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The importance of canine leishmaniosis in non-endemic areas, with special emphasis on the situation in Germany

Die Bedeutung der kaninen Leishmaniose in nicht endemischen Gebieten unter besonderer Berücksichtigung der Situation in Deutschland

Norbert Mencke

Summary

This review article summarizes the situation of canine leishmaniosis in Germany. Published case studies on infections with *Leishmania* (*L.*) *infantum* in either humans or dogs are analyzed. Diagnosed cases of infections by *Leishmania* spp. in humans and animals are not a notifiable disease in Germany or other European countries. Taking this into consideration one may assume that there might be a significant gap between the analyzed and reported cases and the infectious status within the country. The reported case studies and results from surveys indicate that the majority of all *L. infantum* infections are acquired during travelling in endemic regions, predominantly the Mediterranean region. However there are cases reported from human infections and growing number of cases in dogs, where the case history may indicate an autochthonous infection within Germany, a country within a non-endemic region. The current data from entomological field studies proved the presence of two phlebotomine sand fly species. *Phlebotomus* (*P.*) *mascittii*, an anthropophilic sand fly species and *P. perniciosus* a proven vector of *L. infantum*. The impact from a growing leishmania-positive dog population within Germany, the distribution of at least two sand fly species, one with vector potential in the light of climate change and other non-vectorial transmissions are summarized.

Keywords: *Leishmania infantum*, canine leishmaniosis, Germany, non-endemic regions, human visceral leishmaniosis

Zusammenfassung

Im vorliegenden Beitrag wird die Situation der kaninen Leishmaniose in Deutschland vorgestellt. In dem Review geht es um die beschriebenen Fälle von Infektionen mit *Leishmania* (*L.*) *infantum*, dem Erreger der viszeralen Leishmaniose des Menschen und der kaninen Leishmaniose des Hundes. Infektionen mit *L. infantum* sind weder in der Human- noch in der Veterinärmedizin anzeige- oder meldepflichtige Infektionserkrankungen in Deutschland sowie anderen europäischen Staaten. Somit muss davon ausgegangen werden, dass es zwischen den publizierten Fällen und dem tatsächlichen Vorkommen es Erregers erhebliche Unterschiede geben könnte. Die beschriebenen Fälle in der Human- wie auch Veterinärmedizin betreffen in der überwiegenden Anzahl Erkrankungen, bei denen es zu einer Infektion während des Aufenthaltes in einem *Leishmania*-endemischen Gebiet, vorzugsweise dem Mittelmeerraum, gekommen ist. Es sind aber auch Einzel-Fallbeschreibungen publiziert worden, bei denen es zu einer Infektion mit *L. infantum* in Deutschland, einem nicht endemischen Gebiet gekommen sein könnte. Die Infektionen mit *L. infantum* in Deutschland werden den Infektionen in den benachbarten europäischen Ländern gegenübergestellt. Der derzeitige Stand der Schmetterlingsmücken-Forschung in Deutschland hat nachgewiesen, dass es autochthone Vorkommen von *Phlebotomus mascittii*, einer anthropophilen Schmetterlingsmückenart, und *P. perniciosus,* einem nachgewiesenen Überträger von *L. infantum,* gibt. Die Bedeutung einer ständig zunehmenden *Leishmania*-positiven Hundepopulation, das Vorkommen von Schmetterlingsmücken mit Vektorpotenzial bei gleichzeitiger Klimaveränderung und anderen nicht vektorgebundenen Übertragungswegen werden zusammengefasst.

Schlüsselwörter: *Leishmania infantum*, kanine Leishmaniose, Deutschland, nicht endemische Gebiete, menschliche viszerale Leishmaniose

