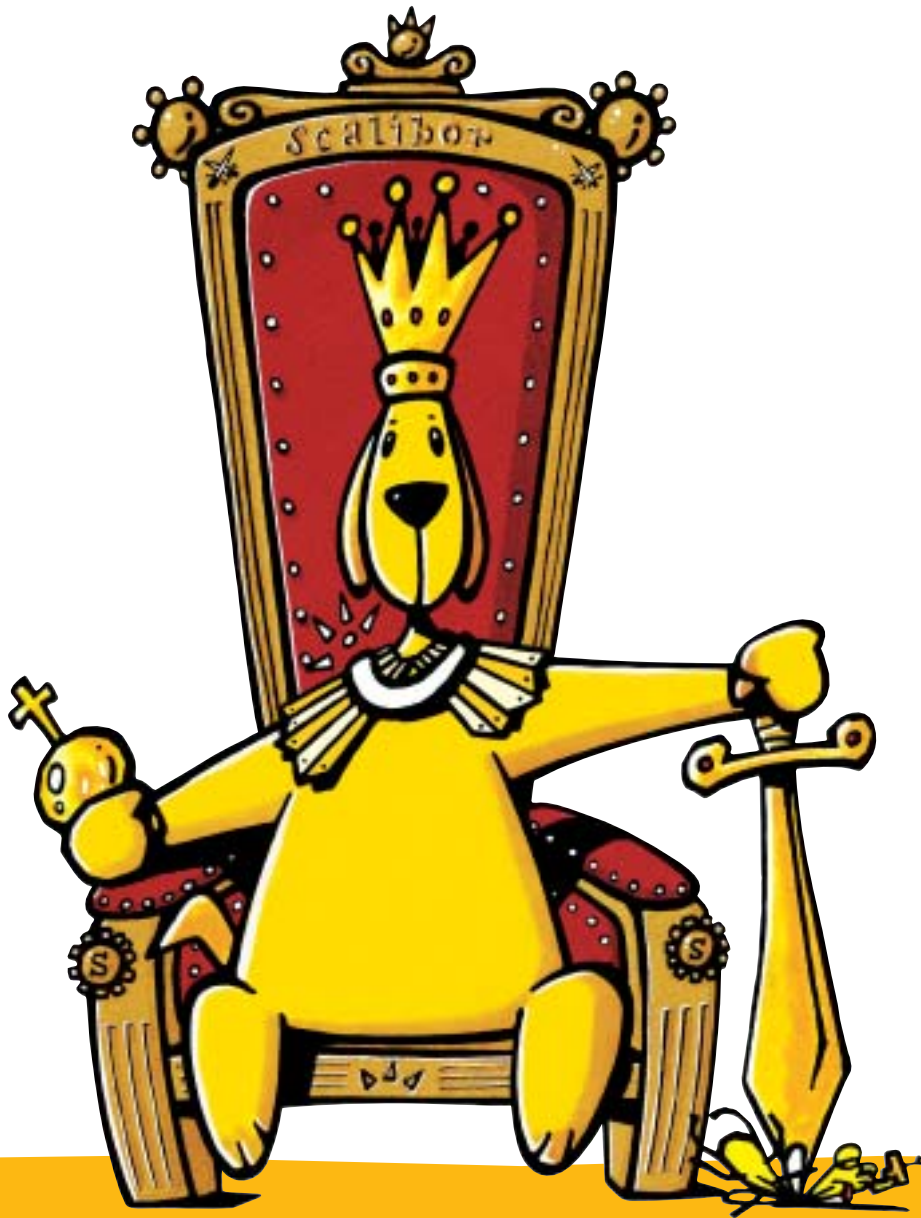




# Summaries of Presentations during the Leishmaniasis Seminar

Worldleish 2, Crete, Greece - 20 to 24 may 2001



# Autochthonous canine leishmaniosis in the united kingdom

Trees, A.J.<sup>1\*</sup>, Howman, P.J.<sup>2</sup>, Bates, P.<sup>1</sup>, Noyes, H.A.<sup>1</sup>, Pratlong, F.<sup>3</sup>, Blakely J.<sup>1</sup>, Niles, J<sup>1</sup>, and Guy, M.W.<sup>1</sup>

<sup>1</sup>Liverpool School of Tropical Medicine and Faculty of Veterinary Science, University of Liverpool; <sup>2</sup>Redside Vets, Kidderminster; <sup>3</sup>Laboratoire de Parasitologie, Université de Montpellier

\*Presenting and corresponding author:

Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, U.K.; trees@liverpool.ac.uk; fax: 44 151 709 3681.

## Introduction

Canine leishmaniosis is transmitted by sandflies of the genera *Phlebotomus* and *Lutzomyia* in the Old World and the New World respectively. There are one or two historic records of non-phlebotomine transmission both between dogs and from dogs to humans but such transmission has appeared to be infrequent. In Britain phlebotomine sandflies do not occur (there is a solitary record from the Channel Islands in 1923) but in 1994 a case of autochthonous transmission was briefly reported (Harris, 1994). This involved a resident dog which shared the same household as one imported from Europe with known leishmaniosis. Subsequently we encountered a case of leishmaniosis in a British dog which had no history of foreign travel. Details of this case, the implications in the light of the Pets Travel Scheme (PETS) and in the context of recent findings of widespread *Leishmania* infection in hounds in the northern USA are presented.

## Case history

A mixed breed, female dog (named 'Susie') was purchased from a rescue centre in Kidderminster near Birmingham, England, in 1991, when approximately one year old. From then onwards she presented with a series of complaints. Chronic vomiting resolved after an inconclusive exploratory laparotomy. Ventral pruritic dermatological lesions, excessive dandruff and crustiness at the tips of both pinnae developed over the next two years. A small interdigital swelling appeared which resolved with treatment, followed by swelling of the eyelids which persisted with remissions due to treatment. Other ocular lesions developed and in 1994 ulcers appeared on all four feet. In 1996 there developed dysphagia and a swollen pharynx, nodules on the head and neck and generalised swellings of the limbs with severe ulceration of one limb.





### **Diagnosis and parasite isolation**

From a nodule biopsy in February 1996 leishmaniosis was diagnosed by a commercial laboratory based on the identification of amastigotes in stained material. Daily 'Glucantime' injections (5ml intramuscularly) were given from 23<sup>rd</sup> March for eight days. Susie's condition continued to deteriorate. On the 19<sup>th</sup> April, Susie was examined at the clinic of the Faculty of Veterinary Science, University of Liverpool but died three days later. Blood examination revealed profound lymphopenia but the CD4 ratio was not depressed. From two nodules removed under local anaesthetic, *Leishmania* parasites were isolated in culture. A variable region of the small subunit ribosomal RNA (SSUrRNA) gene was amplified by PCR and digested with restriction enzymes that generate *Leishmania* specific restriction fragment length polymorphisms - RFLPs (Noyes, Perenz Camps and Chance, 1996). The RFLP profile was consistent with *Leishmania* spp. Isoenzyme characterisation at the Laboratoire de Parasitologie, Montpellier, identified the isolate as *Leishmania infantum* MON-1 zymodeme.

### **Discussion**

*Leishmania infantum* infection was diagnosed on the basis of clinical presentation, histology, parasite isolation, RFLP analysis and isoenzyme characterisation. The dog had had two owners between which it had been in a rescue centre. Both the owners testified that the dog had never been abroad. That being the case, the likely source of infection was at the rescue centre. Transmission must have been effected without phlebotomine sandflies. Although cases of autochthonous transmission in the U.K. appear to be rare, both this case and that reported earlier (Harris, 1994) occurred when quarantine was in place and hence the importation of dogs from endemic leishmaniosis areas was relatively limited. We and other centres in the U.K. encountered a few cases (probably <10) per annum of leishmaniosis in imported dogs in the pre-PETS era. Thus the fact that any autochthonous transmission has been observed is remarkable and actually suggests that transmission without recognised vectors might occur quite frequently, notably if there is close contact between dogs. The recent finding of high prevalences of *Leishmania* infection in hound packs in New York state and the northern USA (see Enserink, 2000) has highlighted this possibility although the means of transmission have yet to be determined. Now that there is much freer movement of dogs from mainland Europe into the U.K. (over 14,000 dogs in the first year of the PETS scheme), the likelihood of autochthonous transmission will undoubtedly rise. Research is needed to determine the transmission mechanisms involved in such cases.

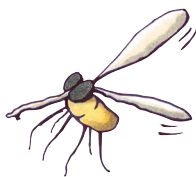


## References

Enserink, M. (2000). *Science*, **290**, 1881.

Harris, M.P. (1994). *Veterinary Record*, **135**, 339.

Noyes, H.A., Perez Camps, A. and Chance, M.L. (1996). *Molecular and Biochemical Parasitology*, **80**, 119.





