2013).

3.2.5. Conclusion on topical insecticides

The topically applied pyrethroid insecticides tested are effective in reducing the proportion of *Leishmania*-infected dogs compared with untreated dogs. Their efficacy has been demonstrated under experimental conditions and in controlled field studies providing mass treatment effect. It is uncertain whether the same efficacy of insecticides can be obtained in individual dogs when application is their owners' responsibility.

3.3. Prophylactic medication

Domperidone (Leishguard®) has been proposed for use as prophylactic medication against CanL. The drug is a dopamine D2 receptor antagonist which has been reported to have immunostimulant properties via the stimulation of prolactin secretion which acts as a pro-inflammatory cytokine (Gomez-Ochoa et al., 2009; Sabate et al., 2014).

There is only one peer-reviewed publication on domperidone as a prophylactic drug against CanL. The drug (Leishguard®) is administered at 0.5 mg/kg daily during 30 consecutive days every four months. A statistically significant protective effect of the drug against clinical disease was observed (Sabate et al., 2014). Further independent assessments are required to fully evaluate the efficacy of domperidone as a prophylactic drug against infection and disease. Furthermore, the efficacy of domperidone in preventing CanL should be assessed in naive dogs from non-endemic areas and those with limited past exposure. Indeed, the drug has been so far evaluated in endemic areas, where previously acquired immunity to *Leishmania* can play a synergic protective role.

Table 6: Effect of domperidone administration on the proportion of dogs infected with *L. infantum*

Description of intervention	Control group		Intervention group		RR (95 % CI)	1 – RR (95 % CI)	Reference
	Positive [(a)]	Negative [(b)]	Positive [(a)]	Negative [(b)]			
Domperidone	22	24	5	39	0.24 (0.10, 0.57)	0.76 (0.43, 0.90)	Sabate et al. (2014)

(a): Dogs tested positive by serological tests.
(b): Dogs tested negative by serological tests.

3.3.1. Adverse effects of domperidone

Following administration of domperidone, commonly observed side effects are mild gastro-intestinal disturbances (diarrhoea, vomiting) and galactorrhoea (Sabate et al., 2014). It should be noted, however, that this drug has displayed cardiotoxicity in humans (Lertxundi et al., 2013) and, for this reason, its safety is currently under re-evaluation by the Pharmacovigilance Risk Assessment Committee (PRAC) at the European Medicines Agency (EMA). Thus, further evaluation of domperidone safety in dogs is required.

3.4. Effects of preventative measures on animal welfare

Following the elicitation of knowledge from six selected CanL experts, it was concluded that topical insecticides and prophylactic medication had the lowest impact on dog welfare and are the most publicly acceptable control measures. Vaccination was considered to have a slightly higher impact on animal welfare (based on four responses) because of higher frequency and the duration of side effects (Mattin et al., 2014).

3.5. Discussion and conclusions on preventative interventions

3.5.1. Assessment of study validity

The risk of methodological shortcomings in the reviewed studies was assessed by the procurement (Mattin et al., 2014). The assessment was based on the Cochrane Handbook for systematic reviews of interventions (Higgins and Green, 2008) and included several domains (e.g. methods of randomisation, presence of incomplete data, study duration). Risk of biases was identified for most studies, including the most efficacious interventions in each intervention category. Therefore, the results of these studies need to be interpreted with caution. The report highlighted that well-designed, adequately powered RCTs are needed to fully assess the benefits conferred by preventative measures.

3.5.2. Efficacy of interventions

The reviewed studies support the use of the following measures to prevent *L. infantum* infection and/or disease in both endemic and non-endemic areas:

- topical insecticides: deltamethrin collars; 65 % permethrin, 10 % imidacloprid and 50 % permethrin spot-ons;

- vaccination with CaniLeish® (other vaccines tested in the reported studies may be as efficient, but CaniLeish is the only vaccine commercialised in the EU);

- possible use of prophylactic medication with domperidone, with reservations as to its efficacy in immunologically naive dogs, and regarding potential long-term toxicity (based on data in humans).

Combinations of preventative measures, such as vaccination and application of topical insecticides, are likely to increase the efficacy of prevention, and they are often recommended by practitioners for dogs living in endemic areas or dogs travelling to endemic areas (Miró et al., 2008). However, no RCTs that would prove the benefits of combining preventative measures have been conducted yet.

4. Performance of diagnostic tests and treatments

Following consultation with the EC, it was agreed, in the context of this mandate, to also examine "test and exclusion" and "test and treatment" as options for mitigating the probability of introduction of *L. infantum* into free areas. Both options highly rely on performance of diagnostic tests. In addition, the second option relies on the efficacy of a long-term parasitological cure through medical treatment. Therefore, two systematic reviews were carried out. One reviewed the sensitivity and specificity of the available diagnostic tools for detection of *L. infantum* infections in dogs; a second review assessed the efficacy of existing pharmaceutical treatments in inducing parasitological cure of *L. infantum* infections in dogs.

A detailed description of the systematic review protocols can be found in the external technical report submitted to EFSA (O'Connor et al., 2015).

4.1. Performance of the currently available diagnostic tests for detection of *L. infantum* infections in dogs

4.1.1. Existing diagnostic tests

Diagnosis is usually performed to confirm disease in a dog with clinical signs or clinicopathological abnormalities compatible with CanL. However, detection of infection may also be pursued for early detection of dogs heading towards progression of disease, for research studies, for screening clinically healthy dogs living in endemic regions, to prevent transmission by blood transfusion, to avoid importation of infected dogs to non-endemic countries and to monitor response to treatment. Different diagnostic procedures and interpretations of test results might be used accordingly, depending on the purpose of the diagnostic investigation (Solano-Gallego et al., 2009).

Accurate diagnosis of clinical leishmaniosis often requires an integrated approach consisting of thorough physical examination, clinicopathological tests and specific diagnostic assays. The main specific assays include microscopic demonstration of parasites in cytological preparations or histopathological specimens, serology, culture of the organism in appropriate media or detection of parasite DNA using molecular methods.

Leishmania amastigotes can be detected via cytology of cutaneous lesions, spleen, lymph nodes, bone marrow and, less commonly, other tissues or body fluids, such as joint, cerebrospinal and abdominal fluids. Detection of amastigotes by cytology is frequently unrewarding because of a low to moderate number of detectable parasites present, even in dogs with full-blown clinical disease (Roura et al., 1999b; Moreira et al., 2007). *Leishmania* parasites may also be visualised in histopathological formalin-fixed, paraffin-embedded biopsy sections of the skin or other infected organs (e.g. lymph nodes). Definite identification of parasites within tissue macrophages may require an immunohistochemical staining method to verify the presence of *Leishmania*.

The isolation in culture of parasites from infected tissues is not suitable for rapid diagnosis. Parasite culture is used more often for research purposes (Solano-Gallego et al., 2009).

Various serological methods for the detection of anti-*Leishmania* antibodies have been developed, such as the indirect immunofluorescence assay (IFA), ELISA, direct agglutination assays (DAT), rapid tests and Western blotting (Gomes et al., 2008; Miró et al., 2008; Solano-Gallego et al., 2009). The diagnosis of CanL can be made by the detection of specific serum antibodies (IgG) using quantitative serological techniques, such as IFAT and ELISA. In general, good sensitivities and specificities are gained with these methods for the diagnosis of clinical CanL (Miró et al., 2008). Rapid serological tests are easy to use and provide qualitative results in the clinical setting. These kits usually have good specificity, but their sensitivity is variable, and their performance is still not optimal (Mettler et al., 2005). High antibody levels are usually associated with clinical disease and a high parasite burden (Reis et al., 2006; dos-Santos et al., 2008) and, for this reason, they are conclusive of a diagnosis of leishmaniosis, provided dogs have not been previously vaccinated (Moreno et al., 2012; Oliva et al., 2014). The presence of lower antibody levels is not necessarily indicative of an active infection and needs to be confirmed by other diagnostic methods such as PCR, cytology or histology (Solano-Gallego et al., 2009). Serological cross-reactivity with different pathogens is possible with some serological tests, especially those based on whole parasite antigens. Cross-reactivity has been mainly reported with other species of *Leishmania* (Barbosa-De-Deus et al., 2002; Ferreira Ede et al., 2007; Porrozzi et al., 2007), and with *Trypanosoma cruzi* (Barbosa-De-Deus et al., 2002). However, these parasites do not infect dogs in Europe.

Detection of parasite-specific DNA in tissues by PCR allows sensitive and specific diagnosis. Different assays have been developed which include conventional PCR, nested PCR and real-time PCR, and target either genomic DNA (gene encoding the small-subunit ribosomal RNA (rRNA), the internal transcribed spacer of the ribosomal operon) or kinetoplastic DNA (kDNA). Assays based on kDNA appear to be the most sensitive for direct detection in infected tissues (Gomes et al., 2006; Miro et al., 2008) because of the high copy number of this target. PCR can be performed on DNA extracted from tissues, blood, body fluids or even from histopathological specimens.

The most useful diagnostic approaches for investigation of infection in sick and clinically healthy infected dogs include (1) detection of specific serum anti-leishmanial antibodies by quantitative serological techniques and (2) demonstration of the parasite DNA in tissues by applying molecular techniques (Solano-Gallego et al., 2009). Thus, the systematic review on CanL diagnostics performed for this mandate has focused on PCR and quantitative serology. The "gold standard" methods consisting of direct detection or culture of parasites from tissues, is highly specific but of low sensitivity. In most observational studies, no reference test is available which would identify the truly infected animals, thus allowing calculation of sensitivity estimates. This is particularly the case with cross-sectional studies where different diagnostic tests are often used in parallel. In order to obtain sensitivity estimates for serology and PCR, it was thus decided to examine two types of studies in which truly infected dogs may be more easily identified, namely challenge studies and longitudinal studies.

4.1.2. Sensitivity estimates from longitudinal studies and experimental infections

4.1.2.1. Longitudinal studies

The objective of the systematic review was to summarise the relative diagnostic sensitivity and specificity estimates of serological tests versus PCR assays reported in studies of naive dogs in areas where *L. infantum* infection is endemic. The searches yielded 3 865 references on diagnosis of CanL, from which diagnostic test characteristics could potentially be calculated for one or more of the following assays of interest for CanL: PCR, IFAT or ELISA. Only studies evaluating tests in dogs were included and studies evaluating *L. mexicana*, or *L. braziliensis* were excluded.

After screening title and abstracts for relevance, 243 were identified as diagnostic test evaluation studies, and at the second level of screening 18 were considered longitudinal studies. The 18 articles were then assessed based on the full text and 7 were considered relevant to the review.

As very few studies used either the same assay or the same sample as the referent, it was not possible to combine the results of the studies into a summary with measures of sensitivity or specificity (see Table 6 in O'Connor et al., 2015).

For example, (Gramiccia et al., 2010) reported that 17 out of 17 dogs tested positive with PCR on the buffy coat, whereas none of them tested positive with PCR on the conjunctiva. After spending about a year in the endemic area, 3 dogs out of 17 tested positive with PCR on the buffy coat, and these 3 dogs tested also positive with PCR on the conjunctiva. None of the three dogs was seropositive by IFAT after a year in the endemic area, indicating a relative sensitivity of PCR on conjunctiva of 100 % and 0 % for IFAT, respectively, compared with the sensitivity of PCR on buffy coat.

Otranto et al. (2013) estimated a relative sensitivity of 54 %, 36 % and 73 % for PCR on bone marrow samples, PCR on conjunctiva and IFAT, respectively, compared with PCR on skin samples of dogs, after spending about five months in the endemic area.

Oliva et al. (2006), reported that, as the number of positive dogs diagnosed by PCR on bone marrow in a cohort of 43 initially negative dogs increased over a period of two years in an endemic area, the relative sensitivity of the IFAT also increased, going from 33 % in the beginning of the period, to 77.8 % compared with the PCR on bone marrow at the end of the period.

From the three examples it is clear that, as the rate of PCR-positive results increased over time in the cohorts, the relative sensitivity of the other tests also increased. PCR detects the parasite earlier in infection than serology. This is because the development of a humoral immune response requires an antigenic stimulation that builds up as the parasite load increases, and also requires time before serum antibodies are detectable. Thus, most estimates of relative sensitivity of serological assays were < 10 % of the sensitivity estimates for PCRs early in the study, and increased to almost 80 % of the sensitivity estimates for PCRs at the end of the study. As these are relative sensitivities, the real sensitivity lays below these values. In the majority of studies, high relative specificities of 100 % were estimated (O'Connor et al., 2015). The evaluation of *Leishmania* tests by latent class analysis, using two different test principles (serology and PCR) on two populations with high and low prevalence rates, could be helpful in order to obtain more accurate sensitivity and specificity estimates.