Introduction

One of the most important canine vector-borne diseases (CVBD) is canine leishmaniosis caused by the protozoan parasite *Leishmania* (*L.*) *infantum*. *L. infantum* is transmitted by the sand fly, a crepuscular and nocturnal bloodfeeder of the subgenus *Larroussius*, genus *Phlebotomus* (*Diptera, Psychodidae, Phlebotominae*). Canine leishmaniosis and other CVBDs have gained more importance due to the impact which climate change (Hemmer et al., 2007; Fischer et al., 2010) and travel with or re-homing of pet dogs (Trotz-Williams and Trees, 2003) have caused onto the distribution of the vector, the pathogen or the occurrence of the disease. Concerning canine leishmaniosis, travelling with or translocation of dogs from endemic to non-endemic regions is currently a European phenomenon; the impact onto the unprotected naïve dog population is not fully understood. *Leishmania* transmitted by phlebotomine sand flies have, since ancient times, been reported for the European Mediterranean region and regarded to be endemic. Endemic due to the fact that both, infected dogs and vector sand flies are present. The prevalence and even more the incidence of the disease differ significantly from region to region and are depending on biotic and abiotic factors. Today with increased numbers of dogs kept as companion animals, dogs travelling frequently with their owners, the absence of boarders within continental Europe due to the Schengen Treaty and most important the translocation of dogs from endemic to previously non-endemic regions, does change our view on canine leishmaniosis significantly. Besides the life threatening disease for the dog, leishmaniosis must be regarded as an important human zoonosis. Human visceral leishmaniosis (VL) caused by *L. infantum* may cause severe disease and even death especially among children and immunosuppressed adults.

Beside the defined vector – host transmission, there are a number of cases from around the world where visceral leishmaniosis, *Leishmania* infections of wildlife or domestic animals other than dogs are recorded while the route of transmission remains unclear. Here the current status of canine leishmaniosis, phlebotomine sand fly vectors and human visceral leishmaniosis is reviewed for Germany, with an outlook on some other Central- and Northern European non-endemic countries.

Definition of "endemic" canine leishmaniosis region

Even in the known endemic regions and countries around the Mediterranean Sea, such as Greece, Italy, Southern France, Spain and Portugal, the basis for declaring these areas endemic are two fold, on the one hand it is dogs diagnosed positive, either serologically, parasitologically or clinically, on the other hand the presence of known vector sand fly species. The vector capacity of sand fly species present in endemic regions are well studied for species such as *Phlebotomus* (*P.*) *perniciosus*, to name the most important one, however information on other sand fly species is limited (Killick-Kendrick, 1999). Investigations on the sand fly species present in a given area and the prevalence of *L. infantum* in these sand flies are limited. Thus the definition of the disease being endemic is more or less based on the presence of infected hosts, the dog. In contrary one can define regions or countries as non-endemic where sand fly vectors are absent and the confirmed cases represent *L. infantum* infections acquired abroad. In this respect the situation in Germany is uncertain, because sand fly species capable to be true vectors have been trapped, even if so in small numbers (Naucke and Pesson, 2000; Schmitt, 2002; Naucke and Schmitt, 2003), and individual canine cases have been published that give evidence of an autochthonous infection (Gothe, 1991; Naucke and Schmitt, 2003). The same accounts for human VL cases with at least one well confirmed case for Germany (Bogdan et al., 2001). Thus the clear differentiation between endemic and non-endemic regions may change in the future.

Sand fly distribution in Northern Europe

Worldwide more than 700 sand fly species have been described and 23 species within Europe (Killick-Kendrick, 1990). Information is limited on the distribution of sand flies in continental Europe outside the endemic regions. In the European endemic regions according to Killick-Kendrick (1999) six sand fly species are closely linked to canine leishmaniosis, these are *P. perniciosus*, *P. ariasi*, *P. perfiliewi*, *P. neglectus*, *P. langeroni* and *P. tobbi.* The vector capacity of three further species in the endemic regions are supposed, these are *P. longicuspis*, *P. kandelakii* and *P. syriacus.* In non-endemic regions of Europe however only two species thereof have been recorded, *P. perniciosus* and *P. perfiliewi* and one additional species *P. mascittii.* The vector capacity of *P. perniciosus* and *P. perfiliewi* is well described, the competency of *P. mascittii* as vector of *L. infantum* is currently unknown, but suspected. *P. mascittii* has been recorded from regions in South-Western Germany (Naucke and Pesson, 2000) and Belgium (Depaquit et al., 2005). *P. perniciosus* has also been recorded from Southern Switzerland (Knechtli and Jenni, 1989), while no sand flies have been trapped in Suisse regions north of the Alps. In a paper by Marett (1923) sand flies have also been recorded as far north as the Channel Island Jersey. The capacity to extend into new habitats has been described for *P. perniciosus* and *P. neglectus* in Northern Italy, a previously non-endemic sand fly region (Capelli et al., 2004; Maroli et al., 2008). There, the increase in abundance of the sand fly species corresponded well with the increase of canine and even human cases. For *P. perfiliewi* the spread as far north as the 49th degree of latitude has been postulated by Naucke (2002). The impact of regional climate on both the vector sand fly and the pathogen has recently been studied for Germany (Fischer et al., 2010). In addition a temperature of 10°C is necessary to initiate the life cycle within the invertebrate and rising temperature is continuously shortening the time needed for completion. The shortest time for maturation within the invertebrate vector is at least six days. The 10°C year isotherm limiting development in the phlebotomine sand fly vector may today be reached as far north as the Channel Islands or the city of Cologne in Germany. Fischer et al. (2010) concluded from their investigation, that temperature seems to be suitable for the sand fly vector across large regions of Germany, in contrast to *Leishmania* where temperature constraints may defer the establishment of the parasitic disease. Fischer et al. (2010)