## Emergence of visceral leishmaniasis in dogs in North America.

Schantz P,<sup>1</sup> Steurer F,<sup>1</sup> Jackson J,<sup>2</sup> Rooney J,<sup>1,3</sup> Akey B,<sup>4</sup> Duprey Z,<sup>1</sup> Breitschwerdt E,<sup>5</sup> Rowton E,<sup>2</sup> Gramiccia M<sup>6</sup>

<sup>1</sup> Division of Parasitic Diseases, NCID, Centers For Disease Control and Prevention, Atlanta, GA; <sup>2</sup> Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; <sup>3</sup> Virginia State Department of Health, Richmond, VA;

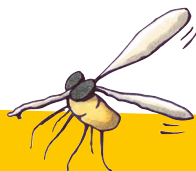
<sup>4</sup> Virginia Department of Agriculture and Consumer Services, Richmond, VA;

<sup>5</sup> College of Veterinary Medicine, North Carolina State University, Raleigh, NC;

<sup>6</sup> Institute of Public Health, Rome (Italy).

### Summary

Beginning in the late summer of 1999, foxhounds at a hunt club in Dutchess Co., NY, developed illness with manifestations that included bleeding, wasting, seizures, hair loss, skin lesions, kidney failure, and swollen limbs and joints; several dogs died. Cytopathologic examination of joint fluid of one of the hounds revealed amastigote forms of *Leishmania* spp. Serodiagnostic testing of foxhounds at the NY kennel revealed a high rate of leishmanial seropositivity (39/93, 42%) and *Leishmania* spp. was isolated from lymph nodes and other tissues of 15 seropositive dogs; the organism was typed at the Institute of Public Health (Rome) as *Leishmania infantum* zymodeme MON1. Tests to demonstrate infection in other breeds of hunting dogs, pet and stray dogs, horses and wild rodents in the vicinity of the affected kennel were negative; however, screening of foxhounds in other states has revealed evidence of more widespread infection. Since March, 2000, sera from more than 10,000 foxhounds and other hunting dogs throughout North America have been tested and positive antibody titers have been detected in 1.8%. At least one seropositive dog was detected in 61 different kennels of foxhounds in 21 U.S. states and 2 Canadian provinces. The organisms isolated from 40 hounds in multiple states and provinces were typed in Rome and determined to be *L. infantum* MON1. The routes of transmission in these dogs are unclear. Phlebotomine species exist in most of these areas, however, vector transmission has not been demonstrated and some epidemiologic characteristics of the infection do not support (a hypothesis of) vector transmission. Serotesting of pet dogs (n=455) and wild canids (n=291), many of them from geographic localities close to (kennels with) infected foxhounds have not revealed evidence of infection. Foxhounds commonly live in close contact with each other and mixing of dogs from hunt clubs in different states is common; therefore, direct dog-to-dog transmission may occur. To date there have been no cases of autochthonous human visceral leishmaniasis reported in the United States.



## **Introduction**

Visceral leishmaniasis is a parasitic infection caused by protozoa of the *Leishmania donovani* species complex and transmitted naturally by the bite of an infected female sand fly. The sand fly becomes infected when taking blood from an infected host, the most important of which are dogs and human beings (WHO, 1990). The infective stages injected by the sand fly are engulfed by host white blood cells (macrophages) and multiply throughout the body causing enlargement and dysfunction of many organs. The classical form of visceral leishmaniasis in humans is known as "kala azar", and is severe and usually fatal if untreated. An estimated 500,000 new cases occur each year in parts of the world where transmission commonly occurs; ninety percent of cases of visceral leishmaniasis in the world are diagnosed in Bangladesh, India, Nepal, Sudan and Brazil (WHO, 1990); however, in this past decade an epidemic of visceral leishmaniasis has occurred in southern Europe mainly among injecting drug users who are also infected with HIV (WHO, 1997). Visceral leishmaniasis is rare in the United States; it is occasionally diagnosed in persons and animals returning from other countries where the disease is endemic.

## **Recognition of the Outbreak**

Beginning in the late summer of 1999, foxhounds at a hunt club in Dutchess Co., NY (Hunt Club A), developed illness with manifestations that included bleeding, wasting, seizures, hair loss, skin lesions, kidney failure, and swollen limbs and joints; there were several deaths. Cytopathologic examination of joint fluid of one of the hounds revealed amastigote forms of *Leishmania* spp. This was confirmed at autopsy of several dogs, and organisms were isolated and grown in culture. Serum and EDTA anticoagulated blood was collected from 93 American or Crossbred Foxhounds and analyzed for antibodies to *L. donovani* antigens by indirect immunofluorescence assay (IIF) (Kagan, 1980) and molecular evidence (PCR) of infection to visceral leishmaniasis. Initial antibody testing revealed an overall seroreactivity (IIF titer  $\geq 1:64$ ) of 47.3% (44/93). Infection was corroborated with PCR and/or by histopathology in 65% of 40 hounds examined by both methods (Gaskin et al., in press). Antibody assay and PCR were then performed on samples from the investigated kennel every two months for the next six months. An additional 11 foxhounds were determined to be infected suggesting continued transmission, however, the lengthy incubation period of visceral leishmaniasis confounds ascertainment of the precise time point of infection. Aspiration or biopsy of lymph nodes and other tissues of 15 seropositive dogs resulted in isolation of *Leishmania* spp. from all 15. Sera from 7 horses resident at Hunt Club A were drawn on March 28; testing of these sera for antibodies by IIF were negative as were the results of PCR for DNA (PCR performed at WRAIR). Rodents (3 white-footed deer mice) trapped on the premises of the Hunt Club A (March 28-29) were negative when examined for evidence of leishmania by PCR, culture and histopathologic studies. Serum specimens were drawn on March 31 from 6 persons employed at the Hunt club who had frequent and close con-



tact with the foxhounds; these sera were negative for leishmania antibodies in the IIF. Serologic testing to demonstrate infection in hunting dogs in two neighboring hunt clubs in Dutchess County, NY, were negative; these included 29 beagles that had been kenneled together with the infected foxhound pack during several months of each of the 2 previous years prior to the recognition of the outbreak in Hunt Club A and 63 foxhounds kenneled approximately 7.5 Km distant from Hunt Club A.. Serologic testing of stray and pet dogs (n=67) surrendered to a local Dutchess Co. animal shelter between May and October, 2000 failed to identify other dogs seropositive for leishmania.

It was not known how and when this infection was introduced into the Hunt Club A foxhounds nor how it was being transmitted within the pack. There were no previous reports of autochthonous leishmaniasis in dogs or other animals or humans in NY state. As is common practice among foxhound hunters, the Hunt Club A foxhounds had been transported to other states for training and competition, including, in recent years, Virginia, Georgia, Alabama and Pennsylvania. During these sojourns hounds from different clubs from different states have extensive contact with other hounds and areas recently occupied by other hounds during competition and rest. Beginning in April 2000, serologic testing for leishmaniasis was extended to foxhounds, other breeds of dogs and wild canids in other U.S. states and Canadian provinces to determine the possible occurrence of leishmaniasis in these hosts. The objectives of the investigation included 1) measuring the seroprevalence and distribution of visceral leishmaniasis in dogs and wild canids in North America, 2) determining the mode (s) of transmission and spread and 3) assessing the possible risk to humans.

## Results of Continuing Investigation

**Serologic screening of hunting dogs:** Serologic screening of foxhounds and other hunting dogs in numerous U.S. states and Canada revealed evidence of more widespread infection. During 12 months through March, 2001, sera from 10,531 dogs from 201 hunt club kennels in 35 states and Canada (excluding those at Hunt Club A in NY) had been tested by IIF and 152 (1.8%) reacted at high titers ( $\geq 1:64$ ) usually associated with active infection (Table 1). One or more dogs seroreactive at these titers were detected in 69 kennels in 21 states and 2 Canadian provinces (Fig. 1).

**Isolation and characterization of infective organism:** Tissue specimens collected by biopsy or at autopsy of seropositive dogs in 6 U.S. states and Ontario Province (Canada) placed in NNN medium (Evans, 1987; Jackson et al., 1989) yielded growth of *Leishmania* promastigotes (Table 2). These included an isolate from a foxhound in Oklahoma obtained in 1980 (Jackson and Fox, 1981). Most of the culture positive dogs have had IIF titers in the higher range



( $\geq 1:256$ ) but two of the culture-positive hounds had titers as low as 1:32. All of the isolates were typed as *L. donovani* sensu lato by the isoenzyme procedure of Kreutzer and others (1980, 1987). Samples of the organisms were sent to an international reference laboratory in Rome, Italy, for characterization by the methods of Gramiccia and others (1989; 1992). Isolates from 30 foxhounds were determined to be *Leishmania infantum* zymodeme MON1, the most common strain of the *L. donovani* complex isolated from human patients with visceral leishmaniasis in countries of the Mediterranean region (Table 2); results on other isolates are pending.

**Serologic screening of pet dogs:** Sera from 455 dogs not associated with the foxhounds have all tested negative (IIF titers  $< 1:16$ ) (Table 1). This canine sample included 70 different breeds from 40 states submitted to CDC since 1997 for testing to meet requirements for entry to foreign countries requiring such testing.

