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Risk of Infection with *Leishmania* spp. in the Canine Population in the Netherlands

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Teske E, Knapen F van, Beijer EGM, Slappendel RJ: Risk of infection with *leishmania* spp. in the canine population in the Netherlands. Acta vet. scand. 2002, 43, 195-201. – The dog is the main reservoir of *Leishmania infantum,* the causative agent of visceral leishmaniasis (VL) in humans in Southern Europe. In order to identify the risk of dogs from a *Leishmania* non-endemic area traveling to a *Leishmania*-endemic area becoming infected and the risk of transmitting infection to humans in non-endemic areas an investigation was performed, in which the results of a questionnaire were combined with the results of a serologic survey.

The questionnaire was sent to 1478 at random chosen families in the Netherlands. Of the 59.0% responders 28.0% had one or more dogs and 4.8% of these dogs had visited Southern Europe during the summer period of that year. On a total population of 1,200,000 dogs in the Netherlands, this means that each year some 58,000 dogs are at risk of being exposed to a *Leishmania* infection in Southern Europe.

During the period 1990-1992 blood was collected for serology in 1911 dogs presented to the Utrecht University Clinic because of clinical problems not related to leishmaniasis, of which 434 had been in Southern Europe in the foregoing years. None was serologically positive. From these data it can be deduced that the highest chance to obtain leishmaniasis during a vacation in Southern Europe is mathematically less than 1/434 or less than 0.23%. Serology was also performed during the period 1989-1993 in 597 dogs that had been in Southern Europe and were suspected of leishmaniasis. Titers were positive in 145 of these samples. Sixty-four of these dogs were born in the Mediterranean and had been imported into the Netherlands. Excluding these imported dogs, it was calculated that at least 0.027% of the 58,000 dogs yearly taken to Southern Europe during holidays become infected with Leishmania. In order to establish the risk of disease transmission for people in close contact with an infected dog, serum samples of owners and house mates of 37 dogs with leishmaniasis were tested. All 112 sera tested negative. It was concluded that the risk to get leishmaniasis was between 0.027% and 0.23% for the dog when taken to Southern Europe during vacation, and that the risk for owners in non-endemic areas to get leishmaniasis from an infected dog is minimal.

Leishmaniasis; dog; non-endemic; infection risk; questionnaire; serology.

Introduction

Leishmania infantum is the causative agent of visceral leishmaniasis (VL) in humans in Southern Europe. The dog is the main reservoir of this parasite (*Bettini & Gradoni* 1986). Infected dogs may develop VL themselves and are a potential source of contamination to both other dogs and man. Sandflies are essential in the transmission of the disease. The prevalence of leishmania infection in endemic areas in the Mediterranean area varies from one country to another. The prevalence in dogs has been reported to rarely exceed 10%, while in the same

areas contamination of human native inhibitants does not exceed 1%-2% (*Slappendel* 1988). No breed, age or sex predeliction has been reported in Europe. In Northern Europe, where appropriate sandflies are lacking, canine VL is diagnosed almost exclusively in animals that have traveled or resided in *Leishmania*-endemic countries.

Clinical signs of leishmaniasis in the dog may vary and may develop in a period of months to several years after the infection. The most common clinical signs are decreased endurance, weight loss, lymphadenopathy, and skin problems. The skin problems are variable, but usually include hyperkeratosis, presenting as excessive scaling of the epidermis, especially on the nose, around the eyes and at the pinna, and thickening, depigmentation and chapping of the nozzle and the foot pad. Systemic signs may include hemorrhagic diathesis, paraglobulinemia, uremia and anaemia (*Slappendel & Ferrer* 1998).

Many reports of canine leishmaniasis in non-endemic countries have been published (*Bindseil et al.* 1985, *Slappendel* 1988, *Bravo et al.* 1993, *Edelhofer et al.* 1995, *Gothe* 1990, *Gothe et al.* 1997, *Johansson et al.* 1998). Its prevalence in these sandfly-free regions is unknown, but may be of interest to veterinarians, pharmaceutical industries, and policy makers in the sector of public health. It may also be of interest to dog owners and veterinarians who want to know what risk a dog runs to obtain leishmaniasis during a temporary stay in an endemic country. In addition, people residing in non-endemic areas may want information about the possible infectivity of dogs with VL to people and other dogs in the absence of sandflies. In dogs, several cases of autochthonous leishmaniasis have been described in non-endemic countries (*Schawalder* 1977, *Gothe* 1990, *Harris* 1994, *Diaz-Espineira & Slappendel* 1997). Disease transmission from dogs to humans in non-endemic countries has not been described so far.