concluded that long-lasting epidemics of human VL are therefore not expected in Germany during the next few decades, while they also left some uncertainty for e. g. an increase in autochthonous cases of leishmaniosis may occur in extremely warm years. Thus, surveillance programmes have been established to investigate on sand fly distribution and the likelihood of VL, like the German program of the Bavarian ministry of health "Vector-borne Infectious Diseases in Climate Change Investigations (VICCI): Project 6: Autochthonous Leishmaniasis in Bavaria: Studies of Vector Prevalence and of Animal Reservoirs". Aspöck et al. (2008) discussed a scenario different to the sole climate change impact on sand fly distribution. They concluded that the Holocene warm postglacial periods 6500 and again 4500 year ago may have been favourable for Mediterranean species, including sand flies to extend into Central Europe. Most of these invading species have died out again when temperatures decreased, however sand fly species like *P. mascittii* may have remained in defined habitats like the upper Rhine valley. This hypothesis may be supported by the recent finding of *P. mascittii* in Germany (Naucke and Pesson, 2000; Schmitt, 2002; Naucke and Schmitt, 2003) and Belgium (Depaquit et al., 2005) and an earlier finding of *P. larroussei* near Strasbourg (Callott, 1950).

Sand fly distribution in Germany

The German situation is different to e. g. Switzerland where the Alps divide the country into a northern, non-endemic and a southern, endemic region. The same accounts for Austria. In both countries the regions south of the Alps are without topographical borders to endemic Northern Italy. In contrast, France consists of endemic Mediterranean regions and more northern non-endemic-regions without geographical boundaries. For Germany the consideration non-endemic for canine leishmaniosis however does not mean that Phlebotomine sand flies are not present in certain regions within the country. Especially the Rhine valley may be favourable for sand flies due to the habitat and the climatic conditions. Naucke and Pesson (2000) reported for the first time the presence of *P. mascittii* using CDC light-traps at three different locations along the Rhine in Southern Germany in the state of Baden-Wuerttemberg. A detailed description of *P. mascittii* habitats in Germany was published by Schmitt (2002) and summarized by Naucke and Schmitt (2003). They recorded this sand fly species along the Rhine as far north as Offenburg with an eastern boundary of the Black Forest. According to their findings 125 specimen of *P. mascittii* were trapped along the Rhine Valley. In addition, *P. perniciosus* was recorded for Germany by the same authors (Naucke and Schmitt, 2003) from the Rhineland-Palatinate village of Gehrsweiler. Naucke and Schmitt (2003) mentioned anecdotal sporadic cases of canine leishmaniosis including one unpublished canine case from a veterinary clinic in the vicinity where the first *P. perniciosus* sand flies have been trapped. No detailed information is provided for evidence to link sand fly presence and clinical cases. Naucke et al. (2008) recorded the northern extension of sand flies trapped in the village of Bremgarten (47°55′05.3″) (Naucke and Pesson, 2000) and further north in Baden-Baden (48°44′42.2″) (Naucke, 2007) till recently around the Moselle city of Cochem (50°19′41.2″)(Naucke et al., 2008).