**Serologic screening of wild canids:** Serum specimens of 291 wild canids were negative for leishmanial antibodies (IIF titers  $< 1:16$ ) (Table 1). These included 130 canids native to southern states (FL, GA, SC, NC, TN, TX, VA) and 161 from central or western states (IL, IN, OH, WY).

## Discussion

While visceral leishmaniasis caused by protozoal agents of the *L. donovani* species complex is well-established in parts of Asia, Africa, and southern Europe, and Central and South America (WHO, 1990), it has not been reported with regularity in humans or other animals in North America. Most cases of visceral leishmaniasis in North America have been diagnosed in canine or human patients previously resident in or which had traveled to countries in which the infection is known to be enzootic (Huss & Ettinger, 1992; Bravo et al., 1993; Lester & Kenyon, 1996). However, there have been sporadic reports of canine visceral leishmaniasis in North America in which there was no history of foreign travel, including a pet Basenji in Texas (Sellon et al., 1993), an English Foxhound maintained in a closed research colony at the Ohio State University (Swenson et al., 1988), an American Foxhound from a kennel in Oklahoma (Anderson et al., 1980), and a toy poodle kept as a house pet in Maryland (Eddlestone, 2000). In these reports, the infected dogs had not traveled outside of the United States, and neither a source of infection nor an insect vector were ever identified.

The parasite infecting dogs in North America is *Leishmania infantum*, a species widely disseminated in the Mediterranean countries where it is enzootic in wild and domestic canines (Dereure et al., 1999). In these regions the parasite is an important cause of morbidity and, more rarely, mortality in



dogs and humans to which it is commonly transmitted by sand flies of the family Phlebotomidae (Killick-Kendrick, 1990). This species of *Leishmania* was not previously believed to be "enzootic/endemic", i.e., transmitted under natural conditions in nature, in North America. However, infection by this organism has been imported repeatedly into the United States in pet dogs returned with families of U.S. citizens, including military personnel stationed temporarily in bases in foreign locations within known *Leishmania infantum*-endemic areas, e.g., Italy and Spain. The clinical and epidemiologic findings reported here document that visceral leishmaniasis is established in hunting foxhounds in a vast region of eastern North America extending from Florida state northward to Ontario province, Canada, and from the Eastern coastal regions to Kansas and Oklahoma in the West. (Fig. 1). *Leishmania* spp. isolated from individual foxhounds from multiple points throughout this broad geographic range were identified by isoenzyme analysis as *L. infantum* zymodeme MON1 (Table 2) suggesting that the common origin of the infective agent was the Mediterranean region (Gramiccia et al., 1989; 1992). The finding that the same strain of the protozoan was present in a foxhound from Oklahoma in the early 1980s (Jackson & Fox, 1981) suggests that the infection has been enzootic in foxhounds for at least 20 years and that it has been spread widely in North America since that time.

The routes of transmission among these dogs remain unclear. Sand fly (Phlebotomine) species closely related to those known to be capable of transmitting *Leishmania* spp. infections exist in most of these areas (Young & Perkins, 1984), however, vector transmission has not been demonstrated. Four species of *Lutzomyia* in the US are known to be mammalian feeders. Of these *Lu. anthorpha* is rarely anthropophilic, but plays a major role in transmission of *L. mexicana* among rodent reservoir hosts and may be responsible for occasional transmission to man along with the more anthropophilic species *Lu. diabolica*. These species have only been reported in Texas, a state in which our survey did not detect seropositive foxhounds. *Lu. cruciata* has been collected around the eastern Georgia-Florida border but appears to be highly autogenous. Females that fed on a human volunteer died without developing mature eggs (Young and Perkins, 1984). *Lu. shannoni* is widespread in the eastern U.S. ranging from Louisiana and Missouri to the Atlantic Ocean and up along the coast and plains regions as far as New Jersey. Extensive surveys for sand flies have not been done in the Mid- and North-Atlantic states except in North Carolina where *Lu. shannoni* was collected in CDC light traps from the Atlantic coast to the eastern edge of the Appalachian Mountains (Harrison B, personal communication). The range of *Lu. shannoni* overlaps the locations of many of the hunt clubs that have seropositive dogs. The vector capacity of this sand fly has not been confirmed, however, anterior stage infections developed after a group of these sand flies fed on a dog infected with *L. infantum* (Lawyer P, personal commu-

nication). If *Lu. shannoni* is capable of transmitting *L. infantum* it may play a role in transmission of this pathogen from dog to dog, to feral animals and, possibly, to man.

Some epidemiologic findings do not support the assumption that the infection is being transmitted by insect vectors. Vector transmission would be expected to result in exposure of dogs of many breeds as well as other susceptible host species. However, serodiagnostic testing of beagles that had been kenneled together with the foxhounds of Hunt Club A in NY state for several summers prior to and including the summer of recognition of the extensive occurrence of visceral leishmaniasis in the foxhounds indicated that the beagles remained free of the infection. Similarly, serum specimens collected from 63 foxhounds kenneled within 10 km. of Hunt Club A were all negative for antibodies to leishmaniasis as were sera of 67 pet and stray dogs collected from multiple locations of Dutchess Co., N.Y. In other states, serologic testing of pet dogs (n=455) and wild canids (n=291), many of them from geographic localities close to infected foxhounds, have not revealed evidence of infection in these potentially susceptible animals (Table 1). Although surveys of potential reservoirs of leishmaniasis other than foxhounds are still limited and incomplete, the accumulating evidence suggests that enzootic visceral leishmaniasis in North America is largely limited to foxhounds.

Preliminary observations suggest that direct (dog-to-dog) transmission via exchange of blood and secretions may be occurring. Direct transmission has been rarely documented, however, it may occur relatively commonly although unrecognized because of the difficulty of distinguishing it from transmission by vectors. Transplacental transmission or direct contact during coitus and parturition can result in transmission between breeding pairs and to offspring (Nuwayri-Salti & Khansa, 1985; Eltoun et al., 1992; Mancianti & Sozzi, 1995). A number of experimental studies have demonstrated that direct transmission of *Leishmania* sp. from an infected host to another is possible, by various means of direct contact (Melby, 1991). The abundant presence of amastigotes in macrophages in the superficial cutaneous lesions of infected dogs has been often noted and an infected dog may be more likely than other hosts to predispose to direct exchange of infective organisms. Foxhounds commonly live in close contact with each other and can be aggressive in play and competition. Mixing and exchanging (drafting) of hounds from hunt clubs in different states is common; therefore, practices common to the sport provide opportunities for transport of infection between states and even from the U.S. to Canada are standard practice.

It is also possible that foxhounds have unique susceptibility to the infection and/or practice subtle behavior that maximizes the possibility of exchange of blood and secretions with other foxhounds. In the few months since recognition of the presence of infection in foxhounds we have become aware of at

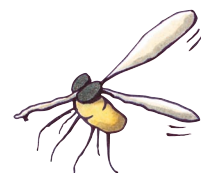




least 3 separate instances of presumptive direct dog-to-dog transmission in the U.S. involving pet dogs imported from endemic areas of Europe and transmitting the infection to other dogs with which they were housed in the U.S. Instances of direct dog-to-dog transmission have been documented in northern Europe (Slappendel & Teske, 1999). Understanding the primary modes of transmission is essential for controlling the disease in foxhounds. The different possible modes of transmission have important implications also for further intra- and extra-species transmission, including the possible risk of human exposure.

To date there have been no documented autochthonous cases of visceral leishmaniasis in humans in the United States. Although direct transmission from an infected dog to humans has never been reported, it is speculated to be possible, and immunocompromised persons would be theoretically at greatest risk. Serologic screening and medical evaluations of humans considered to be "at risk" for this infection, i.e., kennel workers and foxhound hunters is currently in progress.

A recent U.S. Senate report entitled "Expect the Unexpected..." described the specter of emerging diseases in the 21st century and the difficulties of recognizing and adequately responding to such challenges. This title is an apt description of the ongoing investigation of visceral leishmaniasis in dogs in North America. Indeed public health scientists did not anticipate the widespread occurrence and active transmission of this infection. The introduction of this infection into North America from European or other potential source countries reveals once again our nation's vulnerability to introduction of exotic infections introduced by incoming pet, livestock or wildlife animals crossing our borders. It has become apparent that there are continuing logistical problems in responding to outbreaks of infectious diseases in animals that have potential, but unconfirmed, dangers for humans. Communicating surveillance data and coordinating prevention responses to outbreaks in animals, especially of newly recognized infections that do not appear to affect humans, may not occur rapidly enough to facilitate adequate quarantine and control of transmission. The administrative and funding mechanisms necessary to launch a timely and adequately coordinated response are seriously challenged, especially when the disease is found in an animal that has no agricultural importance and when preliminary findings indicate no evidence of transmission to humans. To date this infection appears limited to foxhounds and possibly other hunting dogs associated with foxhounds. There is no current evidence of vector transmission which may account for its apparent confinement to hunting dogs of the foxhound breed. Nevertheless, sand flies closely related to those known to be competent vectors of *Leishmania* spp. do occur within the geographic areas where these infected dogs are kept and it would seem plausible that the parasite may ultimately adapt to trans-



mission by these potential vectors. Should this occur this potentially zoonotic infection would certainly extend its geographic and host range and become a public health problem. The identification of visceral leishmaniasis as an introduced infection of potentially serious public health significance should serve as the catalyst for enhancement of our nation's preparedness for responding to such threats.