4.1.2.2. Challenge studies

The objective of the systematic review was to review diagnostic test characteristics of PCR assays and serological assays (ELISA or IFAT) from studies that use experimental models of CanL. The detailed review protocol is provided in the external technical report provided to EFSA (O'Connor et al., 2015).

From the 3 865 references on CanL diagnostics obtained by the search 513 citations were retrieved after removing duplicates which contained either the term "challenge" (73), "experiment" or "induced" (98). Of these citations, 62 articles described at least one of the assays requested (PCR, ELISA or IFAT) and 18 articles described the use of PCR and either ELISA and/or IFAT and were published after 1990. Only 14 articles provided individual test results in animals, or estimates of sensitivity or specificity of the tests. Most of these studies were vaccine trials and used only few, non-immunised control animals from which data could be extracted. Furthermore, all studies used either different routes of infection, different doses, different life stages or zymodemes of *L. infantum* parasites, different tissue samples and targets of PCR or serological tests and different cut-off values. In addition, animals were tested at different times post inoculation. Thus, a meta-analysis, providing a single summary estimate of the sensitivity and the specificity of the different diagnostic tests could not be carried out.

Data extracted from the articles are shown in Appendix D. PCR on bone marrow or lymph node tissue appear to be the most sensitive and specific for the diagnosis of leishmaniosis (Strauss-Ayali et al., 2007; Maia et al., 2007; Daneshvar et al., 2010). PCR on whole blood and buffy coat appears to be less sensitive. Conjunctival swab PCR has been shown to be accurate in the diagnosis of seropositive

dogs with clinical leishmaniosis and in subclinically infected dogs (Strauss-Ayali et al., 2004). As in the longitudinal studies, the rate of ELISA and IFAT-positive animals increase over the study period, although highly divergent outcomes are obtained, mainly because of the different targets and cut-off values used in the tests in the different studies.

These findings of the systematic review are confirmed by other studies which were not included in this systematic review (because the studies were not longitudinal studies of naturally infected dogs) (Mathis and Deplazes, 1995; Reale et al., 1999; Roura et al., 1999a, b; Solano-Gallego et al., 2001, 2007; Fisa et al., 2001; Lachaud et al., 2002; Manna et al., 2004; Nasereddin et al., 2006; Moreira et al., 2007; Ferreira Sde et al., 2008; Lombardo et al., 2012). In addition, PCR on spleen and skin appears to be also sensitive and specific for the diagnosis of leishmaniosis (Reis et al., 2013; Solca et al., 2014; Courtenay, 2014)

Again, the use of latent class analysis in evaluating the diagnostic tests can be recommended to obtain more accurate estimates of the diagnostic test sensitivity at a particular stage of the infection.

4.2. Efficacy of available treatments for *L. infantum* infection in dogs

Treatment of CanL essentially relies on the use of three pharmaceutical drugs, namely meglumine antimoniate, allopurinol and miltefosine, which may be combined with each other and may be used under different treatment regimes.

Meglumine antimoniate (Glucantime®, Merial) and miltefosine (Milteforan®, Virbac) are considered leishmaniacidal whereas allopurinol (several products marketed used from veterinary or human medicine) is considered leishmaniastatic. These drugs have different modes of action. Meglumine antimoniate is a pentavalent antimonial which selectively inhibits leishmanial enzymes required for glycolytic and fatty acid oxidation. Miltefosine is an alkylphosphocholine membrane-active ether–lipid analogue. Allopurinol is a hypoxanthine compound metabolised by *Leishmania* spp. to produce an analogue of inosine, which is incorporated into leishmanial RNA, causing faulty protein translation and inhibition of parasite multiplication (Baneth and Shaw, 2002; Noli and Auxilia, 2005). Meglumine antimoniate is injected subcutaneously whereas miltefosine and allopurinol are administered orally to dogs. Treatment usually consists of administering either meglumine antimoniate or miltefosine for a month, in combination with allopurinol, and then continuing therapy with only allopurinol for at least six months, and frequently for longer periods or even for the lifetime of the treated dog in moderate disease (clinical stage II or higher) (Solano-Gallego et al., 2009, 2011). Otherwise dogs with moderate disease are likely to relapse. Therefore, short-term treatments are not applied in dogs with moderate disease. Treatment of dogs with leishmaniosis that has not progressed into a severe form often results in cure from clinical disease; however, dogs may remain carriers of *L. infantum* and disease relapse may occur (Solano-Gallego et al., 2011). No treatment or only short-term treatment is administered in dogs with mild disease and good prognosis (clinical stage I).

4.2.1. Randomised controlled trials

A systematic review was carried out to review studies comparing treatment efficacy of meglumine antimoniate, allopurinol or miltefosine on infection with *L. infantum* in dogs in Europe, using a randomised controlled study design. Details on the research protocol and search strategy can be found in the external technical report provided to EFSA (O'Connor, 2015)

Of 3 168 references found by the search strategy, 40 were identified as potentially relevant comparative trials of efficacy for one of the interventions of interest after title/abstract screening. Few studies performed a 360-day follow-up period; which was the minimum duration of follow-up agreed before the onset of the review. Thus, the follow-up period criterion for inclusion was reduced to 180 days to increase the amount of evidence available. Following full-text screening, 12 references met the criteria for data extraction. The manuscripts originated from six European countries: Italy (four studies), Spain (two studies), France/Italy/Spain (one multicentre study), Germany (two studies), France, Greece (two studies), and the Netherlands (1 study).

There was a high degree of heterogeneity between the studies in each outcome category because of differing initial health statuses of dogs, treatment protocols, length of follow-up periods, tissues sampled at follow-up, tests used to monitor infection following treatment (e.g. PCR or cytology to measure parasite load), and metrics of results reported (e.g. there is no standard metric to report parasite load). Therefore, it was not possible to perform a meta-analysis and to provide a summary estimate of efficacy. Based on the Cochrane Collaboration's tool for assessing risk of bias (the Cochrane Collaboration, 2011), all studies included in this scoping review had at least one domain in their study design with an "unclear" or "high" risk of bias.

The six studies performing PCR on blood, conjunctiva, lymph nodes and/or bone marrow during the follow-up, reported very low proportions of dogs that were PCR negative at the end of the follow-up period. In 9 of the 10 groups included in the 6 studies, and treated with different treatments, the parasitological cure as assessed by PCR ranged from 0 to 0.45, with a follow-up period ranging from half a year to 2 years, and there was an average of 2 out of 10 treated dogs which were negative at the end of the follow-up period (Pennisi et al., 2005; Saridomichelakis et al., 2005b; Plevraki et al., 2006; Miró et al., 2009; Ariti et al., 2013). In one of the 10 groups, in the study by Neogy et al. (1994), all the dogs were negative by cytology at the end of the treatment with meglumine antimoniate after a follow-up period of 6 months.

One study (Miro et al., 2011) reported high proportions of dogs being negative by bone marrow parasite culture and xenodiagnosis at the end of the treatment and follow-up period of half a year. The dogs were treated with meglumine antimoniate for one month and/or allopurinol for half a year.

Four studies, in which parasite load was estimated before and after treatment, reported a decrease in parasite load after treatment and a follow-up period of around half a year, with one or more of the above-mentioned pharmaceutical drugs (Neogy et al., 1994; Oliva et al., 1998; Plevraki et al., 2006; Miro et al., 2009). Plevraki et al. (2006) reported a significant decrease in parasite load after treatment with allopurinol, which was not observed in the groups receiving a placebo. Nonetheless, none of these dogs were negative by PCR on bone marrow at the end of the follow-up period of half a year.

It should be noted that none of the above studies has performed quantitative PCR on skin samples, which would have been a good surrogate marker for infectiousness to sand flies (Courtenay, 2014).

In conclusion, drug therapy for CanL appears to mainly slow down the progression of infection, decrease infectiousness and improve clinical manifestations by reducing parasite loads in infected tissues. No treatment (drugs and regime) tested so far has demonstrated 100 % efficacy in the long-term elimination of the parasites.

4.2.2. Other study designs

A study carried out by Alvar et al. (1994) showed that four dogs, which were naturally infected with *L. infantum* and also infectious to sandflies before treatment, were not infectious to the vectors for at least a few months following treatment with meglumine antimoniate for 20 days and allopurinol for 30 days. Furthermore, treatment with meglumine antimoniate resulted in a temporary improvement of the clinical condition of the dogs, but parasitological cure was uncommon after 10 months of follow-up after treatment.

A similar "latency" pattern had been described by Gradoni et al. (1987) in two dogs, treated three times with meglumine antimoniate according to the following schedule: 10 days on treatment, 10 days off-treatment and 10 days on treatment. In the initial period after the first treatment, the infection rate to sandflies fed on both dogs decreased drastically, and the worst clinical signs disappeared. However, in the subsequent period, the infection rate to sandflies increased again. Parasites isolated from dogs before and after treatment were iso-enzymatically identical.

Although both studies were not RCTs, they confirm the above conclusions, that treating infected dogs can improve their condition and temporarily decrease infectiousness. However, infected dogs which have been treated with anti-*Leishmania* drugs may still be infectious during their lifetime and transmit CanL to naive dogs.

4.3. Discussion on the performance of diagnostic tests and treatments

The effectiveness of a test and exclusion strategy is tied to the sensitivity of the diagnostic test(s) used for screening dogs. Based on the literature search, there is considerable uncertainty associated with the diagnostic test sensitivity. The parallel use of appropriate PCR and serological technique(s) would increase the likelihood of detecting infected dogs, than if only one technique was used, and is therefore recommended.

Drug therapy for CanL appears to mainly slow down the progression of infection, decrease infectiousness and reduce clinical manifestations by reducing parasite loads in infected tissues. No treatment (drugs and regime) tested so far has demonstrated 100 % efficacy in the long-term elimination of the parasites

The benefits of either "test and treatment" or "test and exclude" strategies in reducing the probability of introduction and establishment into CanL free areas are assessed in Section 5.

5. Assessment of the risk that the infection would become established in free areas of the EU if *L. infantum* were introduced by infected dogs (TOR 3)

5.1. Definitions

Definitions on establishment, endemicity and persistence of vector-borne infections have been discussed and internationally accepted in the risk assessment framework for emerging vector-borne diseases (Central Veterinary Institute, 2014). These definitions were adapted for this mandate on CanL, respecting also the possibility that non-vectorial transmission may occur.

- Establishment of infection: local transmission of the imported pathogen from vector to host and vice versa, leading to the temporal presence of at least one indigenous infectious host and at least one indigenous infectious vector.

For modelling purposes, "establishment" was interpreted as the vectorial transmission of *Leishmania* infection from the index case to a new dog in a hypothetical contact network of dogs, in a previously CanL free area with sandflies. "Establishment in an area" is then the establishment in at least one independent contact network composed of one or more independent contact networks, in a previously CanL free area with sandflies. The period for which the probability for the possible establishment was modelled was three years.

- Spatial spread: increasing number of indigenous infections over space.

- Persistence of infection: the situation in which infection remains present in a dog population for multiple vector seasons and/or multiple disease generations.

- Endemicity: the situation in which infection persists in a population larger than a kennel or a household. Endemicity implies both persistence and spatial spread of infection.

5.2. Introduction into free areas without competent vectors

Leishmania-infected dogs have travelled into many areas devoid of competent vectors around the world, yet there is no report that this may have been followed by spread and persistence of *Leishmania* infection in these areas. In some cases, very limited spatial spread and local persistence (e.g. at the

level of a kennel or a household) has occurred as a result of incidental non-vectorial transmission as described in Section 2.6.2. However, no CanL endemic situation has yet been observed in such areas.

5.3. Introduction into free areas with competent vectors

5.3.1. Methodology

5.3.1.1. Description of the model

A mathematical, stochastic model was developed in order to assess the probability of CanL introduction via an infected dog, and the probability of establishment in a previously free area, given the existence of competent vectors in that area.

The model is individual-based for the dog populations and uses vectorial capacity to generalise the potential of the sandfly population to transmit the disease to dogs under the assumption of independence from the prevalence of infection in sandflies (Espejo, Costard and Zagmutt, 2014).

The modelling framework comprises three steps, estimating:

1. The probability of introduction (index case module)

   The probability of introducing an infected dog into a non-endemic area with sandflies is calculated for two possible pathways of introduction:

   - dogs returning to a non-endemic area with sandflies, after staying for a given period of time and becoming infected in an endemic area (e.g. travel with household) (P_{Inf});
   - dogs born in an endemic area and moved into a non-endemic area with sandflies, following adoption or purchase (P_{Inf} CA).

The difference between these two scenarios resides in the period of time during which dogs are exposed to sandflies, which affects the probability of becoming infected.

2. The probability of establishment in a network of dogs in a free area (P_{Est}).

3. The overall probability of establishment in a previously free area composed of one or more independent contact network of dogs ($P_{Estregion}$).