Human visceral leishmaniosis in non-endemic Europe

The zoonotic nature of *L. infantum* highlights the importance to view the disease in human and veterinary medicine with great caution, especially in non-endemic region to initiate any early intervention in preventative medicine. Therefore a short excursion on human VL is included. Individual human cases have been published for all Continental European countries sharing borders with Germany and many others including the UK and Ireland. For the UK, a 20-year retrospective study published by Malik et al. (2006) following 39 patients with imported VL from Mediterranean countries and HIV infection. The authors addressed the necessity to have VL as a reportable disease. Rare clinical cases and low prevalence of morbidity is often in clear contrast to high seroprevalence in these regions. In immunocompromised patients however increasing cases are recorded with clinical symptoms from endemic regions such as Spain or Southern France.

Human visceral leishmaniosis in Germany

All human case reports have to be considered with caution, due to limited information on anamnesis and even more diagnostic methods. Early reports of travel related human infections go back to the 1960ies, when Gartmann and Kiessling (1963) reported a case of cutaneous leishmaniosis. Kala-Azar thus VL was reported first from a child by Reimers et al. (1965). For the former East German State, human cases of VL were reported by Hölzer and Kupferschmidt (1986). In 2003, Harms et al. reported results of a two years retrospective evaluation of reported leishmaniosis cases. From 2000 to 2002 a total of 70 cases were reported, of these 43 cutaneous or mucocutaneous and 27 visceral cases. Detailed data was available for 58 patients (35 cutaneous or mucocutaneous and 23 visceral). Interestingly 18 of the 23 VL patients acquired the infection during vacation in the Mediterranean region including Portugal. In six of the 18 cases children of the age of eleven or younger were infected. In addition Bogdan et al. (2001) reported a detailed case study of an autochthonous *L. infantum* MON 1-zymodene infection in a 15 month old boy from a place near to the city of Aachen that had never spend any time in an endemic region outside Germany. Leishmaniosis is not listed as a reportable disease under the German legislation ("Infektionsschutzgesetz"). The Robert-Koch-Institute, however, functions as a centre for surveillance of infectious diseases in Germany including VL and other human leishmanioses.

Human visceral leishmaniosis in neighbouring countries

Information available on human VL is based on data published from individual clinical cases. These contain

either citizen of the respective country that returned home with an infection acquired in an endemic region or people from respective endemic areas that have migrated to the country. Due to the large number of reported cases, some with unclear anamnesis, only examples are listed here solely on VL caused by *L. infantum* and acquired from countries around the Mediterranean region. From Poland a case of a five year old polish child that acquired VL after a vacation in Turkey (Owoc-Lempach et al., 2007), in Belgium a fatal VL in an HIV patient (Wallemacq et al., 2009), for the Netherlands a case of a five year old boy with acquired VL after vacation in Northern Italy (van Vliet et al., 2006). In Czech Republic a total of eight acquired cases of VL were published (Chalupa et al., 2001) for travellers returning from East Africa, Croatia and Southern Italy. Reports on VL acquired in a foreign country has been published for Denmark on a child with VL acquired in the Mediterranean (Peterslund et al., 1982) and in conjunction with HIV (Balslev et al., 1991). In Austria a case of VL was reported (Stoiser et al., 1998) in a 41 year old patient with travel history in Sicily.

Canine leishmaniosis in Germany

Canine leishmaniosis is not a reportable disease under German veterinary legislation. Information is thus based on publications from either individual case reports or data of surveys conducted. To structure the German situation it seems appropriate to separate case studies from surveys.