**Acknowledgements:** Many individuals have provided information, materials or other assistance useful to this investigation and they are gratefully acknowledged. They include: Mr. Dennis Foster, Masters of Foxhounds Assoc., Leesburg, VA; Mr. Spencer Marks, Dutchess County Dept. of Health, Poughkeepsie, NY; Dr. Susan Trock, NY State Dept of Agriculture and Markets, Albany; Dr. Millicent Eidsen, NY Department of Health, Albany; Dr. Victor Nettles, SE Cooperative Wildlife Diseases Project, U Georgia, Athens, GA; MAJ Glenn J. Wortmann, WRAIR;

## References

Anderson DC, Buckner RG, Glenn BL, MacVean DW (1980) Endemic Canine Leishmaniasis. *Vet Path* 17: 94-96

Anon. (1997) Difficult dermatologic diagnosis. *JAVMA* 210:181-2.

Bravo FLA & Brenneman KA. (1993) Canine leishmaniasis in the United States. *Comp Cont Educ Pract Vet* 15: 699-708)

Dereure J, Pratlong F & Dedet J-P. (1999) geographical distribution and the identification of parasites causing canine leishmaniasis in the Mediterranean Basin. *Canine Leishmaniasis: An Update. Proc Inter Can Leish Forum, Barcelona*, pp. 18-25.

Eddlestone SM.(2000). Visceral leishmaniasis in a dog from Maryland. *JAVMA* 217:1686-88.

Evans DA. *Leishmania*. In: Taylor AER, Baker JR eds. *Methods of Cultivating Parasites in vitro*. Orlando: Academic Press, 1987:52-75.

Gaskin AA, Schantz P, Jackson J, Birkenheuer A, Gramiccia M, Tomlinson L, Levy M, Steurer F, Kollmar E, Hegarty BC, Breitschwerdt EB. Visceral leishmaniasis in a New York Foxhound kennel. *J Vet Int Med* (submitted)

Gramiccia M., Gradoni L & Angelici M.C. (1989). Epidemiology of Mediterranean leishmaniasis by *Leishmania infantum*: isoenzyme and kDNA analysis



for the identification of parasites from man, vectors, and reservoirs. In: Leishmaniasis. The current status and new strategies for control (Hart DJ. ed.). Plenum Press, NewYork, NATO ASI Series, Ser. A, Vol. 163, pp. 21-37.

Gramiccia M., Gradoni L & Troiani M. (1992). HIV-Leishmania co-infections in Italy: isoenzyme characterization of Leishmania causing visceral leishmaniasis in HIV patients. Trans.R.Soc.Trop. Med.Hyg., 86:161-163.

Huss BT, Ettinger SJ (1992) Visceral Leishmaniasis, Rocky Mountain Spotted Fever and von Willebrand's Disease in a Giant Schnauzer. J Amer Anim Hosp Assoc 29: 221-225.

Jackson, J.E., and Fox JC (1981). Rapid identification of a *Leishmania* sp. from the U.S.A. and preliminary drug sensitivity screening using radiorespirometry. Proc. **International Symposium on Nuclear Techniques in the Study and Control of Parasitic Diseases in Man and Animals**. Pp. ? Vienna, IAEA-AM

Jackson JE, Talley JD, Tang DB. (1989). An *in vitro* micromethod for drug sensitivity testing of *Leishmania* . Am J Trop Med Hug 41:318-30.

Kagan IG (1980) Serodiagnosis of Parasitic Diseases, pp. 724-750. In: E.H. Lennett, A. Balows, W.J. Hausler & J.P. Truant (Eds), Man Clin Microbiol, 3<sup>rd</sup> Ed., Am Soc Microbiol, Washington D.C.

Killick-Kendrick R. (1990). Phlebotomine vectors of the leishmaniasis: a review. Med Vet Ent 4:1-24.

Kreutzer RD, Christensen HA (1980) Characterization of *Leishmania* spp. by isozyme electrophoresis. Am J Trop Med Hyg 29:199-208.

Kreutzer RD, Souraty N, Semko ME. (1987) Biochemical identities and differences among Leishmania species and subspecies. Am J Trop Med Hyg 36:22-32.

Lester SJ, Kenyon JE (1996) Use of allopurinol to treat visceral leishmaniasis in a dog. J Am Vet Med Assoc 209:615-617.

Mancianti F, Sozzi S. (1995) Isolation of *Leishmania* from a newborn puppy. Trans R Soc Trop Med Hyg 89:402.

Mckenzie K. A study of the transmission of canine leishmaniasis by the tick, *Rhipicephalus sanguineus*, and an ultrastructural comparison of the promastigote. Stillwater, OK: Oklahoma State University, 1984.

Schaer M, Meyer DJ, Young DG. (1985) A dual infection of *Leishmania* and *Ehrlichia canis* in a dog. *Comp Cont Educ Pract Vet* 7:531-534.

Sellon RK, Menard MM, Meuten DJ, Lengerich EJ, Steurer FJ, Breitschwerdt EB (1993) Endemic visceral leishmaniasis in a dog from Texas. *J Vet Int Med* 7:16-19.

Slappendel RJ, Teske E. A review of canine leishmaniasis presenting outside endemic areas. *Canine Leishmaniasis: An Update. Proc Inter Can Leish Forum* 1999;54-59.

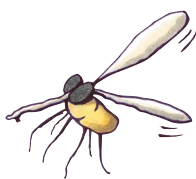
Swenson CL, Silverman J, Stromberg PC, et al.(1988) Visceral leishmaniasis in an English Foxhound from an Ohio research colony. *JAVMA* 193: 1089-1092.

WHO (1990). Control of the Leishmaniasis. Report of a WHO Expert Committee. Geneva:World Health Organization, Technical Report Series, no. 793.

WHO (1997). Leishmania/HIV co-infection. *Weekly Epidemiologic Record* 72:49-54.

Yamaguchi RA, French TW, Simpson CF (1983) *Leishmani donovani* in the synovial fluid of a dog with visceral leishmaniasis. *J Am Anim Hosp Assoc* 1983;19.

Young DG and PV Perkins. 1984. Phlebotomine Sand Flies of North America (Diptera: Psychodidae) *J. Am. Mosq. Cont. Assoc.* 44: 261-304.





**Table 1.**  
**Distribution of serum antibody titers<sup>1</sup> for *Leishmania donovani* antigens in hunting dogs, pet dogs and wild canids, North America, 2000.**

**Number reactive at serum dilutions.**

| Population tested                   | Titers       | <1:16 | >1:16 | >1:32 | >1:64 | >1:128 | >1:256 | >1:512 |
|-------------------------------------|--------------|-------|-------|-------|-------|--------|--------|--------|
| Hunting dogs<br>n = 10,531          | No. sera     | 9159  | 1372  | 566   | 181   | 121    | 71     | 45     |
|                                     | cumulative % | 87.0  | 13.0  | 5.4   | 1.7   | 1.2    | 0.7    | 0.4    |
| Pet dogs <sup>2</sup><br>n = 455    | No. sera     | 455   | 0     | 0     | 0     | 0      | 0      | 0      |
| Wild canids <sup>3</sup><br>n = 291 | No. sera     | 291   | 0     | 0     | 0     | 0      | 0      | 0      |

1. Indirect immunofluorescence assay.
2. Includes 70 different breeds of dogs from 40 states.
3. Includes 130 foxes and coyotes native to southern states (FL, GA, SC, NC, TN, TX, VA) and 161 translocated from central or western states (IL, IN, OH, WY).

**Table 2. Isolation and classification of *Leishmania* spp. from Foxhounds in North America, 1980-2000**

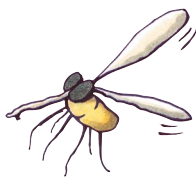
| Premise <sup>1</sup> | State         | Year | No. of isolates <sup>2</sup> | <i>Leishmania donovani</i> complex <sup>3</sup> | <i>Leishmania infantum</i> MON1 <sup>4</sup> |
|----------------------|---------------|------|------------------------------|---|--|
| Kennel 1             | Oklahoma      | 1980 | 1                            | 1   | 1  |
| Kennel 2             | New York      | 2000 | 15                           | 15  | 15   |
| Kennel 3             | Virginia      | 2000 | 3                            | 3   | 3  |
| Kennel 4             | Virginia      | 2000 | 3                            | 3   | 3  |
| Kennel 5             | Virginia      | 2000 | 1                            | 1   | 1  |
| Kennel 6             | Kentucky      | 2000 | 1                            | 1   | 1  |
| Kennel 7             | Michigan      | 2000 | 1                            | 1   | 1  |
| Kennel 8             | Ontario (CAN) | 2000 | 5                            | 5   | 5  |
| Kennel 9             | Maryland      | 2000 | 1                            | 1   | 1  |
| Kennel 10            | Michigan      | 2000 | 1                            | 1   | 1  |
| Kennel 11            | Ontario (CAN) | 2000 | 3                            | 3   | 3  |

<sup>1</sup> Each premise represents a kennel housing foxhounds owned by a fox hunting organization.