The above probabilities were first estimated in a baseline scenario, where no mitigation measures are implemented and the results are presented below (Section 5.3.2). The model has been run in situations where different preventative measures are applied on dogs travelling from endemic to non-endemic areas, and the reduction of these probabilities achieved by each preventative measure is presented as an outcome of the model in Section 6.

5.3.1.2. Limitations and assumptions of the model

<u>Limited scope of the model</u>

The model simulates the probability of "establishment", i.e. at least one new indigenous case resulting from the introduction of an infected dog into a fully susceptible population, over a three-year period. The model does not take into account other modes of transmission, which may play a role at a local level. Furthermore, the model does not assess "persistence" as defined in the introduction to this section, nor does it estimate the long-term (e.g. > 3 years) prevalence in a newly affected area.

Limitations due to uncertainties and difficulties to parameterise the model

Several parameters used to estimate P_{Inf}, P_{Est} and $P_{Estregion}$ contained high uncertainty. The sensitivity analysis indicated that the most influential parameters for P_{Inf} were the "period of the year" when the trip to an endemic area was taking place, followed by the "duration of the travelling period" (Table 7). The most influential parameter for P_{Est} was the time of the year when the index case became infected and the season was the key driver of the probability of establishment in previously CanL-free areas.

Further, uncertainties in the number of female sandfly bites per day, the daily rate of transition from latent to infectious sandflies were estimated using expert opinion. In addition, the prevalence of CanL infectious dogs in endemic areas influences P_{Inf} and $P_{Estregion}$. In the absence of data on the prevalence of infectious animals in endemic areas, this parameter was estimated using the prevalence of clinical CanL and since subclinical infected dogs may occasionally be infectious, it is likely that the prevalence of infectious dogs was underestimated.

The parameters used in the model are shown in Table 7.

Table 7: Biological parameters used in the model

Parameter	Short explanation [(a)]	Modelling	Probability distribution	Distribution parameter [(b)]	Sources
Transmission parameters					
Time to transition from latent to infectious (σ)	Distribution of the time from dog infection (from sand flies) to infectiousness (ability to transmit the infection to sandflies, days)	Variability	Weibull	Shape: 1.34; scale: 396.95	Oliva et al. (2006)
Time to transition from infectious subclinical to infectious clinical (ρ)	Distribution of the time for dogs to go from the infectious subclinical to infectious clinical state (days)	Variability	Weibull	Shape: 4.3; scale: 233	Oliva et al. (2006)
Vectorial capacity					
Number of female sandflies per dog (m)	Number of female sandflies per dog	Uncertainty	Gamma	Shape:866; scale: 0.0019	Expert opinion, based on Rossi et al. (2008), Velo et al. (2005)
Number of female sandflies bites per day (a)	Number of female sandfly bites per day	Uncertainty	Normal	μ: 2.03;σ: 0.29	Expert opinion, based on Rossi et al. (2008), Velo et al. (2005)
Transition rate from latent to infectious sandflies (τ)	Daily rate of transition from latent to infectious sandflies	Uncertainty	Normal	μ:6.5;σ: 0.53	Expert opinion Anonymous (2013)
Daily mortality rate of female sandflies (μ)	Daily survival probability of sandflies	Uncertainty	Empirical samples with equal weight	21d;42.5d	Expert opinion Anonymous (2013)
Characteristics of environment					
Proportion of *Leishmania*-infected dogs that are infectious	Prevalence of illness in endemic areas (%)	Uncertainty	Mixture of two equally weighted Betas	Beta (α: 30, β: 1 048), Beta (α: 20, β: 400)	Galvez et al. (2010), Miró et al. (2012), Papadopoulou et al. (2005), Aoun et al. (2009)
Prevalence of infection in endemic areas	Prevalence of infected dogs in endemic areas (%)	Uncertainty	Posterior Bayesian estimation of true prevalence	–	Leontides et al. (2002), Keck and Dereuer (2003), Baldelli et al. (2011)
Length of the winter season	Number of days when the environmental conditions are not favourable to the reproduction of sandflies (days)	Scenario analysis	Fixed	ICM: 150; TM: 90	Oliva et al. (2006)
Period of the year travelling	Period of the year when travel to the endemic area is performed	Variability	Uniform	Minimum: 0; maximum: 365	Assumes that travel can happen any day with equal chance
Durations of travelling days	Number of days per trip to endemic areas per year (days)	Variability	Empirical (histogram frequencies)	1–3 days: 20.7 %; 4–7 days: 38.1 %; 8–14 days: 6.3 %; 15–28 days: 11.1 %; 29–91 days: 3.6 %; 95–365 days: 0.2 %	EuroStat (2013)

Parameter	Short explanation [a]	Modelling	Probability distribution	Distribution parameter [b]	Sources
Mitigation measures					
Repellent use (p')	Proportion of a certain dog population (e.g. region, country) that uses repellent (%)	Scenario analysis	Fixed	NA	Scenario analysis
Repellent efficacy	Proportional reduction of sandfly bites on dogs using repellent (%)	Uncertainty	PERT	PERT 0.51, 0.84, 0.98	Espejo, Costard and Zagmutt (2014), Wylie et al. (2014a), Tables 5 and 6
Diagnostic test sensitivity	Sensitivity of the tests	Uncertainty	Fixed	0.5	The systematic review did not provide a precise estimate of test sensitivity (see Section 4.1.2). A 50 % sensitivity was chosen by the experts for illustrative purposes for the model, being an average sensitivity for testing dogs of all ages by parallel testing using PCR and serology
Vaccine use (θ)	Proportion of dogs in a certain population (e.g. region, country) that have been vaccinated (%)	Scenario analysis	Fixed	NA	Scenario analysis
Vaccine efficacy	Proportion of the vaccinated dogs population that will not become infected when exposed to infectious sandflies (%)	Uncertainty	PERT	63.4 % (CI: 6.9 %; 85.6 %) *(active infection)*	Oliva et al. (2014)
Dog's life					
Mortality rate (δ)	Dogs' life expectancy distribution (days)	Variability	Weibull	Shape: 2.467; scale: 4 468.8	O'Neill et al. (2013)
Age	Age distribution dogs at any given time (days)	Variability	Log-normal	μ: 1 898; σ: 1 241	Galvez et al. (2010)
Number of dogs travelling per household	Distribution of the number of dogs per household	Variability	Zero-truncated Poisson	lambda: 0.74	Slater et al. (2008)
Time to replacement	Time for a new replacement dog to be brought home/introduced into the home after a dog is lost/dead (days)	Variability	PERT	Minimum: 3; maximum: 3 650; mode: 120	McCutcheon and Fleming (2002)
Probability of replacement (α)	Proportion of households that replace a lost/dead dog (%)	Uncertainty	Beta	Alpha: 52; beta: 53	McCutcheon et al. (2002)

(a): Parameters expressed in days were transformed to weeks for the transmission module.
(b): ICM, Index Case module; NA, not applicable; PERT, ;TM, transmission module.
Modified from Espejo, Costard and Zagmutt (2014).

Limitations due to key assumptions

Several key assumptions were made for this modelling work. Vectorial capacity was used as a parameter in the model and it was assumed that the infection reaches an equilibrium in the sandfly population quickly after the introduction of an infected dog in the contact network of a non-endemic area with sandflies. This is a reasonable assumption because the vector dynamics develop on a much shorter timescale than the infection in dogs.

The vectorial capacity was first calculated using information on sandfly–host interactions from known endemic areas, such as sandfly density and transmission parameters. As these endemic areas are high transmission areas, this assumption corresponds to the "worst-case scenario". Thereafter, two scenarios that were judged to be more realistic for the non-endemic areas were created, using 10 % or 50 % of the vectorial capacity of the endemic areas to model the probability of establishment in a network of dogs, and in a region (network of dog networks) where competent vectors may be present, but at lower densities.

5.3.2. Results

5.3.2.1. Probability of one dog returning infected from a trip to an endemic area (P_{Inf})

When no mitigation measures were implemented, the mean probability that one dog travelling to CanL endemic areas during the active vector season, would become infected was estimated to be 8 %, with a 95 % confidence interval of 3 % to 16 %. The model assumed an equal chance of travelling on each day of the year.

5.3.2.2. Probability of one infected dog imported from an endemic area (P_{InfCA})

This was considered as equivalent to the prevalence of *Leishmania* infection in the country of origin. The prevalence estimates were based on seroprevalence surveys and ranged from 7 to 18 %.

5.3.2.3. Probability of establishment within a contact network of dogs (P_{Est}) in a non-endemic area with competent vectors

P_{Est} following the introduction of one infected dog into a network in a non-endemic area with competent vectors was 72 % (64–81 %) in the worst-case scenario, where the vectorial capacity in the non-endemic area was considered 100 % of that of endemic areas.

When assuming that vectorial capacity in the non-endemic areas would be half of the vectorial capacity estimated for the endemic areas, P_{Est} following the introduction of one infected dog into a contact network was still as high as 70 % (63–74 %). When vectorial capacity in the non-endemic area was assumed to be 10 % of vectorial capacity estimated for the endemic areas, then P_{Est} would still be 47 % (19–66 %), following the introduction of one infected dog in a dog network..

These results suggest that *Leishmania* infection is very likely to become established into a contact network of dogs, following introduction of an infected dog in an area with competent vectors, even when vectorial capacity in the non-endemic areas of introduction is half or less than half vectorial capacity in endemic areas.

5.3.2.4. Probability of establishment in several contact networks in an area ($P_{Estregion}$)

The probability of establishment in a region (Figure 2) depends on the probability of establishment in a single network of dogs, the prevalence in the endemic areas in which the travelling dogs have stayed, and the number of infected dogs introduced. The results in Figure 2 show that for the baseline scenario the averaged probability of establishment in a region is close to 1 if the number of imported animals is larger than 300, even if vectorial capacity in the free area is 50 % or 10 % of the vectorial capacity in an endemic area.

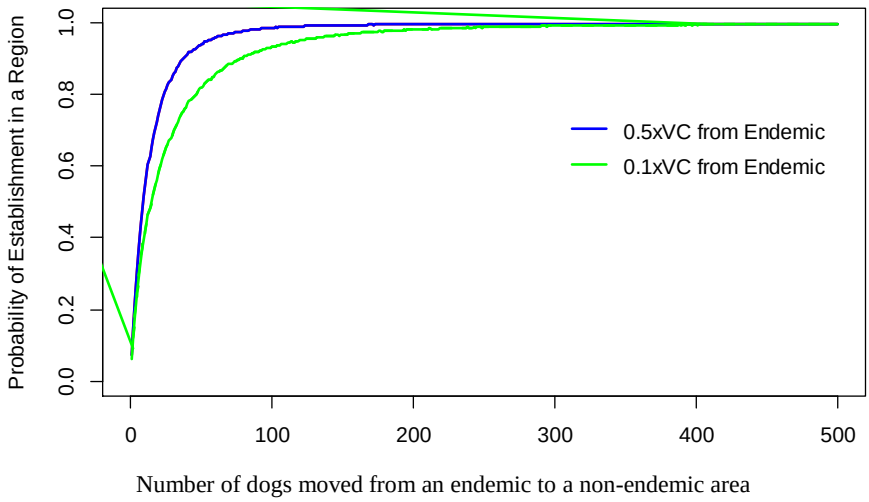


Figure 2: Average probability of establishment of CanL in a free region considering different number of dogs moved into the free region with a vectorial capacity of 0.1 and 0.5 compared with an endemic area

5.4. Discussion and conclusions

The average probability of CanL establishment following the introduction of infected dogs in previously disease-free areas where competent vectors are present was estimated by a mathematical model. The model established three scenarios reflecting varying vectorial capacities in non-endemic areas, mainly because of varying densities of sandfly vectors. In the worst-case scenario, where the vectorial capacity is considered 100 % of that of endemic areas and no mitigation measures are applied on dogs moved from endemic areas, the model yielded high probabilities for both CanL introduction and establishment within a contact network of dogs. This is in agreement with field studies where susceptible dogs were introduced in dog contact networks in endemic areas (Dye et al., 1993; Oliva et al., 2006), if similar exposure times are simulated.

Overall, the model assessed the average probability of establishment as very high for disease-free EU regions with competent sandflies where infected dogs are introduced, with the average probability of establishment in a region approaching 1 if more than 300 dogs are moved into one or more independent networks in the region, irrespective of the vectorial capacity considered.

One should bear in mind that although P_{Est} for a non-endemic region may be very high, the prevalence in that region in case of CanL introduction and establishment may take any value, from extremely low to high, mainly depending on vector abundance.