Case studies

The first published information (Saar, 1969) in Germany published pictures of a diseased dog. No further conclusion may be drawn from this paper. Two cases where reported by Koch (1986) and in the following year the therapy of an imported case (Reusch and Reiter, 1987). Another canine leishmaniosis case with dermatological signs where *L. infantum* was identified in a biopsy was recorded by Luft (1990). Gothe (1991) described one dog from Cologne region and another dog from the Southern German city of Landsberg/Lech. Both dog owners report, neither have the dogs or the bitch and hound travelled outside of Germany nor had these dogs contact to infected dogs. Reasonable suspicion may be allowed whether these dog owners' statements elucidate the full case history. In a publication summarizing the results of hemodialyis in twelve dogs with acute renal failure one dog was listed with late stage clinical signs of canine leishmaniosis (Dörfelt et al., 2007). The dog in this study acquired the pathogen outside Germany. Another clinical case was published including two Golden Retrievers from the state of Brandenburg in North-Eastern Germany (Kellermeier et al., 2007). Both dogs were diagnosed positive. One, a four year old male Golden Retriever, never left Germany. However this dog over extended periods of time was in close contact with another dog that frequently travelled between Germany and Spain. The diagnosis was based on the clinical appearance, despite the fact of a low titre in quantitative serology. The second dog diagnosed positive was a seven year old female Golden Retriever. Interestingly the bitch delivered a litter bred from the infected male one year prior to the clinical onset. The litter remained serologically negative. Another autochthonous case was published by Moritz and Steuber (1999). Further published data are available from the 1990ies (Gothe et al., 1997; Moritz et al., 1998) where the authors listed case studies of canine leishmaniosis acquired in endemic regions and diagnosed in veterinary clinics within Germany.

Surveys

The first published survey for Germany (Glaser and Gothe, 1998a; 1998b) showed that more than 12% of puppies were not born in Germany. Travel across German borders, even before the Schengen Treaty was estimated to be nearly 3000 dogs and 1/3 of these crossing borders frequently. An extensive survey among veterinary practices was published by Zahner and Bauer (2004). In 2002 the authors contacted 7500 small and mixed veterinary practices in Germany with a questionnaire to gain information on the situation of *Leishmania* positive dogs in Germany. The authors achieved a low 5.2% return rate (391 answers). The clinicians reported 724 cases from their clinical databases over the years 1996 to 2001. The numbers increased dramatically over these years from 54 cases in 1996 to 258 cases in 2001. The dogs seeking veterinary consultation were either imported from endemic areas or have travelled to endemic areas. Twelve veterinarians reported in the questionnaires cases from within Germany; however the authors stated, an intensified phone interview could not justify these cases as autochthonous. The results reported from an investigation among dog owners and follow up serological testing was conducted in 2003 by Mettler et al. (2005). 291 dogs were included in the serological testing, all of these dogs have either been introduced from *Leishmania* endemic Mediterranean regions (87%), predominantly from Spain (178 dogs), or travelled with their owners to endemic regions. The authors report 111 dogs (38%) with positive *L. infantum* ELISA titres. Menn et al. (2010) reported the results from a serological investigation comparing 4681 dog blood samples from within Germany with 331 from Portugal. From the total 4681 samples, 4226 were collected from imported dogs derived from various endemic regions, predominantly from the Mediterranean. Another group of 87 German dogs included in the survey had a travel history in the Mediterranean. An *L. infantum* antibody titre was recorded for 569 or 12.2% of the investigated dogs. An additional interesting finding of this survey was, that 502 dogs (10.7%) were co-infected with two different vector-borne pathogens at the same time and that 201 dogs (4.2%) were even co-infected with more than two vector-borne pathogens. The latest survey reporting the investigation of CVBD in German traveling dogs was published by Hamel et al. (2011). The authors tested a total of 997 German dogs with travel history in the year 2004 to 2008 to *Leishmania* endemic, e. g. Mediterranean but also non-endemic countries, e. g. Denmark or Poland. The overall prevalence of antibodies for *L. infantum* was 3.6% or 25 of the 698 dogs tested. Interestingly two of the 45 dogs with travel history to Austria were tested *Leishmania* positive. Unfortunately these dogs were not followed up, thus detailed informations are missing. In this survey multiple infections were analysed in six dogs. Four of these six dogs were positive for tick-borne *Babesia canis* and *Leishmania*, one dog for tick-borne *Ehrlichia canis* and *Leishmania* and one dog for *B. canis* and *E. canis*. These recent surveys (Menn et al., 2010; Hamel et al., 2011) indicated polyinfections

in dogs with pathogens transmitted by the sand fly or various tick species present within the same habitat.