In order to identify the risk of infection by taking dogs from a non-endemic area to a *Leishmania*-endemic area an investigation was performed, in which the results of a questionnaire were combined with the results of a serologic survey.

Materials and methods

Questionnaire

A questionnaire was sent to 1478 randomized families throughout the Netherlands in November 1989. The families were asked to indicate if they owned any dogs and if so, how many dogs they had. In addition, they were asked if they had taken their dog(s) during their vacation to a *Leishmania* endemic area (*Dereure et al.* 1999) between April and October of that year. A map indicating the countries of concern accompanied the questionnaire.

Serology

Dogs. During the period 1990-1992 blood was collected for serology by venepuncture from dogs that had been referred to the Utrecht University Clinic of Companion Animals (UUCCA) with clinical symptoms not related to leishmaniasis. The owner was also asked if the dog had been in a *Leishmania* endemic area. Samples were also obtained from dogs presented to the UUCCA that were suspected of leishmaniasis, because of clinical signs and a previous stay in Southern Europe with clinical signs possibly related to leishmaniasis. In addition, blood samples were obtained from dogs sent in by practioners to diagnose leishmaniasis during the period 1989-1993, for Utrecht being the only veterinary laboratorium in The Netherlands to determine *Leishmania* titers. Clinical signs included one or more of the following: generalized skin lesions, especially around the eyes and on the dorsal aspect of the nose, ex-

Table 1. Results of DAT serology testing for leishmaniasis in 1911 dogs.

Clinical information	No. of dogs	Positive test	Negative test
No symptoms of VL	1911	1	1910
- visited in endemic area	434	0	434
- not been in endemic area	577	1	576
Suspect of VL been in endemic area	597	145	452

cessive scaling of the epidermis, diffuse hair loss, pronounced weight loss, lymphadenopathy, splenomegaly, and renal failure.

Humans. Owners and other human house mates of dogs with VL residing in the Netherlands were asked to participate on a voluntary base in this investigation. If they agreed, their family doctor collected a blood sample for serology on leishmaniasis. The serology was carried out at the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands, by standard immunofluorescence procedures, using promastigotes of various *Leishmania* species, known as human pathogen (*Harrith et al.* 1989).

Direct agglutination test (DAT). The DAT was performed as described previously (*Harith et al.* 1989, *Oskam et al.* 1996). In short, test serum was serially diluted twofold in V-shaped micro wells (Greiner, Frickenhausen, Germany) with a diluent consisting of NaCl 0.9% (w/v), gelatin 0.2% (Difco Laboratories, Detroit, Michigan) and ß-mercaptoethanol 1.56% (v/v), and subsequently incubated at 37°C for 1 h. Aliquots of freeze-dried leishmania antigen which had been prepared as described by *Meredith et al.* (1995) were freshly reconstituted in 5 ml normal saline (0.9%(w/v) NaCl). Fifty μl of reconstituted antigen was added to each well of the micro well plate containing 50 μl of diluted serum. An 18-20 h incubation period was employed at ambient temperature before the reading of the DAT. The titer was defined as the highest dilution at which agglutination is still visible. This agglutination shows as blue mats or enlarged blue dots in comparison to the compact blue dots present in the negative control wells. The reported sensitivity and specificity of this test is 100% and 98.9%, respectively (*Harith et al.* 1989).

Results

Of the 1478 randomized chosen families in the Netherlands to which a questionnaire had been forwarded, 872 (59.0%) had responded. Of these 872 responders 244 families owned one or more dogs (i.e. 28.0%) including a total of 270 dogs. Thirteen dogs (4.8%) had accompanied their owner to Southern Europe in the foregoing infectious period (April to October) during a shorter or longer vacation. Extrapolated to the 1.2 million dogs living in the Netherlands, this means that yearly approximately 58,000 Dutch dogs visit Southern Europe, hence may be at risk of getting a *Leishmania* infection.