The outcome of the model is consistent with the observation that in most areas of Europe where competent sandfly species are present, CanL is endemic. In areas which are known to be devoid of competent sandflies species (e.g. Scandinavia), CanL is limited to imported cases and has not resulted in endemic situations so far. The most crucial question relates to the limited foci ("pockets") of competent vectors that may be present in particular areas within non-endemic regions of temperate Europe, or in the fringe areas between endemic and non-endemic areas. These foci are not all identified and the presence and abundance of phlebotomine vectors need to be monitored. In such foci, where sandfly populations are likely to have a lower vectorial capacity than in endemic areas because of lower vector densities (hence lower number of sandfly bites per dog per unit of time), the probability of establishment following introduction of an infected dog, remains high, according to the model.

In areas where no competent sandfly species exist, transmission has also been reported in situations where naive dogs were living together with infected dogs, imported from endemic areas, or in pups

born from infected dams, or in animals receiving blood transfusions from infected donors. No endemic situation has ever been reported in areas with no (or few) vector, or vectors with limited competence. If local transmission has occasionally occurred during a few dog generations under such circumstances, the spread and long-term persistence of the disease have not been reported. Although non-vectorial transmission is possible, the expert panel judged that the disease could only become established in a previously free area if competent vectors are present in that area.

In conclusion, *Leishmania* transmission is essentially linked with the presence and abundance of the sandfly vectors. The risk of CanL introduction through infected dogs, and consequent establishment in non-endemic areas, is high if competent vectors are present, even at low densities. Data on sandflies are difficult to collect because of the absence of systematic sampling programmes. Available field data suggest that these insects are spreading northwards in Europe (northern Italy, Austria, Germany and Hungary) and their densities are increasing in some newly colonised areas (Maroli et al., 2008). Owing to the wide distribution of susceptible dogs and the high host–vector contact rates, the main limitation to CanL spread is probably represented by the vectors (Santi et al., 2014). This reinforces the need for improving our knowledge of the vectorial competence of some sandfly species and of the distribution and abundance of known vectors.

6. Evaluation of the efficacy of available preventative measures to protect dogs against *L. infantum* infection, with the objective of mitigating the risk of introduction of the infection into free areas of EU, through movements of infected dogs

A description of available preventative measures and their efficacy based on a systematic literature review was provided in Sections 3 and 4. The current section will assess the effect of preventative measures on reducing the risk of CanL introduction and establishment in a previously free area, using the simulation model described in Section 5.

Mitigation measures were only evaluated for areas at risk, where competent phlebotomine vectors are present. Again, since the vector abundance in the non-endemic areas is not precisely known, three scenarios were developed, assuming either 100 %, 50 % or 10 % of the vectorial capacity in the free areas.

The **target population** on which the preventative measures were tested were the dogs returning or imported from endemic areas.

The **evaluated measures**, potentially mitigating the risk of introduction and/or establishment of CanL in non-endemic areas were the following[4]:

- Vaccination and topically applied insecticides, individually applied or in combination.
- Diagnostic test and exclusion: testing dogs before they enter free areas and refusing to introduce those found infected.

Test and treatment in the endemic area, prior to movement into a non-endemic area, does not appear as an effective and realistic option to mitigate the probability of introduction of CanL into the non-endemic area, as no treatment against *L. infantum* infection can provide permanent elimination of the parasite. Therefore, testing dogs before they enter free areas and treating those found infected was not considered as a scenario for the model.

6.1. Vaccination

Vaccinating dogs before their trip to endemic areas is unlikely to significantly reduce the probability of introducing *Leishmani*a infection upon their return in free areas. Therefore, vaccination as a

measure to prevent introduction was not considered a meaningful scenario to be evaluated by the model and therefore the model only examined the probability of establishment.

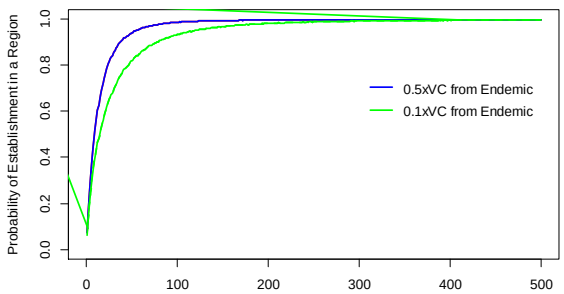
Considering that vaccinating with CaniLeish® may reduce parasite load and infectiousness in those dogs which get infected despite vaccination (see Section 3.1), vaccinating travelling dogs could be expected to reduce the probability of establishment in a region, upon their return. Based on the results of the systematic review, a PERT-distribution around 63.4 % (95 % CI: 6.9 % to 85.6 %) was used in the model as an estimate of the vaccine efficacy for preventing the development of active infection.

In the scenario considering a vectorial capacity of 100 % compared with endemic areas, however, P_{Est} was estimated to be 69 % (43 %–73 %) following the introduction of one infected, vaccinated and almost identical as compared with P_{Est} 72 % (64–81 %) after introducing a dog without vaccination. When a vectorial capacity of 50 % or 10 % was assumed, P_{Est} became 64 % (27 %–72 %) and 32 % (4 %–60 %), respectively.

Thus, the model estimated that vaccination would have only a very limited effect on reducing the probability of establishment of CanL in a network of dogs once an infected, vaccinated dog has been introduced, and this only in areas with low vectorial capacity.

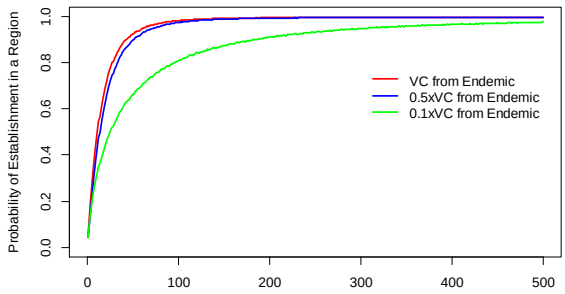
Vaccination of moving or travelling dogs also had a marginal effect on the average probability of establishment of CanL in a dog population in a region (see Figure 3A). The curves show the average probabilities of establishment in a region. It should be highlighted that, when considering the 95th percentile confidence bands, they largely overlap (see Appendix C). If the number of introduced, vaccinated, infected animals is larger than 300, the $P_{Estregion}$ would be close to 1, similar to without interventions. When vectorial capacity was assumed to be only 10 % of the endemic regions, approximately 500 vaccinated, infected animals would have to be introduced to reach an average $P_{Estregion}$ of 100 %.

A. Baseline scenario



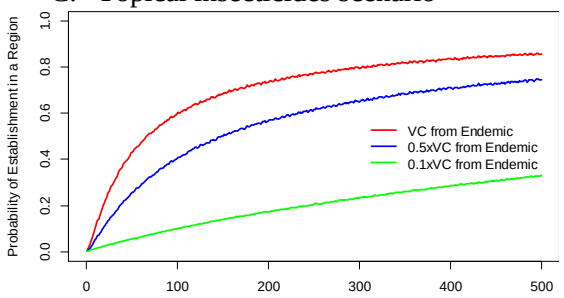
Number of dogs moved from an endemic to a non-endemic area

B. Vaccination scenario



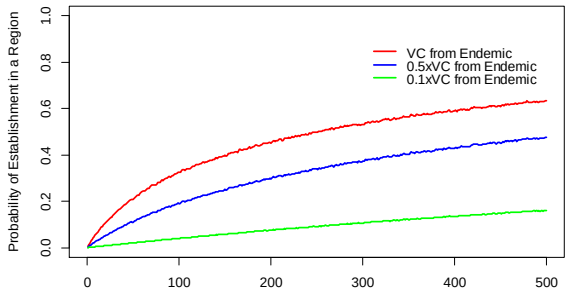
Number of dogs moved from an endemic to a non-endemic area

C. Topical insecticides scenario



Number of dogs moved from an endemic to a non-endemic area

D. Vaccination and insecticide scenario



Number of dogs moved from an endemic to a non-endemic area

Figure 3: Average probability of establishment of CanL in a region, considering different numbers of vaccinated, infected dogs imported or travelling back to the free region and considering different preventative measures applied to travelling dogs. Confidence intervals can be found in Appendix C

6.2. Topical insecticides

The model estimated that when topical insecticides were applied on the travelling dog, the average probability of infection during travel was almost zero (0.03 %, 95 % CI 0–0.3%), representing a 99.6 % reduction compared with not using insecticides.

The application of topical insecticide also had an effect on $P_{Est,}$ after return or import of a dog into a non-endemic area. In the scenario with a vectorial capacity of 100 % (compared with endemic areas), P_{Est} was reduced to 10 % (0–53 %), as compared with 70 % (63–74 %) following the introduction of an infected dog into a contact network without topically applied insecticide. When vectorial capacities of 50 % or 10 % were assumed, P_{Est} became 5 % (0–27 %) and 0.1 % (0–3 %), respectively, after application of topical insecticides on travelling dogs.

Applying topical insecticide on travelling or imported dogs moving from endemic areas also had an effect on the establishment of CanL in a region of the non-endemic area. $P_{Estregion}$ was around 80 % when the number of infected dogs treated with topical insecticide was around 500, considering the same vectorial capacity as in the endemic regions. When vectorial capacity was considered 10 % of that in the endemic regions, the average probability of establishment in the region remained below 40 % if the number of travelling or imported animals was smaller than 500 (see Figure 3C). If the number of animals imported is large (e.g. around 500), no overlapping confidence intervals were observed when comparing the later scenario with the baseline scenario (see Appendix C).

6.3. Combined use of vaccination and topically applied insecticide

The combination of vaccination and application of topical insecticides in travelling dogs further reduced the probability of P_{Est} to 6 % (0.1–35.7 %) in the scenario with a vectorial capacity of 100 % that of endemic regions. When vectorial capacities of 50 % and 10 % were assumed, P_{Est} became 3 % (0–16 %) and 0 % (0–3 %), respectively, if the dog was vaccinated and treated with topical insecticides. This suggests that there could be a synergistic effect of the two preventative measures used together (their combined effect being greater than the sum of their individual effects) (see Figure 3D). As for the topical insecticide applied alone, there were no overlapping confidence intervals observed when the number of animals imported is large, when comparing the low vectorial capacity scenario with the baseline scenario (see Appendix C).

6.4. Test and exclusion prior to entering free areas

In this hypothetical scenario, dogs moved to free areas were tested before entering and denied access when testing positive to evaluate this effectiveness of this potential mitigation strategy. Since the systematic review did not provide a precise estimate of test sensitivity (see Section 4.1.2), a 50 % sensitivity was chosen for illustrative purposes, considering the long latent period and the wide range of susceptibility degrees, which is known to affect the sensitivity of the test.

The effect of test and exclusion on reducing $P_{Estregion}$ was important when very small numbers of dogs (e.g. 10) were moved from an endemic area, but quickly decreased with increasing numbers of travelling dogs. For example, even when 100 % of the travelling dogs were tested (and the positive dogs were excluded), the $P_{Estregion}$ was 86.8 % (0–100 %) for only 100 dogs travelling to endemic areas in the baseline scenario, and it was 100 % for 500 dogs. Figure 4 shows the limited effect of testing and excluding imported dogs on $P_{Estregion}$ compared with not testing the dogs with an assumed test sensitivity of 50 %.