<sup>2</sup> Each isolate from a distinct foxhound host.

<sup>3</sup> Isolate classified as *Leishmania* of the donovani complex by alloenzyme analysis (Kreutzer et al., 1980; 1987).

<sup>4</sup> Isolate classified as *L. infantum* MON1 according to the methods of Gramiccia et al (1989;1992).





# The role of foxes (carnivora: canidae) in the maintenance and transmission of *Leishmania infantum*: implications for peridomestic control.

Courtenay O.\*, Quinnell R. J. <sup>1</sup>, Dye C. <sup>2</sup>

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT.

## Current addresses:

\*Ecology and Epidemiology Group, Department of Biological Sciences, University of Warwick, Coventry, CV4 7A, UK. <sup>1</sup> School of Biology, University of Leeds, Leeds, LS2 9JT, U.K. <sup>2</sup> Communicable Diseases Control, Prevention and Eradication, World Health Organisation, Geneva, Switzerland.

\* Contact: orin.courtenay@warwick.ac.uk

## Introduction

In search of effective strategies to control human infection with *Leishmania infantum* (= *L. chagasi*), it is prudent to quantify the contribution of wildlife hosts to the dog-sandfly-human transmission cycle. Additional hosts could amplify transmission rates, or (re)-introduce infection into parasite free areas. Given either scenario, intervention targeted at only peridomestic reservoirs (e.g. dogs) might be under-estimating the true size of the control problem.

The principal mammalian hosts of *L. infantum* are members of the Carnivora: Canidae (foxes, jackals, wolves), including the crab-eating fox, *Cerdocyon thous*, in Latin America, the red fox, *Vulpes vulpes* in western Europe, and the golden jackal, *Canis aureus*, and *V. vulpes* in the Middle East, all of which have been implicated as additional sources for human infection. Their incrimination is based on isolation of the parasite, or demonstration of anti-*Leishmania* antibodies. Indeed, high infection prevalences are not uncommon (**Table 1**). However, estimates of the transmission potential of these hosts have not been available with which to confirm or reject these propositions. Unlike dogs, for example, the disease in foxes is seemingly rare, reported in only 3/26 (11.5%) crab-eating foxes, and 2/30 (7%) red foxes, with confirmed infections (**Table 1**). This suggests that dogs and foxes may not in fact be equally competent reservoirs.

Until now, only two xenodiagnosis studies had been performed on wild canids, both of which confirmed the ability of crab-eating foxes to infect *Lu. longipalpis* (the sandfly vector) (Deane and Deane, 1954; Lainson *et al*, 1990; Courtenay *et al*, 1996). But with a total sample size of two, those studies





failed to provide sufficient information to quantify their contribution to infection. The data presented here seeks to do just this, on route answering two fundamental questions (i) what is the extent of infectiousness in a naturally infected wildlife host population, and (ii) can the population maintain a transmission cycle independent of infectious domestic dogs.

### **The Marajó Project**

Infection, clinical outcome, and infectiousness to *Lu. longipalpis* (the sandfly vector) was monitored over 24 months in a free-ranging cohort population of 37 crab-eating foxes in rural Marajó, Pará (48°03'W, 00°46'S), located in endemic Brazil. Using a proven system of live (re)-capture, foxes were bled, clinically examined and bone marrow biopsies obtained on 1-4 occasions per fox at an average interval of 4.3 months. Infectiousness was measured by xenodiagnosis where foxes were exposed to laboratory-bred *Lu. longipalpis* in 44 trials (1-3 times each) (Courtenay, 1998). The spatial movements of the fox population was also monitored by radio-telemetry, and contact rates with both peridomestic and sylvatic habitats quantified (Macdonald & Courtenay, 1996; Courtenay, 1998; Courtenay *et al*, 1994; *in press*). To compare the courses of fox and dog infection and infectiousness, similar longitudinal data were collected for a cohort of 126 sentinel dogs (Quinnell *et al*, 1997), and the serological status of resident dogs examined annually (Courtenay *et al*, 1994; Courtenay, 1998).

### **Results**

**Infection and disease.**

By the end of the study, 78% of the 37 foxes presented ELISA antibody titres of 3.35 log units (the intrinsic cut-off titre for dogs), and 38% of 21 foxes were parasite positive by isolation from inoculated culture slopes or hamsters. The proportion of parasite positives in the population increased with age with an asymptote at approximately 30% around 15 months. Seroprevalence profiles based on a number of fitting procedures asymptoted at or near unity at the same age. None of the longitudinal antibody titres declined significantly to indicate serorecovery, and all foxes were apparently healthy. Spatial analysis revealed no differences in movement patterns between paired seropositive and seronegative foxes to indicate debilitating disease of a more chronic nature (Courtenay *et al*, 1994).

### **Infectiousness**

Xenodiagnosis was performed on 26 foxes in 44 trials. This included 20 (80%) of the seropositives (titres  $\geq 3.35$ ), and 5 of 8 parasite positive animals. Of the total 1,469 sandflies dissected from all trials, none were found to be infected.

### **Spatial analysis**

Spatial analysis revealed that Marajó foxes live at low densities of 0.55 fox per km<sup>2</sup> (range: 0.27-0.77), and in sylvatic habitats (savanna and scrub) largely hostile to *Lu. longipalpis* (Macdonald & Courtenay, 1996; Lainson *et al*, 1990). Ninety-two percent of 24 radio-tagged foxes spent an average 38 minutes (0-242 minutes) of their nightly nocturnal activity period in 1-3 endemic villages, where 46% of foxes initiated 80% of all household contacts (Courtenay *et al*, in press). Here they were shown to predate domestic fowl from animal huts known to harbour large numbers of domiciliary *Lu. longipalpis*. All fox and dog isolates were identified as *L. infantum* MON-1 suggesting a common cycle of infection. Although foxes experienced similar seroprevalence rates to sympatric village dogs, we did not detect an association between fox infection and either village contact rates, or vegetation type favoured or disfavoured by *Lu. longipalpis*.

### **Comparison with dogs**

Foxes also experienced similar courses of infection to the sentinel dogs (Figure a and b). In contrast to foxes, 45% (18/40) of these dogs proved infectious to a mean 28% (95% CL. 20-36%) of sandflies; asymptomatic and symptomatic dogs were equally infectious. Dog infectiousness increased with log antibody titre: taken as an expected level of infectiousness for a given antibody titre, foxes were clearly less infectious by comparison (**Figure b**).

### **Species-specific contribution to transmission**

The basic reproductive number  $R_0$ , is an index of transmission and size of the control problem.  $R_0 > 1$  represents the condition for endemic persistence, or more specifically, for an independent single-host (e.g. fox-sandfly-fox) transmission cycle. Applying our empirical parameter estimates to a multi-host transmission model (Rogers, 1988) we can partition the contributions of foxes and dogs to  $R_0$ . In Marajó, we estimated  $R_0 = 8$  (Quinnell *et al*, 1997). We simulate the worst case scenario for foxes by making them equal to dogs in all quantities of the infection process except for infectiousness, thus rendering the test highly conservative. The exercise uses the observed mean infectiousness of dogs  $C_{dog} = 0.28$ , and the upper 95% CL. of mean infectiousness of foxes  $C_{fox}$ , calculated as  $C_{fox} = -\ln(0.05)/N = 0.002$ . Using these values, we find the proportion of  $R_0$  due to foxes is  $R_{0fox} = 0.007$ , and the contribution of dogs is  $R_{0dog} = 1 - 0.007 = 0.993$ . Given the observed value of  $R_0$ , the critical value of mean fox infectiousness  $C_{crit}$ , for foxes to maintain a transmission cycle independent of dogs is  $C_{crit} = C_{dog}/(R_0 - 1) = 0.04$ . This represents a 20 fold increase of the observed value of  $C_{fox}$ . From a different view point, the value of  $R_0$  at which foxes could maintain an independent transmission cycle given their observed level of infectiousness, is  $R_{0crit} = 1/R_{0fox} = 143$ . This value is an order of magnitude greater than empirical estimates of  $R_0$  for Marajó, or elsewhere.