During the period January 1990-January 1993 blood was collected for serology from 1911 dogs presented to the UUCCA which had all kinds of different disorders but were not suspected of VL. Results are presented in Table 1. Only one dog tested *Leishmania* positive, even after repeated testing. This dog had never been in a *Leishmania* endemic area. Of the 1910 *Leishmania* negative dogs 434 dogs had been in South Europe for some time during the 3 years

preceding the blood sampling. From these data it can be deduced that the highest chance to obtain leishmaniasis during a vacation in Southern Europe is mathematically less than 1/434 or less than 0.23%.

During the period January 1989-January 1994 serology was performed in 597 dogs that had visited Southern Europe and showed clinical symptoms possibly related to leishmaniasis. Of these animals, 145 dogs tested *Leishmania* positive. Eighty-one (56%) of these dogs (i.e. approximately 16 dogs per year) had only temporarily resided abroad. The other dogs were born in the Mediterranean and had been imported into the Netherlands. Excluding this latter group of dogs from the risk calculation it can be concluded that at least 16 (0.027%) of the 58,000 dogs yearly visiting southern Europe are infected with *Leishmania.*

Anti-leishmania antibodies were assayed in 112 owners or human house mates of 37 dogs with confirmed leishmaniasis. All tested negative.

Discussion

Little is known about the actual prevalence of canine VL in Northern Europe. Due to the increasing international tourism and migration of people that are accompanied by pets, the number of dogs with VL in non-endemic regions is growing and its occurrence has been reported now in many countries which lack the appropriate sandfly for *Leishmania* (*Bindseil et al.* 1985, *Slappendel* 1988, *Bravo et al.* 1993, *Edelhofer et al.* 1995, *Gothe* 1990, *Gothe et al.* 1997, *Johansson et al.* 1998).

In the present study, some quantitative data have been collected that may be helpful to estimate the infection risk for dogs visiting a *Leishmania* endemic area. A questionnaire was sent to 1478 randomized families of which 58% responded. This percentage is not bad for such a questionnaire, although of course a potential selection bias can never be excluded. According to this questionnaire it was calculated that approximately 58,000 Dutch dogs, i.e. 4.8% of the total dog population in the Netherlands, yearly visit Southern Europe, hence may be at risk of getting a *Leishmania* infection. In a period of 4 years, 81 dogs that had temporarily resided in Southern Europe tested positive with the direct agglutination test (DAT), which is a highly specific and sensitive test to diagnose canine VL (*Harith et al.* 1987, *Harrith et al.* 1989, *Oskam et al.* 1996). By combining this number with the results of the inquiry, it was concluded that VL is caught by at least 0.027% of the 58,000 dogs yearly visiting Southern Europe.

The negative results of the DAT in all of the 434 dogs that had been in an endemic area but visited the UUCCA for clinical symptoms not related to leishmaniasis, leads to the conclusion that chances are mathematically less than 0.23% that a dog visiting Southern Europe gets leishmaniasis. Combining all data, it is concluded that Dutch dogs temporarily visiting Southern Europe have a chance of 0.027% to 0.23% of obtaining canine visceral leishmaniasis. That the odds are more than 0.027% is likely because the diagnosis has doubtlessly been missed in some dogs. However, it is very likely that the number of dogs in which the diagnosis had been missed is relatively small. Almost every dog in which a preliminary diagnosis of leishmaniasis had been confirmed in our clinic by serology or cytology of material sent in by practioners, had subsequently been referred to our clinic for treatment. Moreover, diagnostic tests for canine VL, including cytology and DAT, were almost exclusively performed in our clinic. The calculated maximum of 0.23% seems therefore rational indeed.

The practical significance of these figures is nevertheless limited for the individual dog. The odds that a dog obtains leishmaniasis while accompanying his owner during a vacation in Southern Europe is more subtle than 0.027% to

0.23%. In the present study no differentiation was made between dogs that had been in a region with a high or a low degree of infection, had remained at one location, had traveled around, nor whether they had stayed there for a short or longer period of time. These considerations are important when a veterinarian informs a client about the risk of taking a dog on a vacation abroad. The effect of preventive measures like the use of insecticide impregnant collars may also significantly reduce the infection risk for the individual dog (*Killick-Kendrick et al.* 1997).