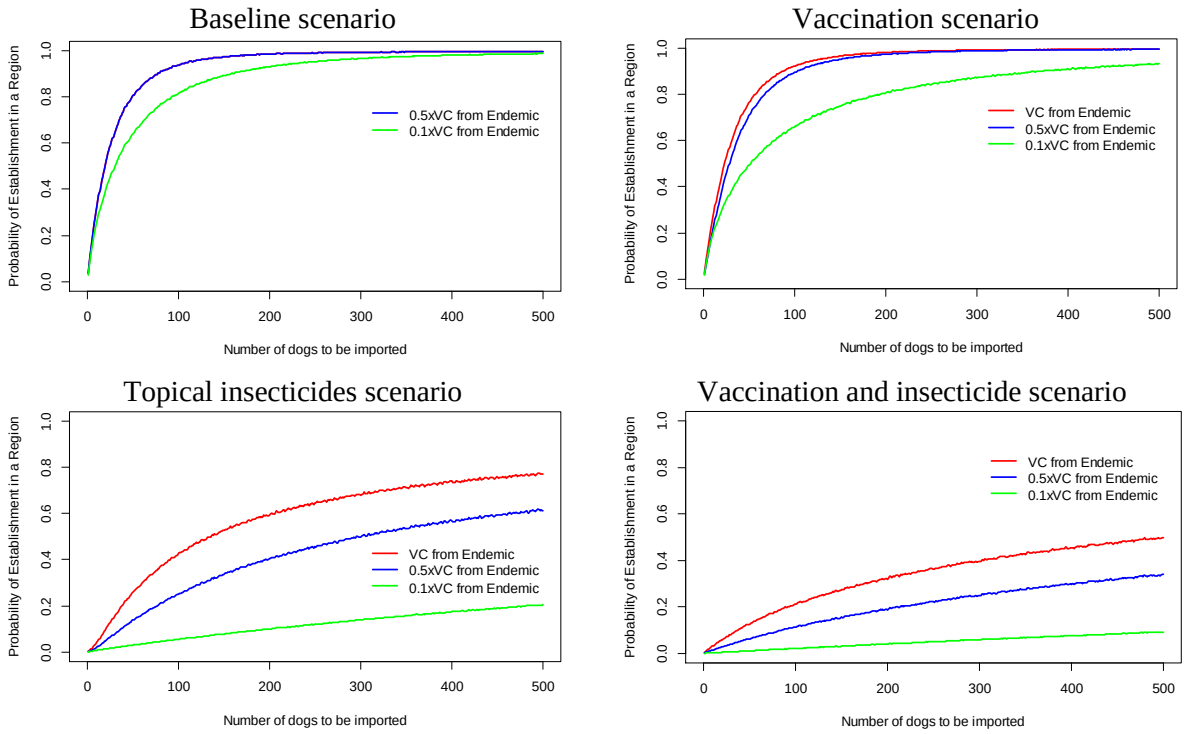


Figure 4: Average probability of establishment of CanL in a region after testing and excluding positive imported dogs, assuming a test sensitivity of 50 %; considering different numbers of infected dogs imported and different preventative measures applied on travelling dogs (see Appendix C for confidence intervals). The **evaluated measures**, potentially mitigating the risk of introduction and/or establishment of CanL in non-endemic areas were the following: (1) vaccination and topically applied insecticides, individually applied or in combination and (2) diagnostic test and exclusion: testing dogs before they enter free areas and refusing to introduce those found infected

6.5. Discussion and conclusions

In a **baseline scenario** without mitigation measures, the high values for P_{Inf} and P_{Est} (a mean of 8 % and 72 %, respectively) resulted in a high probability of establishment in a non-endemic region with competent vectors, with values of $P_{Estregion}$ reaching 100 % when only 300 infected dogs had been moved into a free region.

P_{Inf} and P_{Est} could be reduced by mitigation measures, applied separately or in combination. The most effective mitigation measure, when correctly applied on all the dogs, was **topically applied insecticide**, with a P_{inf} of 0.03 % and P_{Est} of 10 %, 5 % and 0.1 % for a vectorial capacity of 100 %, 50 % and 10 %, respectively, in the non-endemic area.

Vaccination of dogs prior to travelling to endemic areas had a limited effect on the probability of establishment in a non-endemic region, and this effect seems more apparent when vectorial capacity decreases. The use of topical insecticide and vaccination in travelling dogs had a synergistic effect in reducing P_{Est} and $P_{Estregion}$ after their return to a non-endemic area. Again, this effect was higher in areas where a low vectorial capacity of the vectors was assumed.

Regarding **the testing (with a theoretical scenario of sensitivity = 0.5) and exclusion measure**, the model indicated that the probability of establishment in at least one dog population in a previously CanL-free area remained high when a large number of dogs were moved, even when all dogs were tested. Testing dogs before their introduction has a low value if applied shortly after exposure to infected sandflies. This is mainly because of the fact that it takes several months to establish a positive test.

The model indicated that for a limited number of dogs (< 10), test and exclusion may be an applicable option. An example could be when dogs that are born in endemic areas or have stayed in those areas for long periods, were to be clinically examined and tested before they are moved into a free area, and if diagnosed as *Leishmania*-infected, would be denied movement. This would be mainly applicable in the context of adoptions or commercial purchase, and the measure would have special value for dogs introduced into kennels for breeding purposes, as introduction of infected males or females may result in local spread of *Leishmania* infection via non-vectorial, venereal and vertical transmission (see Section 2.5.2).

Dogs that have stayed in endemic areas for a longer period of time, should be tested for absence of *Leishmania* infection by PCR and serology before they are approved for breeding or as blood donors.

In conclusion, efforts to prevent CanL introduction and establishment in non-endemic areas via measures on dogs travelling to and from or imported from endemic areas, may include the use of preventative measures, such as topical insecticides alone or in combination with vaccination, before and/or during their trips to endemic areas.

A high-risk scenario is when dogs from endemic areas known to be infected visit or stay in non-endemic areas, especially if competent vectors may be present.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

TOR1 (epidemiology and impact)

- CanL is endemic in the European countries or regions surrounding the Mediterranean where disease distribution matches that of the phlebotomine vectors.
- On average, around 10 % of dogs in endemic countries are seropositive for *L. infantum*, with wide variations between territories.
- Studies conducted in endemic areas have given much higher prevalences than serology, with up to 80 % of the dog population being PCR-positive.
- Infection in the canine population in endemic areas of Europe is widespread and the prevalence of infection in dogs is much higher than the fraction that shows clinical illness or seroconversion.
- In central European countries, knowledge about the presence of competent vectors and the presence of endemic CanL is limited.
- Data on **sandflies** are limited because of the absence of systematic sampling programmes and expertise.
- Available field data suggest that sandflies are spreading northwards in Europe and their densities are increasing in some newly colonised areas.
- No CanL endemic situation has been observed in areas without competent vectors, suggesting that none of these transmission routes appears to sustain infection in a large population (i.e. larger than that of a household or a kennel).
- In northern European countries, where competent vectors have not been found, "imported" cases in dogs with a history of travelling from endemic areas and CanL foci in households or in kennels have been described. These foci can last for several years because of non-vectorial transmission.
- Once infected, a sandfly remains infected for life, that is, on average, two to three weeks. Vertical transmission of *Leishmania* has not been reported in sandflies.

- Infection spreads quickly and extensively among the dog population when environmental conditions for transmission are optimal (vectors and contacts).
- All seropositive *L. infantum*-infected dogs, whether they express clinical disease or not, are potential sources of infection for vectors and may transmit the parasite.
- The role of wild mammals as reservoirs has not been fully demonstrated. Black rats, wild rabbits and hares may contribute to maintaining *L. infantum* circulation in some areas of southern Europe.
- The impact of infection with *L. infantum* on the health and welfare of dogs depends on its severity, which ranges from subclinical to very severe, including euthanasia.
- The average incidence of visceral leishmaniosis reported in humans in southern Europe ranges between 2 and 134 cases per year and per country, and the cutaneous forms caused by *L. infantum* range between 1 and 50 cases.
- Most infections in humans with *L. infantum* are asymptomatic. Risk factors for clinical disease include young age, HIV infection and other immunosuppressive states.

TOR2 (efficacy of available preventative measures)

- No CanL **vaccine** developed so far is able to confer full protection against infection or disease. However, some vaccines, such as CaniLeish®, the only vaccine authorised in the EU, do provide partial protection against active *L. infantum* infection and clinical disease in dogs.
- The efficacy of **topically applied insecticides** has been demonstrated under experimental conditions and in controlled field studies providing a mass treatment effect. It is uncertain whether the same efficacy of insecticides can be obtained in individual dogs when application is their owners' responsibility.
- Limited data are available on the efficacy of **prophylactic medication** with domperidone in endemic areas. Furthermore, data on treatments of immunologically naive dogs and its potential long-term toxicity are lacking and this area needs further investigation.
- In most observational studies, no reference test is available which would detect all the truly infected animals, thus hampering calculation of sensitivity estimates.
- Drug therapy for CanL appears to mainly slow down the progression of infection, decrease infectiousness and improve clinical manifestations by reducing parasite loads in infected tissues, but no treatment (drugs and regime) tested so far has demonstrated 100 % efficacy in the elimination of the parasites.

TOR3 (probability of establishment in free areas)

- Owing to the limited available knowledge on factors such as vector competence and abundance, dog distribution and movements, the average probability of introduction and establishment of CanL in a theoretical dog network or a network of networks was estimated, assuming the presence of competent vectors in some areas in a CanL-free area.
- The model assessed the average probability of disease establishment, defined as the local transmission of from vector to host and vice versa, leading to the temporal presence of at least one indigenous infectious host and at least one indigenous infectious vector. The probability of establishment was very high in these areas.
- Even in areas where sandfly populations are likely to have a lower vectorial capacity than in endemic areas, e.g. in some foci with low vector densities, the average probability of establishment following introduction of an infected dog remains high, according to the model.

- Although the average probability of establishment in a non-endemic region with competent sandflies may be very high, according to the model, the prevalence in that region in the event of CanL introduction and establishment may vary from extremely low to high, depending mainly on the vectorial capacity.

- Owing to the wide distribution of susceptible dogs and the high host–vector contact rates, the main limitation to CanL spread is represented by the vectors. This reinforces the need for knowledge of the vectorial competence of some sandfly species and of the distribution and abundance of known vectors.

- Results from the model indicated that the probability of introduction and establishment can be reduced by mitigation measures, separately or in combination. The most effective mitigation measure to reduce the probability of introduction and establishment of CanL was topically applied insecticide.

- The model indicated that vaccination of dogs prior to travelling to endemic areas had only a limited effect on the probability of establishment in a non-endemic region, and this effect seems more apparent when the vectorial capacity and the number of imported dogs were low.

- The use of topical insecticide and vaccination in travelling dogs had a synergistic effect in reducing the probability of establishment in a dog network and in reducing the probability of establishment in a region after their return to a non-endemic area, according to the model. Again, this effect was more marked in areas where a low vectorial capacity of the vectors was assumed.

- Testing dogs before their introduction into a non-endemic area is of limited value if applied shortly after exposure to infected sandflies. This is mainly because of it takes several months after exposure before testing gives a positive result.

- Test and treatment in the endemic area, prior to movement into a non-endemic area, will reduce disease risk in individual animals; however, it does not appear to be an efficient and realistic option to mitigate the risk of introduction of CanL into the non-endemic area, as no treatment against *L. infantum* infection can provide permanent parasitological cure.

RECOMMENDATIONS

- Recommendations for preventing introduction:

  – Owners of dogs travelling from free areas to endemic areas should be informed about the risks posed by CanL and potential risk mitigation measures.

  – The most useful diagnostic approaches for investigation of infection in sick and clinically healthy infected dogs include (1) detection of specific anti-leishmanial antibodies in serum using quantitative serological techniques and (2) demonstration of parasite DNA in tissues by applying molecular techniques. To optimise the sensitivity of CanL diagnostics, especially in subclinical dogs, the two techniques should be used in parallel.

  – Dogs born in endemic areas, which are confirmed to be infected with *L. infantum* by an appropriate test, should not be imported from endemic areas into non-endemic areas.

  – Dogs that have stayed in endemic areas for a longer period during transmission season should be tested for absence of *Leishmania* infection by PCR and serology before they are approved for breeding or as blood donors.

- To prevent CanL introduction and establishment in non-endemic areas via measures imposed on dogs travelling to and from or imported from endemic areas, the use topical insecticides is strongly recommended.

- Exclusion of travelling dogs testing positive by means of serology and/or PCR after their return may not be imposed on dog owners. However, the close clinical monitoring of these dogs is recommended, including medical treatment, which will mitigate the risk of disease and its impact on welfare, and which will reduce parasite loads and infectiousness of the dog.

- In addition, when the presence of competent vectors in a free area is known, the use of insecticide collars in those infected dogs in non-endemic areas would further reduce the risk of CanL vectorial transmission.

- Recommendations for further research:

  - The prevalence and incidence of CanL in dogs in different areas of Europe should be estimated more precisely.

  - Parameters driving the transmission of *L. infantum* infection in dogs, humans, other reservoirs and sandflies, including non-vectorial transmission (e.g. the survival of the parasite in blood products), should be quantified.

  - Well-designed, adequately powered RCTs on the efficacy of the preventative measures, such as vaccination and application of topical insecticides, alone and in combination, should be carried out.

  - Surveillance of competent or possibly competent vectors of CanL and their spread and emergence into new areas should be carried out to predict CanL risk of establishment in new regions.

  - Sensitivity and specificity of diagnostic tests for detecting *L. infantum* should be quantified, e.g. by latent class analysis, using two different test principles (serology and PCR).

  - Diagnostics and prognostic tests in dogs should be improved and developed, e.g. biomarkers to differentiate status of infection and infectiousness should be developed.

GLOSSARY

Xenodiagnosis:	diagnostic method by exposing possibly infected animals to a arthropod vector and then examining the vector for possible pathogens it may have ingested.
Crepuscular:	active primarily during twilight (i.e., dawn and dusk).
Onychogryphosis:	hypertrophy and curving of the nails, resulting in a claw-like appearance of the nails
Leishmaniacidal:	having the ability to kill *Leishmania spp.*
Leishmaniastatic:	having the ability to slow the growth of *Leishmania spp.*
Introduction:	entry of pathogen in previously free area
Establishment of infection:	local transmission of the imported pathogen from vector to host and vice versa, leading to the temporal presence of at least one indigenous infectious host and at least one indigenous infectious vector.
Persistence of infection:	the situation in which infection remains present in a dog population for multiple vector seasons and/or multiple disease generations.
Endemicity:	the situation in which infection persists in a population larger than a kennel or a household. Endemicity implies both persistence and spatial spread of infection.