### **Conclusions**

The collective results indicate that despite high infection rates, the crab-eating fox is substantially less infectious than dogs. Unlike dogs, foxes live at very low densities which is not expected to facilitate intra-specific endemic persistence. Further support for this view comes from mathematical simulation which shows that even given the most conservative scenario, foxes are unlikely to maintain a transmission cycle independent of infectious dogs. Consequently, it is village habitats where sandfly and infectious dog densities are greatest where *L. infantum* is likely to spill-over into foxes, not *visa versa*. We predict that controlling peridomestic transmission will reduce infection rates in foxes.

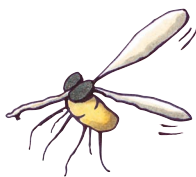
### **Acknowledgements**

All the work conducted here was supported by the Wellcome Trust.

| number parasite positive /N (seropositive/N)  | number of symptomatics / N positives | region/country                 | source  |
|---|--------------------------------------|--------------------------------|---|
| <b>Crab-eating fox <i>Cerdocyon thous</i></b> |                                      |                                |   |
| 11/26 (13/25)                                 | 0/11 (0/13)                          | Marajó, Pará, Brazil           | Silveira <i>et al.</i> , 1982; Lainson <i>et al.</i> , 1990<br>Courtenay <i>et al.</i> , 1994 |
| 8/21 (29/37)                                  | 0/8 (0/29)                           | Marajó, Pará, Brazil           | Courtenay, 1998   |
| 3/23  | 0/3                                  | Belém, Pará, Brazil            | Lainson <i>et al.</i> , 1969; 1987;<br>Lainson and Shaw, 1971                                 |
| 4/33  | 3/4                                  | Sobral, Ceará, Brazil          | Deane and Deane, 1954a; 1955;<br>Deane 1956   |
| 1/11  | 0/1                                  | Corumba, Mato Grosso, Brazil   | Mello <i>et al.</i> , 1988  |
| 7/173   | 0/7                                  | Ceará, Brazil                  | Alençar, 1959; 1961   |
| <b>Red fox <i>Vulpes vulpes</i></b>           |                                      |                                |   |
| 4/5 (4/5)                                     | 0/4 (0/4)                            | Setubal region, Portugal       | Santos <i>et al.</i> , 1996   |
| 9/50 (18)                                     | 0/9                                  | Imperia, Liguria region, Italy | Mancianti <i>et al.</i> , 1994  |
| 4/71 (14/61)                                  | 0/4 (0/14)                           | Setubal region, Portugal       | Abranches <i>et al.</i> , 1982; 1983; 1984  |
| 3/64  | 0/3                                  | Alhama, Spain                  | Marin Iniesta <i>et al.</i> , 1982  |
| 2/99  | 1/2                                  | Cevennes, France               | Rioux <i>et al.</i> , 1968  |
| 2/150   | 1/2                                  | Cevennes, France               | Lanotte, 1975   |
| ≥1/ 68  | 0/1                                  | Grosetto, Tuscany, Italy       | Bettini <i>et al.</i> , 1980; Pozio <i>et al.</i> , 1981b                                     |
| 0/169 (0/22)                                  | -                                    | Alcacer do Sal, Portugal       | Abranches <i>et al.</i> , 1982; 1983; 1984  |
| 0/24 (0/7)                                    | -                                    | Lisbon region, Portugal        | Abranches <i>et al.</i> , 1982; 1983  |
| - (11/16)                                     | - (0/11)                             | Priorat, Tarragona, Spain      | Saladrigas, 1992  |
| - (3/5)                                       | -                                    | Alto-Douro, Arrabida, Spain    | Semiao <i>et al.</i> , 1996   |
| 50/67*  | -                                    | Guadalajara, Spain             | Criado-Fornelio <i>et al.</i> , 2000  |
| 2/19  | 0/2                                  | C. Asia                        | Maruashvili & Bardzhadze, 1966  |
| 1/36  | 0/1                                  | C. Asia                        | Maruashvili & Bardzhadze, 1966  |
| 1/10  | 0/1                                  | Iran                           | Nadim <i>et al.</i> 1978  |
| 1/10 (2/10)                                   | 0/1 (0/2)                            | Iran                           | Edrissian <i>et al.</i> 1993  |
| - (1/22)                                      | -                                    | Israel                         | Baneth <i>et al.</i> , 1998; Baneth & Jaffe, 1999   |
| <b>Golden jackal <i>Canis aureus</i></b>      |                                      |                                |   |
| - (4/53)                                      | -                                    | Israel                         | Baneth <i>et al.</i> , 1998   |
| 1/20  | 1/1                                  | Iran                           | Nadim <i>et al.</i> , 1978  |
| 4/161 (6/48)                                  | 1/4 (1/6)                            | Iran                           | Hamidi <i>et al.</i> , 1982   |
| 2/30 (5/30)                                   | 0/2 (0/5)                            | Iran                           | Edrissian <i>et al.</i> , 1993  |
| ≥5/nd   | -                                    | C. Asia                        | Latyshev <i>et al.</i> , 1961; Lubova, 1973<br>Dursunova <i>et al.</i> , 1965                 |

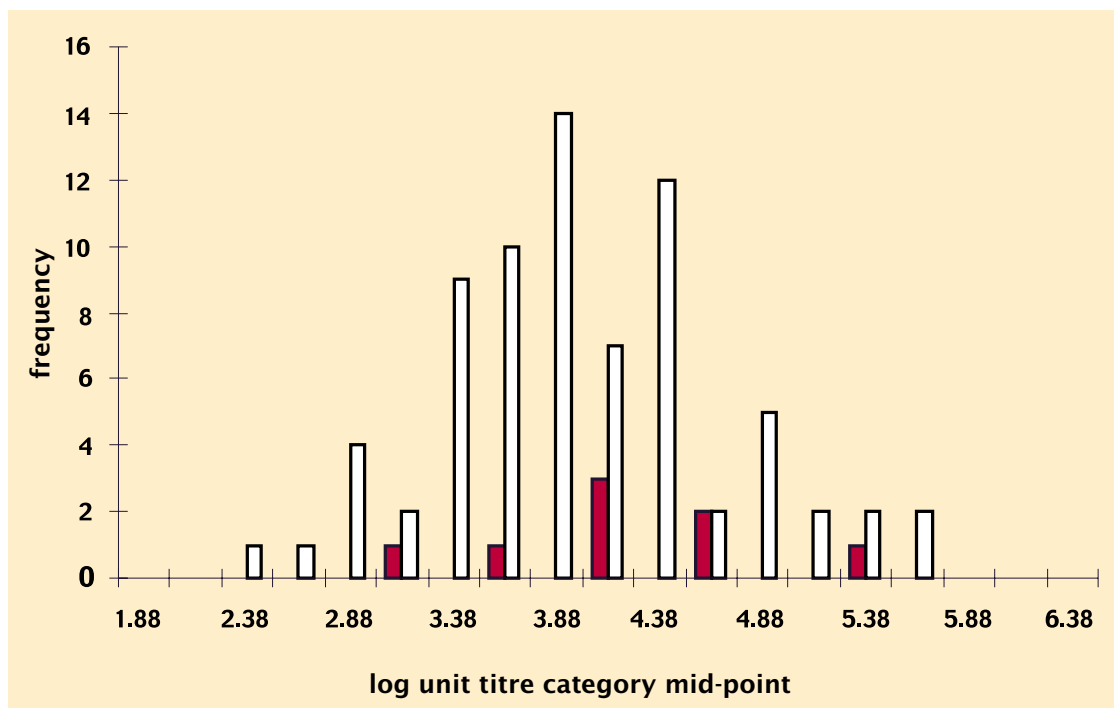
\* molecular diagnosis

Natural infections of *L. infantum* in selected canids in endemic regions.