Another interesting aspect of canine VL in non-endemic areas concerns its possible transmission from dog to people or from dog to dog in the absence of sandflies. Although direct transmission, probably by the bite of an infected hamster or by accidental contact with an infected injection needle has been described in a laboratory technician (*Terry et al.* 1950), no cases have been documented of humans directly infected by dogs in non-endemic countries. Even in the present study none of the owners and house mates of dogs with VL had a positive *Leishmania* titer. This may probably be ascribed to the low susceptibility of healthy adult people to a *Leishmania* infection in comparison to dogs (*WHO*, 1990). Amastigotes have been detected in saliva and urine of infected patients (*Faust & Russell* 1957, *Hubbert et al.* 1975) and although the uptake of amastigotes by an intact gastrointestinal tract is unlikely, it seems quite possible that they are picked up by the macrophages in a mucosal lesion or bite wound. Mechanical contamination via biting flies has also been documented (*Hubbert et al.* 1975). Even though the gastrointestinal tract of ticks may harbor infectious promastigotes (*Sherlock & Santos* 1964, *McKenzie* 1984), the significance of this finding regarding transmission of the diasease remains questionable as it still has to be seen how these infectious promastigotes should infect the dog. Autochtonous cases of canine VL in non-endemic countries have been described in Switzerland (*Schawalder* 1977), Germany (*Gothe* 1990, *Reusch & Reiter* 1987), England (*Harris* 1994), and the Netherlands (*Slappendel* 1988, *Diaz-Espineira & Slappendel* 1997). In the present study one dog that had never been in a Leishmania endemic country tested repeatedly positive with the DAT. In this dog no parasites could be demonstrated cytologically. As this dog did not develop clinical signs of VL, these test results have to be considered false positive. In conclusion, the risk for dogs on getting infected with *Leishmania* when they are accompanying the owners to endemic areas during vacation is between 0.027% and 0.23%. The risk for owners in non-endemic areas to get leishmaniasis from their infected dog seems to be minimal, although the sample size for detecting transmission to humans in this study was rather small.

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Sammanfattning

Risiko for infektion med Leishmania spp. i den nederlandske hundepopulation.

Leishmania infantum orsakar den viscerala formen av leishmaniasis (VL) hos människor i södra Europa. Hunden är huvudreservoar för parasiten. Risken för hundar som lever i ett icke endemiskt område för Leishmania, att smittas av infekterade hundar vid förflyttning till ett endemiskt område, och därmed även utgöra en infektionsrisk för människorna i området, har undersökt genom att kombinera resultaten från en enkätundersökning med resultaten från serologiska prover.

Enkäten skickades ut till 1478 slumpmässigt utvalda familjer i Nederländerna. Av de 59,0% som besvarade enkäten hade 28% av familjerna en eller flera hundar, och 4,8% av dessa hade besökt södra Europa under sommaren det senaste året. Detta betyder att av en totalpopulation av 1.200.000 hundar i Nederländerna, löper 58.000 hundar risk för att exponeras för infektion av Leishmania i södra Europa.

Under perioden 1990-1992 togs blodprov för serologi från 1.911 hundar som besökte universitetskliniken i

Utrecht på grund av kliniska problem relaterade till leishmaniosis. Fyrahundratrettiofyra av dessa hundar hade besökt södra Europa under de tidigare åren. Ingen av dem var serologiskt positiv. Resultaten tyder på att risken för att infekteras med leishmaniosis under semester i södra Europa är mindre än 0,23%.
Serologiska prover togs under perioden 1989-1993 på 597 hundar som hade bevistat södra Europa och som var misstänkta för att ha leishmaniosis. En positiv titer hittades i 145 av dessa prover, vilket ger en lägsta infektionsfrekvens på 29 hundar per år i Nederländerna. Fyrtiofyra procent av dessa hundar var födda i medelhavsländerna och hade importerats till Nederländerna. Av detta drogs slutsatsen att minst 0,027% av de 58 000 hundar som årligen besökte södra Europa under semestern infekterades med Leishmania.
För att undersöka risken för människor i nära kontakt med en infekterad hund att bli smittade med leismaniosis, togs serumprover från djurägare till samt människor sam varit i nära kontakt med 37 infekterade hundar. Samtliga 112 undersökta prover var serologiskt negativa.
Undersökningen visar att risken för hundar, som besöker södra Europa, att infekteras med leishmaniosis ligger mellan 0,027-0,23 %, samt att risken för hundägare i ett icke-endemiskt område att infekteras med leishmaniosis är minimal.

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