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APPENDICES

Appendix A. Distribution of potential vectors of *L. infantum* in Europe

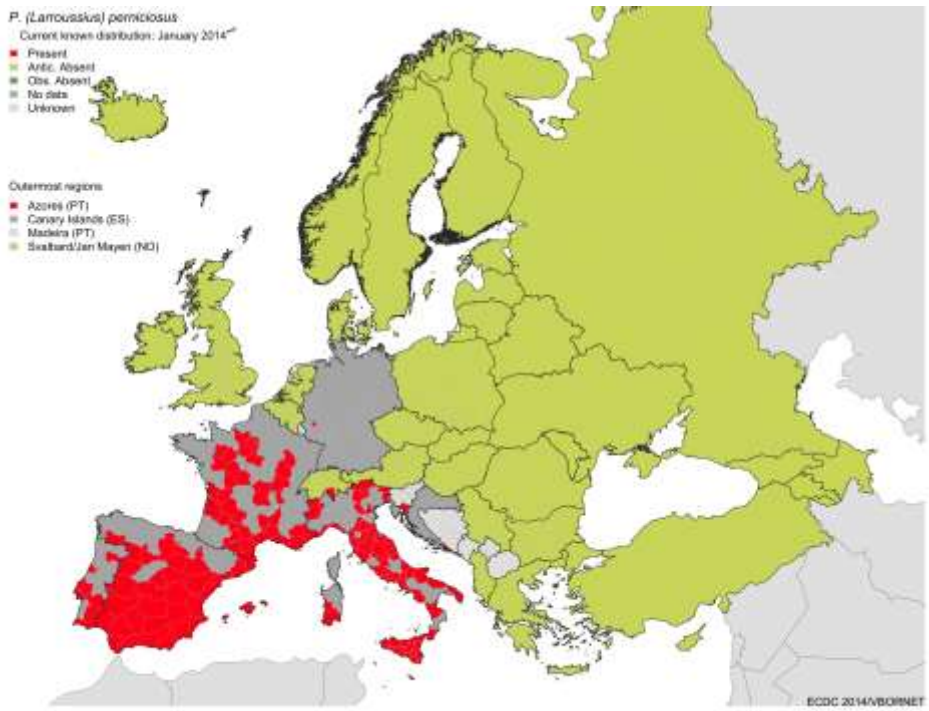

Figure 5: Reported presence of *P. perniciosus* in Europe

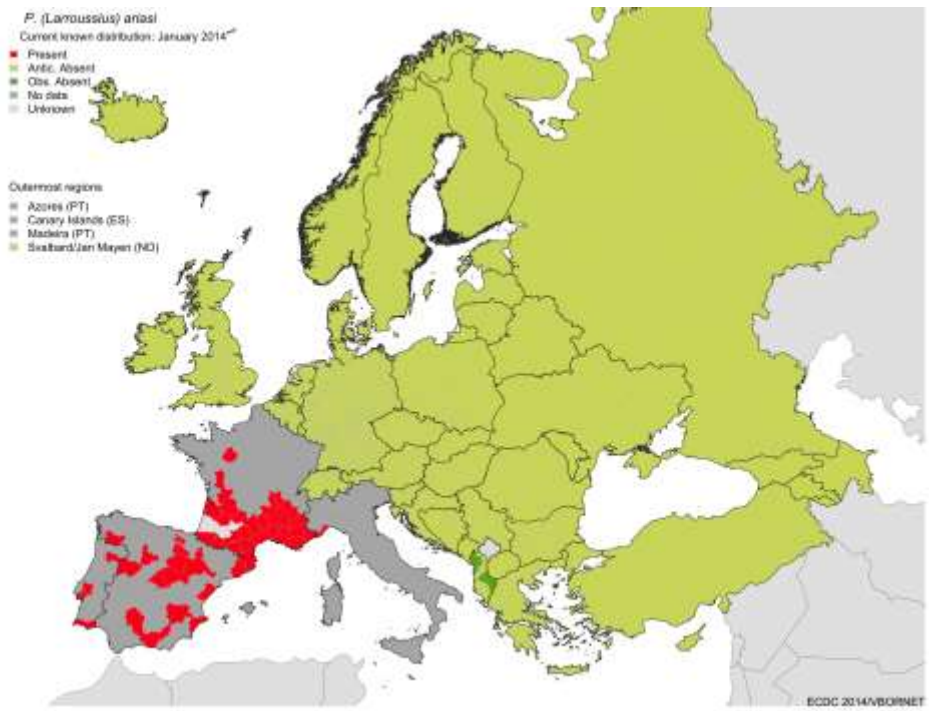

Figure 6: Reported presence of *P. ariasi* in Europe

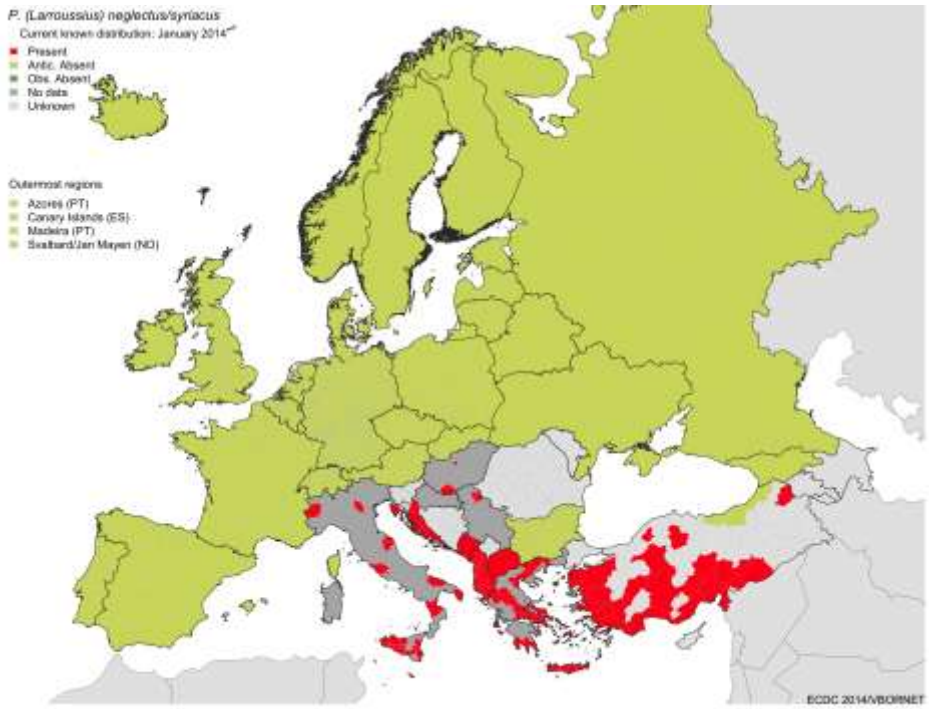

Figure 7: Reported presence of *P. neglectus* in Europe

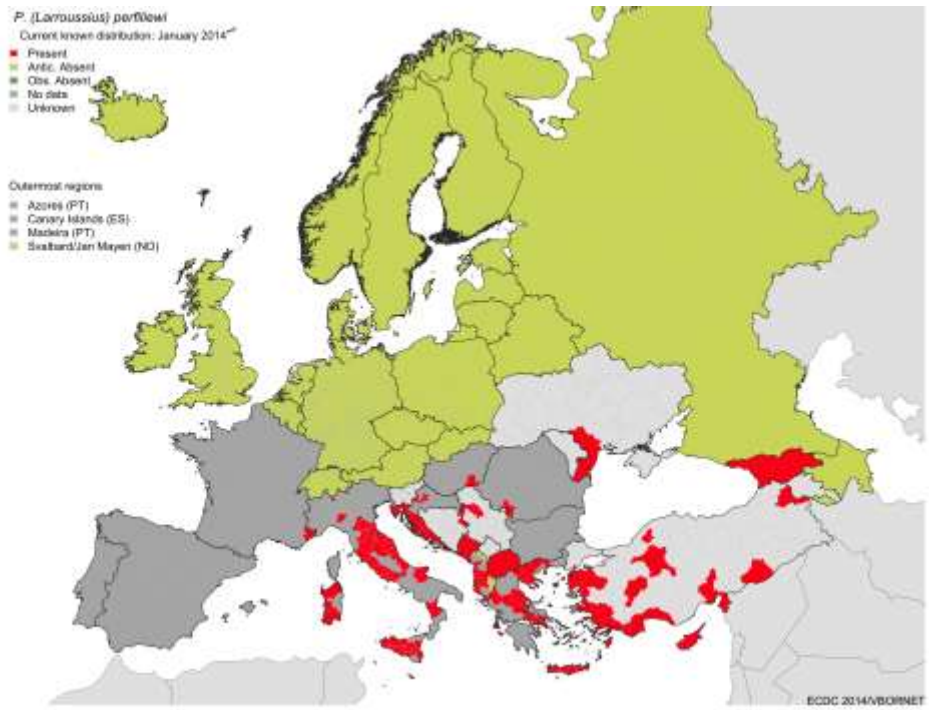

Figure 8: Reported presence of *P. perfiliewi* in Europe

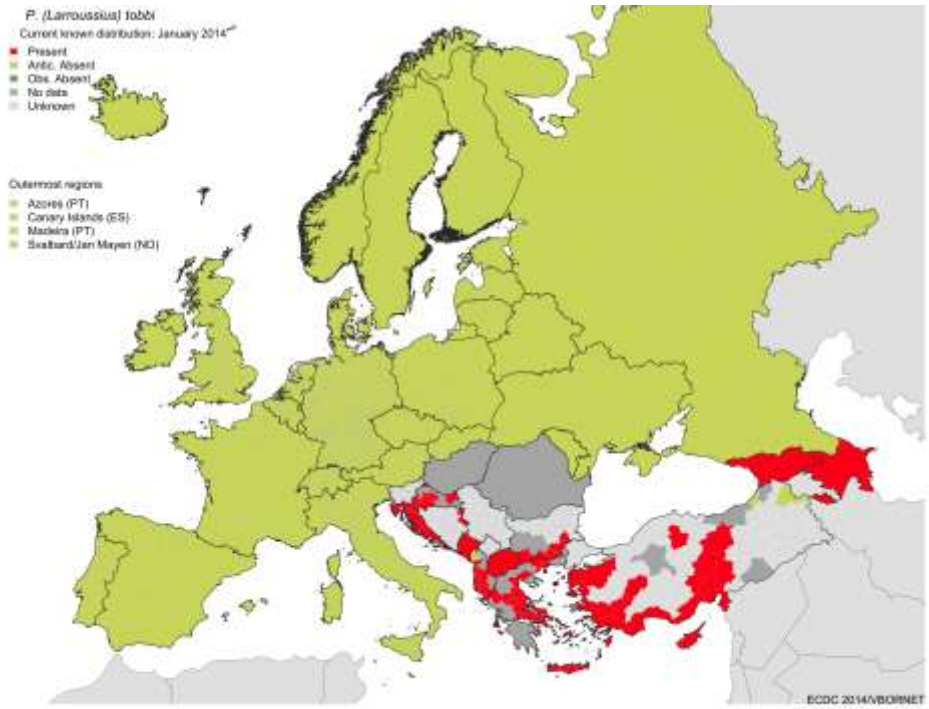

Figure 9: Reported presence of *P. tobbi* in Europe

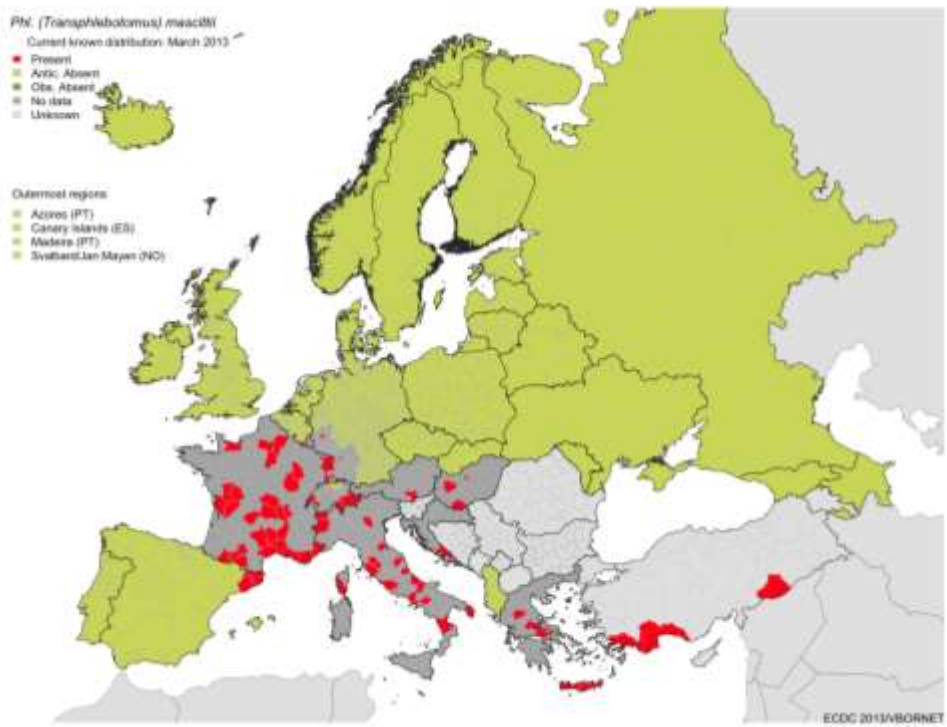

Figure 10: Reported presence of *P. mascittii* in Europe

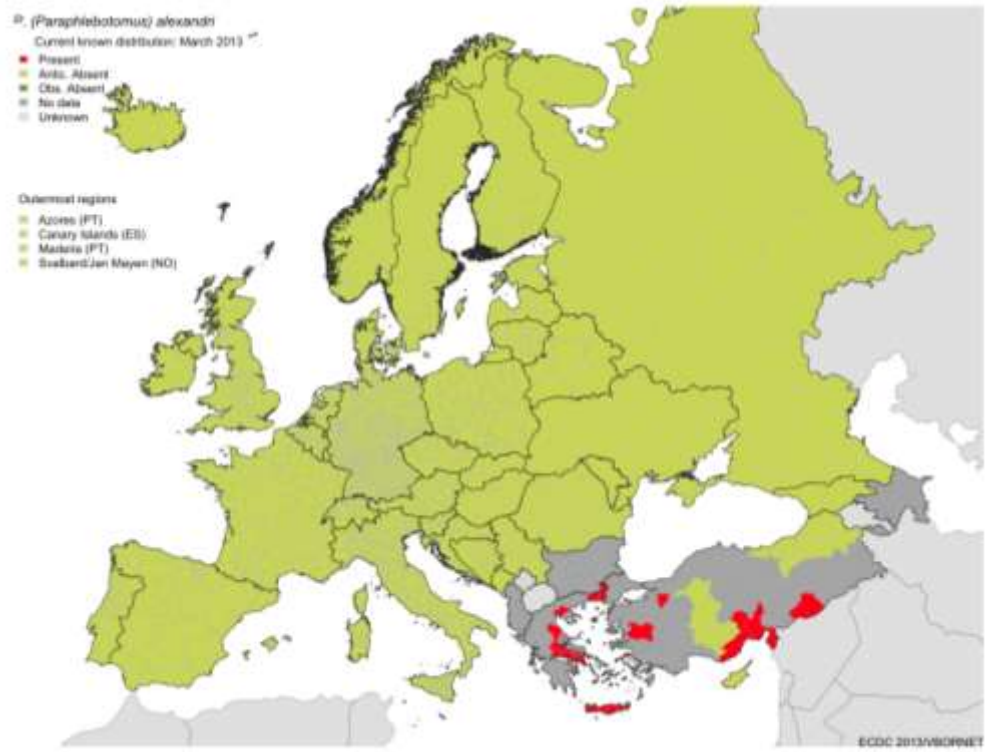