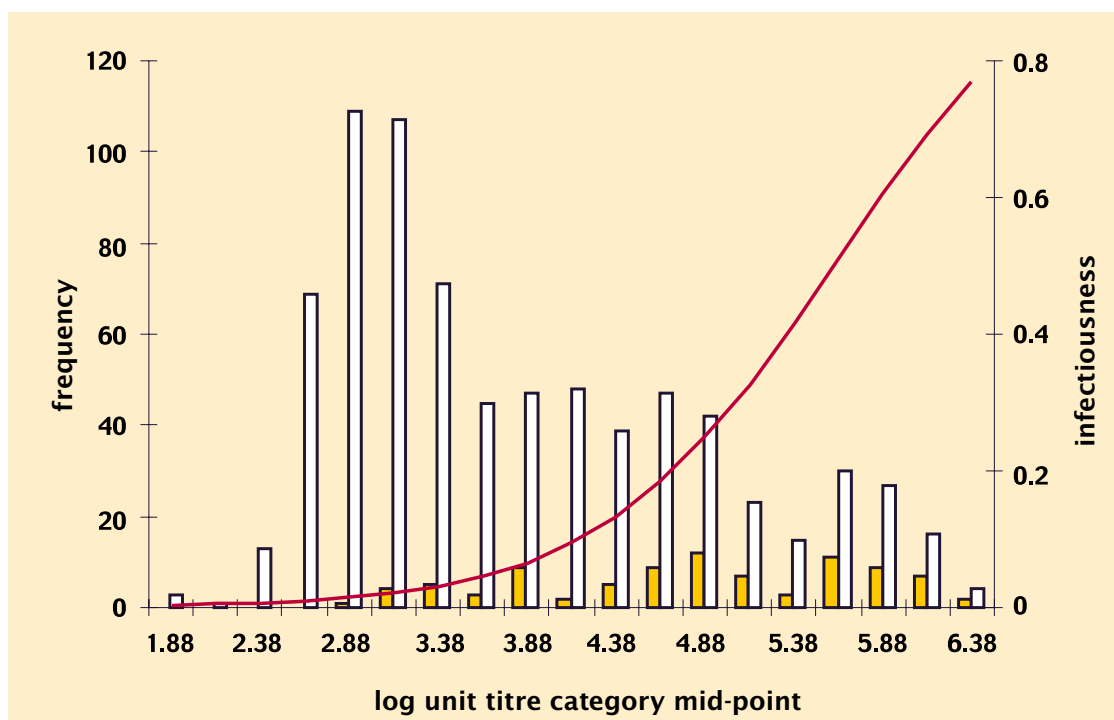




a



b



Frequency distribution of anti-*Leishmania* (ELISA) antibody titres and titres of parasite positive samples (shaded) in (a) foxes, and (b) sentinel dogs. The line represents the best logistic fit of the proportion of *Lu. longipalpis* infected by dogs in xenodiagnosis trials performed at corresponding titres.

## References

Courtenay, O., Quinnell, R. J. & Chalmers, W. S. K. Contact rates between wild and domestic canids: no evidence of parvovirus or canine distemper virus in crab-eating foxes. *Veterinary Microbiology*, (in press).

Courtenay, O. (1998). The epidemiology and control of canine visceral leishmaniasis in Amazon Brazil. PhD thesis, University of London.

Courtenay, O., Santana, E. W., Johnson, P., Vasconcelos, I. A. B. and Vasconcelos, A. W. (1996). Visceral leishmaniasis in the hoary zorro *Dusicyon vetulus*: a case of mistaken identity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90: 498-502.

Courtenay, O., Macdonald, D. W., Lainson, R., Shaw, J. J. & Dye, C. (1994). Epidemiology of canine leishmaniasis: a comparative serological study of dogs and foxes in Amazon Brazil. *Parasitology*, 109: 273-279.

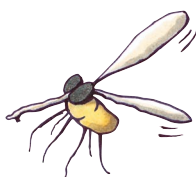
Deane, M. P. & Deane, L. M. (1954). Infecção experimental do *Phlebotomus longipalpis* em raposa (*Lycolopex vetulus*), naturalmente parasitada pela *Leishmania donovani*. *O Hospital*, 46: 651-653.

Lainson, R., Dye, C., Shaw, J. J., Macdonald, D., Courtenay, O., Souza, A. A. & Silveira, F.T. (1990). Amazonian visceral leishmaniasis: distribution of the vector *Lutzomyia longipalpis* (Lutz & Neiva) in relation to the fox *Cerdocyon thous* (L.) and the efficiency of this reservoir host as a source of infection. *Memorias do Instituto Oswaldo Cruz*, 85: 135-137.

Macdonald, D. W. and Courtenay, O. (1996). Enduring social relationships in a population of crab-eating zorros, *Cerdocyon thous*, in Amazonian Brazil (Carnivora, Canidae). *Journal of Zoology* (London), 239: 329-355.

Quinnell, R. J., Courtenay, O., Garcez, L. & Dye, C. (1997). Epidemiology of canine leishmaniasis: transmission rates estimated from a cohort study in Amazonian Brazil. *Parasitology*, 115: 143-156.

Rogers, D. J. (1988). A general model for the African trypanosomiasis. *Parasitology*, 97: 193-212.





## Deltamethrin-impregnated dog collars (Scalibor® ProtectorBand®) protect dogs from sand fly bites.

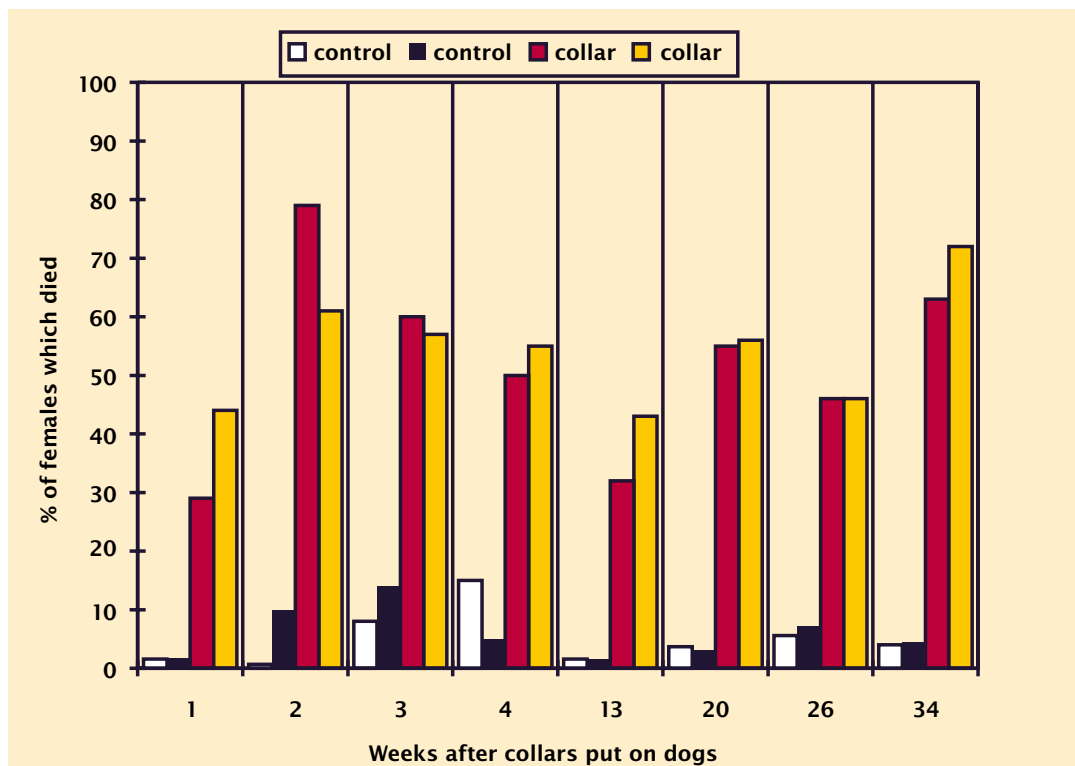
Killick-Kendrick, R. Department of Biology, Imperial College at Silwood Park, Ascot SL5 7PY, UK.

Canine leishmaniasis (CanL) is difficult both to treat and control. Conventional treatment with Glucantime, with or without allopurinol, is expensive and seldom prevents a relapse. Control by culling seropositive dogs gives no more than a temporary fall in prevalence and has many disadvantages. It is opposed by dog owners and, since it requires considerable manpower, it is costly. No vaccine against CanL is yet available. This presentation is a review of recent studies on the deltamethrin-impregnated Scalibor ProtectorBand and an assessment of its potential for the protection of individual dogs against sand fly bites and the control of CanL. Scalibor is a plastic dog collar containing a depot of a complex of deltamethrin and the excipient triphenyl phosphate that permits the insecticide to be slowly released into the lipids of the dog's skin. It covers the complete body of the animal and, unlike conventional dog collars or applied insecticides, the deltamethrin retains its activity on the dog for many months. Scalibor was first shown to give dogs long-term protection (6 months) against tick infestation before being tested with sand flies.

### Laboratory trials on the effects of Scalibor on sand flies.

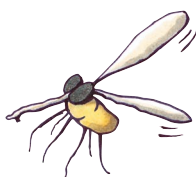
France.