Canine leishmaniosis in neighboring countries and UK

In the Netherlands an autochthonous cases was reported by Diaz-Espineira and Slappendel (1997). A comprehensive report of canine leishmaniosis was published by Slappendel (1988) including 95 cases from within the Netherlands. Data from an earlier survey conducted in 1989 (questionnaire) and serology 1990–1992 were published by Teske et al. (2002). The authors published data based on the response to 1478 questionnaires send to randomly chosen families. 872 reported back with a total of 13 dogs (4.8%) accompanied their owners to endemic regions. Unfortunately, no information was given by the authors on introduction of dogs from endemic regions. The authors calculated from their survey results and the total amount of dogs living within the Netherlands, that each year about 58 000 dogs may accompany their owners during visits in Southern Europe and thus may be at risk to acquire canine leishmaniosis if left unprotected. In addition, a serological survey was performed using sera from 1911 dogs presented to the clinics at the University of Uetrecht during January 1991 to January 1993 for a variety of disorders without any suspicion of canine leishmaniosis. Only one dog was tested positive and this dog had not been travelling to an endemic region. In a different serological survey the authors included sera collected between January 1989 and January 1994 from 597 dogs with travel history to endemic Southern European regions. 145 of these 597 dogs were *Leishmania* positive. 81 of these 145 dogs had a travel history; all other 64 dogs were born in the Mediterranean region and introduced to the Netherlands. The risk to acquire canine leishmaniosis was reported for Denmark as early as 1985 (Bindseil et al., 1985). A Danish wire-haired Pointer returning from a one year long stay in Malaga (Spain) was diagnosed positive with clinical signs 21 months following the return to Denmark. The first report on introduction of canine leishmaniosis to Sweden was published by Johansson et al. (1998). The Austrian situation is documented in two surveys published by Edelhofer et al. (1995) and Leschnik et al. (2008). In both surveys the authors concentrated on dogs with known travel history or dogs been introduced to Austria. The situation in the UK is different to most continental European countries due to their island situation and stringent importation rules. Since the introduction of the Pet Travel Scheme (PETS) in 2000 comprehensive information is available on dogs entering the UK or returning to the UK, with a total of 411 582 dogs recorded between 2000 and January 2008. The number of dogs travelling to the UK has increased year after year (www.defra.gov.uk/animal/diseases). In a recently published survey by Shaw et al. (2009) a total of 257 clinical cases have been investigated. From 183 of these a complete travel history was available. 28 dogs were rescued from kennels in the Mediterranean region and 26 entered the UK with clinical signs of leishmaniosis. The majority had spent at least six months in an endemic region. Interestingly, three dogs were obtained from re-homing centers without any travel history. Furthermore two dogs with canine leishmaniosis were resident on Jersey.

Diagnosis of canine leishmaniosis

Diagnostic methods to detect canine leishmaniosis have evolved over the years, and todays methods have a higher sensitivity and specificity. Nevertheless, recommendations on methods to be used in clinical settings are subject to large numbers of publications with often controversial outcome. A group of experts in the field of canine leishmaniosis named LeishVet reviewed the literature, mirrored published data with own clinical experience and published recently directions for the diagnosis of canine leishmaniosis (Solano-Gallego et al., 2009) and a guideline for practical management (Solano-Gallego et al., 2011). An intensive review on the literature on diagnostics in canine leishmaniosis would be out of focus of the article published here. However, it is important to mention that the cases and surveys for both canine and human leishmaniosis are based on diagnostic methods, which, if reviewed thoroughly today, may not withstand and thus support the originally published diagnosis. Furthermore a paradigm shift has evolved for canine leishmaniosis, made the categories of symptomatic and asymptomatic canine leishmaniosis obsolete. Today it is recommended to differentiate between diseased dogs with clinical signs and those without clinical signs but infected with the parasite. This impacts diagnosis fundamentally. Diagnosis to confirm the disease or infection with *Leishmania* requires different diagnostic techniques. Diagnosis of canine leishmaniosis in diseased dogs with clinical signs should be based on detection of clinicopathological abnormalities to define the stage of disease together with the detection of amastigotes in stained cytological smears. The serological technique recommended for detection of specific serum anti-leishmanial antibodies (IgG) is quantitative serology, e. g. immunofluorescence antibody test (IFAT) or enzyme-linked immunosorbent assay (ELISA). The critical part in IFAT using whole promastigote antigen is the sensitivity in infected while clinical healthy dogs. Another diagnostic technique available today with high sensitivity is the PCR assay. The detection of *Leishmania*-specific DNA by PCR is recommended to identify dogs harboring an infection