Figure 11: Reported presence of *P. alexandri* in Europe

Appendix B. Animal welfare impact on clinical stages of canine leishmaniosis

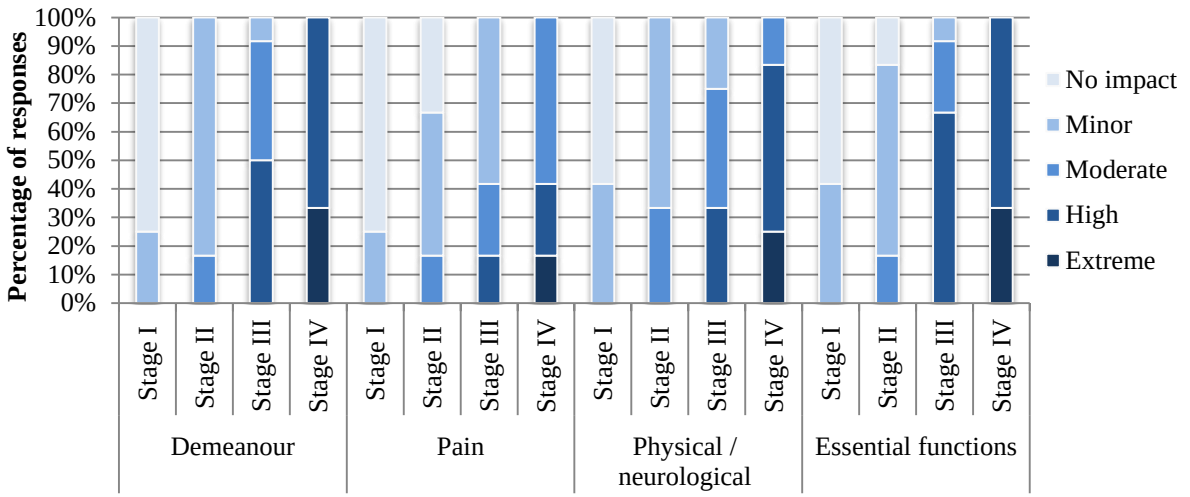

Figure 12: Expert opinion of the animal welfare impact of clinical stages (I–IV) of canine leishmaniosis (responses from six experts)

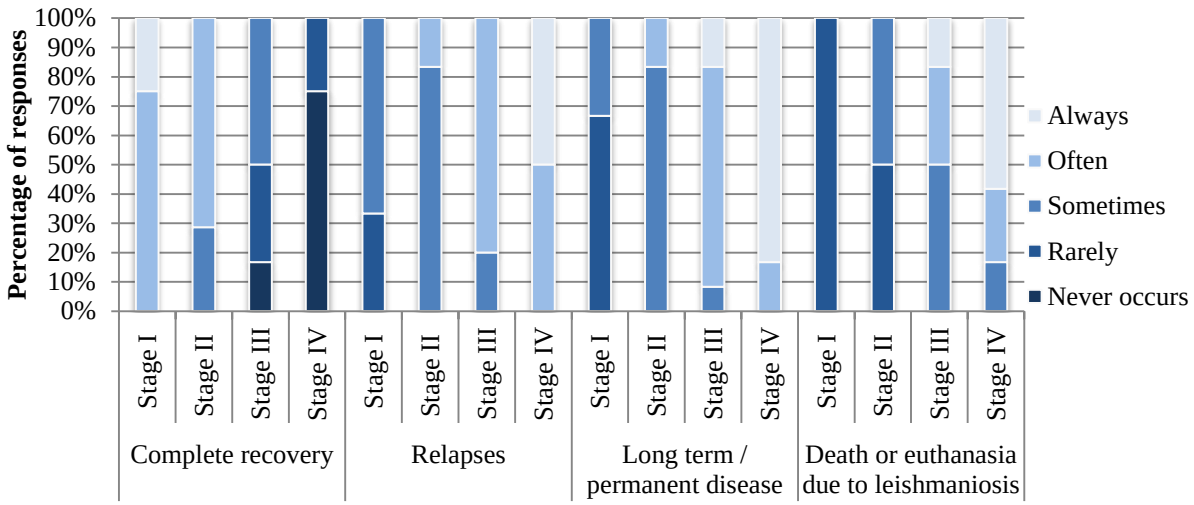

Figure 13: Expert opinion on the likely outcome of clinical stages (I–IV) of canine leishmaniosis (responses from six experts)

Appendix C. Probability of establishment in a region considering different number of dogs imported to the free region and prevalence

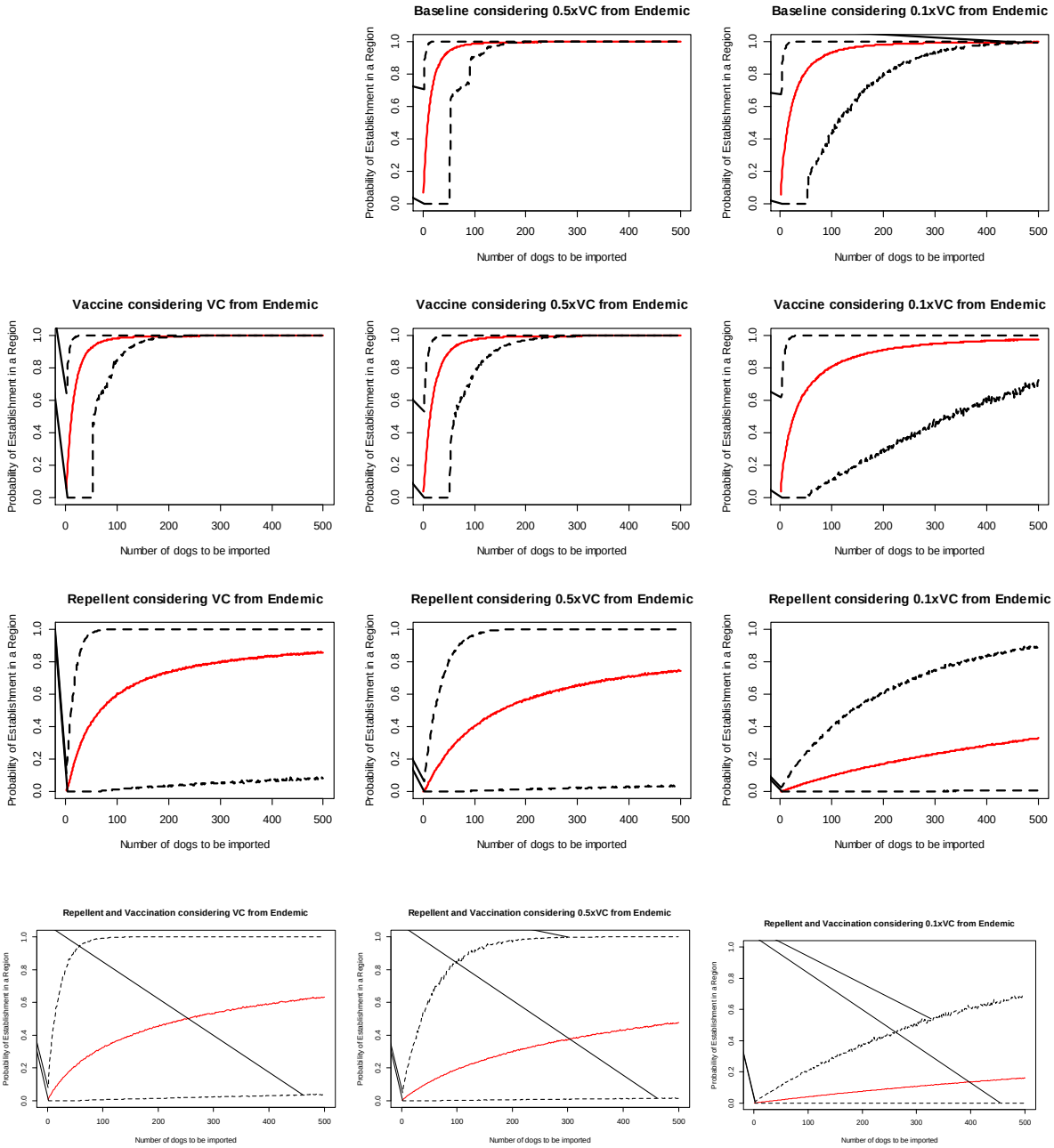

Figure 14: Probability of establishment of CanL considering different number of dogs moved into a CanL-free region (individual scenarios with confidence interval)