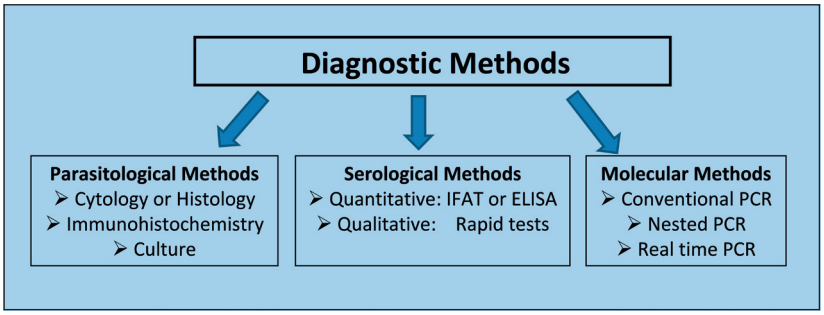

FIGURE 1: *Parasitological, serological and molecular techniques for the diagnosis of canine leishmaniosis (Solano-Gallego et al., 2011).*

while clinically healthy in case of re-homing or prior to use dogs as blood donors, ideally in combination with quantitative serology. In contrast a positive PCR reading alone is not sufficient to initiate anti-*Leishmania* therapy. Today's most common diagnostic methods are summarized in Figure 1.

Wildlife and feral animals

The role of semi-domestic and stray dogs in the perpetuation of canine leishmaniosis in endemic regions is well described. In addition stray cats are frequently reported to be infected with *L. infantum* in endemic regions. In non-endemic regions discussed here, stray dogs are virtually absent, while semi-domestic and stray cats in the contrary are prevalent in large numbers. Besides dogs and cats *Leishmania* infections have been reported from a number of mammalian species either in the wild or under captivity as zoo animals. The *Leishmania* species is not clearly defined in some of these case reports. Infections in rodents have been reported for the black rat (*Rattus rattus*) from Italy (Gradoni et al., 1983). Infection was recorded for the maned wolf (*Chrysocyon brachyurus*) (Curi et al., 2006), interestingly with a higher prevalence than the one in domestic dogs living in the same habitat. One report of a fatal *L. infantum* infection was reported for the gray wolf (*Canis lupus*) from Croatia (Beck et al., 2008). In Central Europe the red fox (*Vulpes vulpes*) is abundant. Red foxes infected by *L. infantum* have been reported from Italy (Mancianti et al., 1994; Dipineto et al., 2007) and Spain (Criado-Fornelio et al., 2000; Sobrino et al., 2008). Even so the dog is the predominant host for *L. infantum*, leishmaniosis has been frequently recorded for domestic cats from endemic regions such as Portugal (Maia et al., 2010), Barcelona, Spain (Tabar et al., 2008), Greece (Diakou et al., 2009) or Italy (Poli et al., 2002; Pennisi et al., 2004). Maroli et al. (2007) could even show, that under experimental conditions laboratory reared *P. perniciosus* get infected while blood feeding on a naturally infected cat. For wild felids, infection in the Iberian lynx (*Lynx pardinus*) has been recorded amongst other wild canids including the red fox in Spain (Sobrino et al., 2008). Canine leishmaniosis in wildlife is limited to occasional findings and individual case reports. No prevalence data is available for wild canids or felids within endemic regions. Taking into account that the red fox has been recorded positive with canine leishmaniosis foxes should be added to any surveillance monitoring programs. The same accounts for domestic and feral cats.