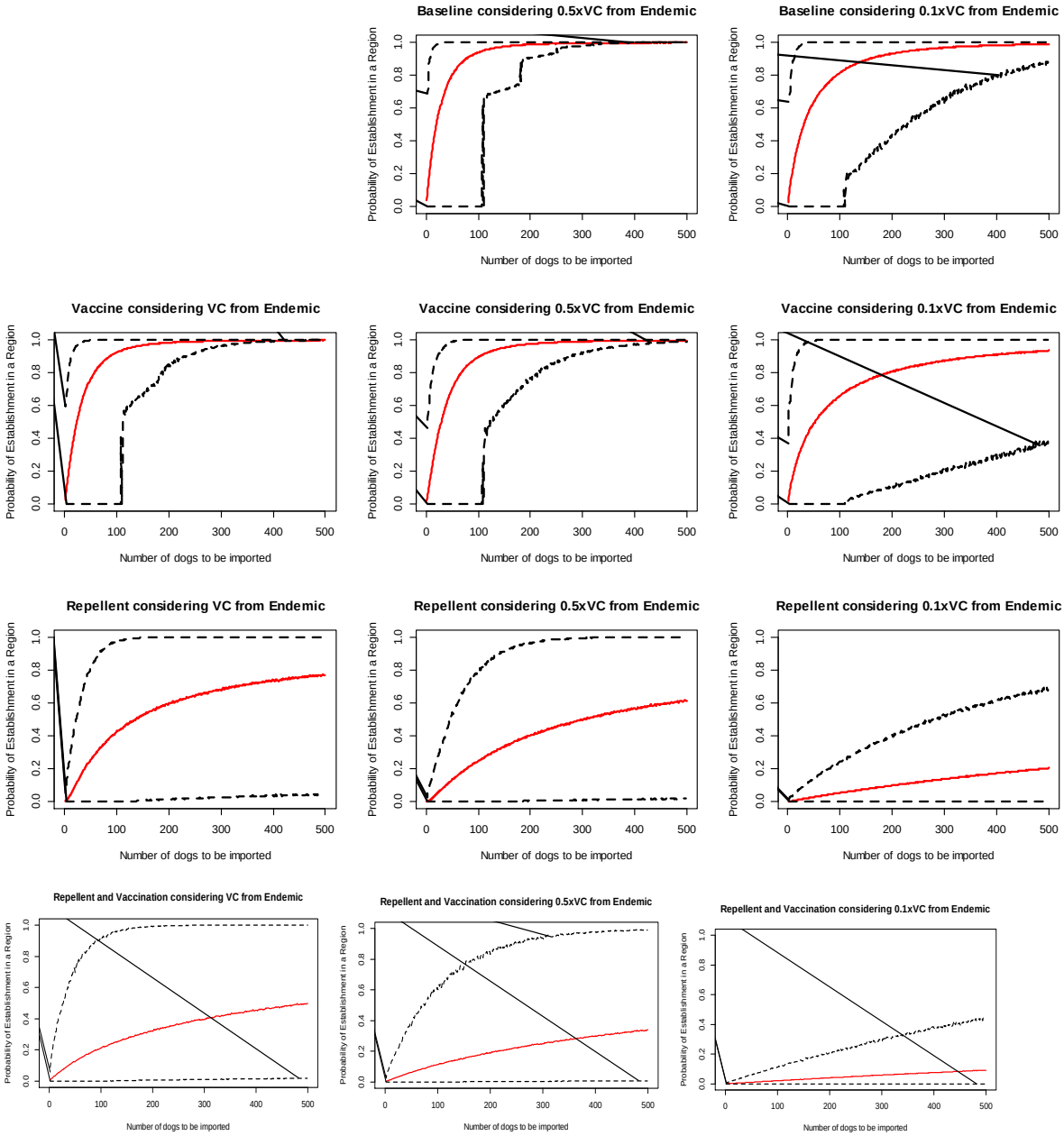

Figure 15: Probability of establishment of CanL considering different number of dogs imported into a CanL-free region after testing dogs, but before entering the free area (individual scenarios with confidence interval, assuming a test sensitivity of 0.50)

Appendix D. Results of the systematic review on the sensitivity of different diagnostic tests to detect *L. infantum* in experimentally infected dogs

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	1	12	5	42	Maia et al. (2010)
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	2	12	6	50	Maia et al. (2010)
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	3	12	9	75	Maia et al. (2010)
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	4	12	12	100	Maia et al. (2010)
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	5	12	12	100	Maia et al. (2010)
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	6	12	12	100	Maia et al. (2010)
ELISA	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	0	12	1	8	Maia et al. (2010)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	1	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	1	3	2	67	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	3	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	3	3	1	33	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	6	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	6	3	2	67	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	9	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	9	3	1	33	Leandro et al. (2001)

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	12	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	12	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	16	3	0	0	Leandro et al. (2001)
ELISA	Serum	A	10^6	Mon-1 (MHOM/PT/93/IMT184)	IV	16	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	5×10^5	MCAN/ES/1996/BCN150	IV	12	7	7	100	Fernandez-Cotrina et al. (2013)
ELISA	Serum	P	5×10^6	MCAN/ES/1996/BCN150	IV	12	10	10	100	Fernandez-Cotrina et al. (2013)
ELISA	Serum	P	5×10^7	MCAN/ES/1996/BCN150	IV	12	8	8	100	Fernandez-Cotrina et al. (2013)
ELISA	Serum	P	10^9	JPCM5 (MCAN/E-S/98/LIM-877)	ID	12	6	6	100	Daneshvar et al. (2010)
ELISA	Serum	P	10^9	JPCM5 (MCAN/E-S/98/LIM-877)	IV	12	6	6	100	Daneshvar et al. (2010)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	1	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	1	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	3	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	3	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	6	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	107	Mon-1 (MHOM/PT/93/IMT184)	IV	6	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	9	3	0	0	Leandro et al. (2001)

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	9	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	12	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	12	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	16	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	10^7	Mon-1 (MHOM/PT/93/IMT184)	IV	16	3	0	0	Leandro et al. (2001)
ELISA	Serum	P	5×10^5	MON1/MCAN/ES/01/LLM996	IV	5	7	7	100	Carcelen, et al. (2009)
ELISA	Serum	P	5×10^5	MON1/MCAN/ES/01/LLM996	IV	11	7	4	57	Carcelen, et al. (2009)
ELISA	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	3	6	1	17	Rodriguez-Cortes et al. (2007)
ELISA	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	5	6	2	33	Rodriguez-Cortes et al. (2007)
ELISA	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	13	6	4	67	Rodriguez-Cortes et al. (2007)
ELISA	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	12	41	NR	98	Rodriguez-Cortes, et al. (2013)
ELISA	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	12	41	NR	78	Rodriguez-Cortes, et al. (2013)
ELISA	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	12	41	NR	76	Rodriguez-Cortes, et al. (2013)
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	0	12	0	0	Maia et al. (2010)
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	1	12	0	0	Maia et al. (2010)
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	2	12	0	0	Maia et al. (2010)

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	3	12	2	17	Maia et al. (2010)
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	4	12	7	58	Maia et al. (2010)
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	6	12	12	100	Maia et al. (2010)
IFAT	Serum	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	5	12	12	100	Maia et al. (2010)
IFAT	Serum	P	5×10^5	MCAN/ES/1996/BCN150	IV	12	7	6	86	Fernandez-Cotrina et al. (2013)
IFAT	Serum	P	5×10^6	MCAN/ES/1996/BCN151	IV	12	10	10	100	Fernandez-Cotrina et al. (2013)
IFAT	Serum	P	5×10^7	MCAN/ES/1996/BCN152	IV	12	8	8	100	Fernandez-Cotrina et al. (2013)
IFAT	Serum	P	5×10^7	MCAN/ES/92/BCN-83/MON-2	IV	12	41	NR	65	Rodriguez-Cortes, et al. (2013)
PCR	blood	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	0	12	0	0	Maia et al. (2010)
PCR	blood	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	3	12	0	0	Maia et al. (2010)
PCR	blood	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	6	12	0	0	Maia et al. (2010)
PCR	Blood	P	5×10^6	MCAN/ES/98/LLM-877)	IV	9	2	1	50	Sima et al. (2005)
PCR	Blood	P	5×10^6	MCAN/ES/98/LLM-877)	IV	9	2	1	50	Sima et al. (2005)
PCR	Blood	P	5×10^6	MCAN/ES/98/LLM-877)	IV	9	2	2	100	Sima et al. (2005)
PCR	Blood	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	4	6	1	17	Rodriguez-Cortes et al. (2007)
PCR	Blood	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	5	6	3	50	Rodriguez-Cortes et al. (2007)
PCR	Blood	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	6	6	3	50	Rodriguez-Cortes et al. (2007)

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
PCR	Blood	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	13	6	3	50	Rodriguez-Cortes et al. (2007)
PCR	BM	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	0	12	0	0	Maia et al. (2010)
PCR	BM	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	3	12	0	0	Maia et al. (2010)
PCR	BM	A	10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	6	12	11	92	Maia et al. (2010)
PCR	BM	A	10^9	MON-1(MCAN/PT/94/IMT 195)	IV	38	3	0	0	Campino et al. (2000)
PCR	BM	P	10^9	MON-1(MCAN/PT/94/IMT 195)	IV	38	2	1	50	Campino et al. (2000)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	0	8	0	0	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	4	8	6	75	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	8	8	5	63	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	12	8	3	38	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	16	8	2	25	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	0	8	0	0	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	4	8	6	75	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	8	8	5	63	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	12	8	3	38	Moreno et al. (2007)
PCR	BM	P	10^8	MCAN/ES/98/LLM-722	IV	16	8	2	25	Moreno et al. (2007)
PCR	BM	P	10^9	JPCM5 (MCAN/ES/98/LIM-877)	IV	12	6	6	100	Daneshvar et al. (2010)

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
PCR	BM	P	10^9	JPCM5 (MCAN/E-S/98/LIM-877)	ID	12	6	5	83	Daneshvar et al. (2010)
PCR	BM	P	5×10^6	MCAN/ES/98/LLM-877)	IV	9	2	1	50	Sima et al. (2005)
PCR	BM	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	4	6	3	50	Rodriguez-Cortes et al. (2007)
PCR	BM	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	5	6	2	33	Rodriguez-Cortes et al. (2007)
PCR	BM	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	6	6	3	50	Rodriguez-Cortes et al. (2007)
PCR	BM	P	5×10^7	MCAN/ES/92/BCN-83/MON-1	IV	13	6	2	33	Rodriguez-Cortes et al. (2007)
PCR	BC	A	10^8	MCAN/IL/2001/LRC-L1021	IV	1.5	6	1	17	Strauss-Ayali et al. (2004)
PCR	BC	A	10^8	MCAN/IL/2001/LRC-L1023	IV	2	6	3	50	Strauss-Ayali et al. (2004)
PCR	BC	A	10^8	MCAN/IL/2001/LRC-L1025	IV	2.5	6	0	0	Strauss-Ayali et al. (2004)
PCR	BC	A	10^8	MCAN/IL/2001/LRC-L1027	IV	3	6	1	17	Strauss-Ayali et al. (2004)
PCR	Con	A	10^8	MCAN/IL/2001/LRC-L1022	IV	2	6	5	83	Strauss-Ayali et al. (2004)
PCR	Con	A	10^8	MCAN/IL/2001/LRC-L1024	IV	2.5	6	6	100	Strauss-Ayali et al. (2004)
PCR	Con	A	10^8	MCAN/IL/2001/LRC-L1026	IV	3	6	6	100	Strauss-Ayali et al. (2004)
PCR	Con	A	10^8	MCAN/IL/2001/LRC-L1020	IV	1.5	6	5	83	Strauss-Ayali et al. (2004)
PCR	LN	A	1×10^7	MON-1 (MCAN/PT/05/IMT-373)	IV	6	12	11	92	Maia et al. (2010)
PCR	LN	A	10^8	MON-1 (MCAN/1994/PT/IMT 205	IV	NR	3	2	67	Santos-Gomes et al. (2003)
PCR	LN	A	10^9	MON-1 (MCAN/PT/94/IMT 195)	IV	38	3	1	33	Campino et al. (2000)

Method	Tissues	Life stage	Dose	Zymodeme	Route	Months PI	Infected (a)	Positive (b)	Sensitivity	Reference
PCR	LN	P	10^9	JPCM5 (MCAN/E-S/98/LIM-877)	ID	12	6	4	67	Daneshvar et al. (2010)
PCR	LN	P	10^9	MON-1(MCAN/PT/94/IMT 195)	IV	38	2	2	100	Campino et al. (2000)
PCR	LN	P	10^8	MON-1 (MCAN/1994/PT/IMT 205	IV	NR	3	0	0	Santos-Gomes et al. (2003)
PCR	LN	P	10^8	MON1/MCAN/ES/01/LLM996	IV	10	5	2	40	Ramos et al. (2009)
PCR	LN	P	10^9	JPCM5 (MCAN/E-S/98/LIM-877)	IV	12	6	5	83	Daneshvar et al. (2010)
PCR	Serum	P	5×10^5	MCAN/ES/1996/BCN150	IV	12	7	7	100	Fernandez-Cotrina et al. (2013)
PCR	Serum	P	5×10^6	MCAN/ES/1996/BCN151	IV	12	10	9	90	Fernandez-Cotrina et al. (2013)
PCR	Serum	P	5×10^7	MCAN/ES/1996/BCN152	IV	12	8	4	50	Fernandez-Cotrina et al. (2013)
PCR	Skin	P	5×10^5	MON1/MCAN/ES/01/LLM996	IV	11	7	1	14	Carcelen et al. (2009)

(a): Number of dogs infected.
(b): Number of dogs positive.
A, amastigotes; BC, buffy coat; Con, conjunctiva fluids; ID, intradermal inoculation; IV, intravenous inoculation; LN, lymph nodes; P, promastigotes