Non-Phlebotomine transmission

Within the endemic Mediterranean region *L. infantum* is transmitted by Phlebotomine sand flies. In recent years other ectoparasites have been described harbouring *L. infantum*. Their vectorial role remains controversial. The role of the Brown dog tick (*Rhipicephalus sanguineus*) in transmission of *L. infantum* was recently summarized by Dantas-Torres (2011). Similar reports have been published for South America, for both the Brown dog tick and the cat flea (*Ctenocephalides felis*) were reported to harbour *L. infantum* (Colombo et al., 2011). Mechanical transmission by other blood feeding arthropods like Triatomine bugs in South America (Cavalcante et al., 2003) and e. g. Tabanids has been discussed. Apart from the discussion about other arthropod vectors of *L. infantum*, transmission was confirmed in dogs without any vector involvement. Intra-uterine infection was recorded in Germany (Moritz and Steuber, 1999) and from Italy (Masucci et al., 2003). Under experimental conditions transplacental transmission was recorded for laboratory mice (Rosypal and Lindsay, 2005). In the same study *L. infantum* DNA was detected in both the placenta and the unborn pups, furthermore veneral transmission occurred from infected female BALB/c mice to an uninfected male. In dogs a case report was published for intrauterine infection (Riera and Valladares, 1996; da Silva et al., 2009). Another route of non-vectorial transmission of *L. infantum* from dog to dog is well confirmed for blood transfusion. This non-vectorial transmission was confirmed under experimental conditions by blood transfusion from an infected foxhound to an anaemic dog (Owens et al., 2001). Blood transfusion was recorded a cause of infection under clinical settings (De Freitas et al., 2006). The North American infection of *L. infantum* in foxhounds without clear confirmation of vector involvement is of interest for other non-endemic regions. *L. infantum* was first recorded in foxhound kennels within a region supposedly free of sand flies (Gaskin et al., 2002; Schantz et al., 2005). Following this initial finding, foxhound kennels spread over the eastern states were recorded positive with *L. infantum* (Duprey et al., 2006). A sand fly species was described, while no information is currently available on the vector capacity of *Lutzomyia vexator* (Ostfeld et al., 2004). Gene mapping traced the origin of *L. infantum* to Europe and relationship with *L. infantum* MON-1 zymodeme (Schantz et al., 2005). Several other non-vectorial routes of *L. infantum* transmission have been discussed, such as dog-fighting similar to *Babesia gibsoni* transmission, dogs licking wounds, oral uptake of blood, needle and veneral transmission, while data supporting these hypotheses are lacking.

Conclusion

There is an urgent need for further research on the distribution of sand flies and their vector capacity on *L. infantum* transmission. The increasing numbers of dogs being relocated for welfare reasons from endemic to non-endemic regions and the numbers of dogs travelling with their owners to endemic regions should foster legislatory bodies to make canine leishmaniosis a reportable disease at least for the non-endemic Central European countries. The same was requested for human visceral leishmaniosis (Malik et al., 2006; Stark et al., 2009). Practicing veterinarians in non-endemic regions need to be aware of canine leishmaniosis and thus should advise dog owners on preventative measure. Furthermore while non vectorial transmission may be possible, the transmission in kennels where positive and naïve dogs may be housed together should be avoided. This situation has been well demonstrated as route of infection in North American foxhound kennels. Blood transfusion as another course of infection must require examination of the blood donors, even more so in non-endemic regions with the potential risk of infected dogs without clinical signs being used as donors. Therefore guidelines need to be in place to rule out any infectious agent in donor dogs. The role of wildlife and feral cats needs to be at

least considered a potential threat and survey should be in place to investigate the capacity of esp. red foxes and feral cats to harbour *L. infantum*.

While a positive association between El Niño cycles and the annual incidence of human leishmaniosis has been demonstrated in Brazil, the changing climate pattern in Europe need to be observed with caution and should be linked to sand fly monitoring to predict disease pattern. Even so *L. infantum* is currently not eminent in Germany, climate change predictions may indicate the probability of local establishment. Within Germany, several examples from the past on surveillance systems based on the legislations of reportable diseases hold a proven record as an early warning system. An implemented system including leishmaniosis would allow a timely recording and thus analysis of the distribution of this important emerging disease (Dujardin et al., 2008, Jansen et al., 2009, Ready, 2010). Surveillance on the clinical situation of leishmaniosis in dogs and humans together with distribution pattern of sand fly in the climatic most favourable regions within Germany as e. g. the upper Rhine valley should be initiated. Overall canine leishmaniosis cannot be regarded any more as a disease of the Mediterranean region only, infection with the zoonotic protozoan parasite *L. infantum* is today and most probably more so in the future of importance within the whole of Europe.

Conflict of interest

The author declares herewith no conflict in terms of professional or personal interests with the content of this review paper.

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