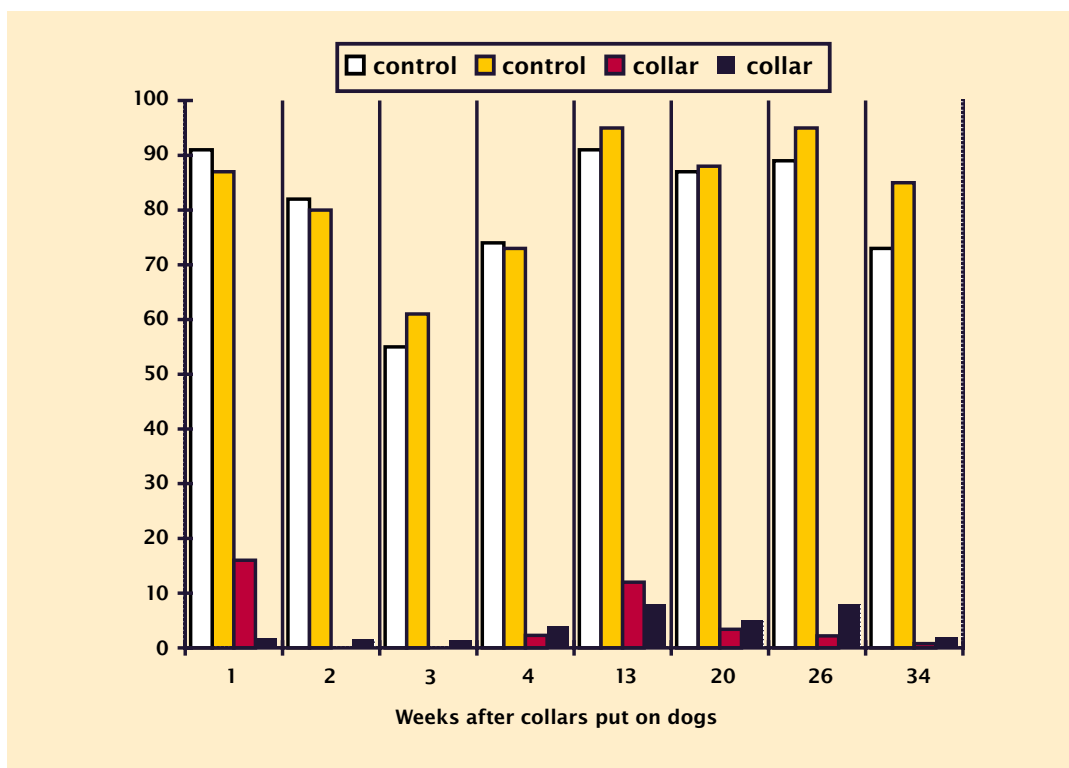
In 1996, the first laboratory trial was done in France<sup>1</sup> with beagles and *Phlebotomus perniciosus*, the most widespread vector of CanL around the Mediterranean Basin. Dogs with and without collars were sedated and exposed in mosquito nets for 2 h to the bites of up to 200 laboratory bred female *P. perniciosus*. Numbers of engorged and dead sandflies were then counted. Live flies were held for ~20 h so that delayed deaths could be recorded and the numbers added to deaths at the end of exposure. Of the flies confined with collared dogs, 28-78% died compared to 1-15% of the flies kept with the control dogs (figure 1).



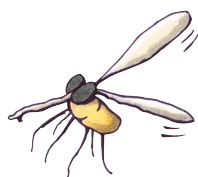
**Figure 1.** Percentages of all female flies dead in the net and ~20 h after confinement with 2 control and 2 collared dogs.(from Killick-Kendrick *et al.* 1997<sup>1</sup>).

Unexpectedly, the collars protected the dogs from a high proportion of sand fly bites for the whole 34 week period of the experiments. Differences between the percentages of flies that engorged on the control and collared dogs are shown in figure 2. Of recaptured flies at weeks 2 to 34, 1,911 females had engorged on the control dogs and 75 on the dogs with collars. Thus, of every 100 flies which fed on control dogs, only 4 had fed on collared dogs, i.e., in the conditions of the tests, the collars protected the dogs from 96% of the bites with the activity maintained up to and including week 34.





**Figure 2.** Percentages of female flies that engorged on 2 control and 2 collared dogs during 2 h confinement (from Killick-Kendrick *et al.* 1997<sup>1</sup>).



## Spain

These results were confirmed by Lucientes<sup>2</sup> in Spain with the same experimental procedures, breed of dogs and species of sand fly. A comparison of the anti-feeding effect of the collars in the two series of experiments is shown in Table 1 from which it can be seen that there is a close correlation in the results.

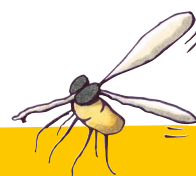
| Weeks after collars were attached | % antifeeding effect (Killick-Kendrick <sup>1</sup> ) | % antifeeding effect (Lucientes <sup>2</sup> ) |
|-----------------------------------|---|--|
| 1                                 | 95  | -  |
| 2                                 | 99  | 90   |
| 3                                 | 99  | -  |
| 4                                 | 96  | 92   |
| 12                                | -   | 98   |
| 13                                | 89  | -  |
| 16                                | -   | 93   |
| 20                                | 95  | 92   |
| 26                                | 94  | 84   |
| 34                                | 97  | -  |

**Table 1**

Comparison of two trials of the anti-feeding effect of Scalibor® against *P. perniciosus* (mean % flies fed on controls less mean % flies fed on collared dogs divided by mean % flies fed on controls).

## Iran

As the two first laboratory trials in France and Spain were both done with beagles and *P. perniciosus*, they gave no information on the protection of dogs with different coats than beagles against the bites of species of sand flies other than the Mediterranean vector. The first indication that the anti-feeding effect was as marked with other systems came from a laboratory trial in Iran<sup>3</sup> with *P. papatasi* and seven guard dogs (breeds unspecified, but assumed to be large dogs of mixed race). Before collars were put on, each dog was sedated and left in a mosquito net overnight (7 h) with 70 - 100 mostly female *P. papatasi* collected earlier the same night from walls inside houses. The next morning, all flies were collected and scored as live or dead, fed or unfed. Live flies were held until 20 h after the start of the tests and were scored again. These initial results were control observations. Protector-





Bands were then fitted to each dog and the assays were repeated 8 days later. The reduction in feeding on dogs with collars was around 80%. The authors conclude that "The observed reduction in blood-feeding of *P. papatasi* on dogs, when fitted with deltamethrin-impregnated collars, confirms the potential value of this simple device for protecting dogs against ZVL." (ZVL was the term used by the authors for canine leishmaniasis or human visceral leishmaniasis caused by *Leishmania infantum*). There was no significant difference in mortality rates in tests done before and after collars were fitted.

### **Brazil**

Further evidence that Scalibor provides the same high degree of protection for dogs other than beagles against bites of various species of sand flies comes from the results of a laboratory trial in Brazil by John David and colleagues<sup>4</sup>. This trial was with mongrel dogs and two species of *Lutzomyia* – *Lu. longipalpis* (a proven vector of CanL and human visceral leishmaniasis in Latin America) and *Lu. migonei* (a probable vector of the human dermatropic parasite *Leishmania braziliensis* of South and Central America that is found in dogs in some places). The experimental procedures were the same as in the French and Spanish trials.

During 7 experiments up to and including 35 weeks, the average mortality among *Lu. longipalpis* confined with the deltamethrin collared dogs was 68%. Only 4% (81/2022) were engorged giving an average anti-feeding effect of 96%. Similar findings were found with *Lu. migonei*. With 2034 sand flies in 7 experiments over 34 weeks, the average mortality was 70.4% and the average anti feeding effect was 96.6%.

### **Field trials of the effect of Scalibor on the prevalence of canine leishmaniasis.**

#### **Italy**

The first pilot field trial of the effect of Scalibor on the prevalence of CanL was done by Maroli and colleagues in a focus of the disease in southern Italy with a seroprevalence of around 15%<sup>5,6</sup>. During two consecutive transmission seasons (June - October), deltamethrin-impregnated collars were fitted to about 70% of an estimated population of 500 dogs owned by the people living in San Sebastiano al Vesuvio. Five nearby towns with roughly the same number of dogs served as the control area. Before each transmission period (i.e. before June), the seroprevalence of CanL in both places was determined by cross-sectional surveys. After each sandfly season (i.e. starting from November), prevalence rates of seroconversions were determined in selected cohorts of the dogs, i.e. adult dogs that were serologically negative before the sandfly season and puppies born after the previous transmission season.

Estimated degrees of protection from CanL were strikingly different in the two years of the study. After the first year of observations, the estimated protection against infection was 50%, although the difference in the seroconversion rates in the two groups was not statistically significant. In contrast, at the end of the second year, the difference in the seroconversion rate between collared and control dogs was highly significant ( $P < 0.001$ ), with an estimated protection against CanL of about 86% in the collared dogs. The difference in the results of the two years was explained by differences in the force of infection over the period of the trial. In the first year, too few of the unprotected dogs seroconverted for there to be a significant difference between the two groups. In the second year, it appears the force of infection was higher than in the first and enough uncollared dogs became infected for a marked difference to be apparent. The authors conclude: 'our results show that the impact of the mass use of deltamethrin-impregnated dog collars on the incidence of CanL may be negligible during low transmission seasons or, probably, in unstable foci of CanL with a permanently low endemicity, but can be very strong when the force of transmission is high.'

### **Brazil**

In Brazil there are currently two ongoing field trials involving several thousand dogs.

### **Discussion and Conclusions**

Synthetic pyrethroids are so efficient at killing insects that their ability at low concentrations simply to interfere with feeding by haematophagous arthropods is sometimes questioned. However, a review of the literature<sup>7</sup> reveals numerous references to an 'anti-feeding effect' or 'repellence' by a wide range of pyrethroids. Control dogs in the trials in Spain and Brazil were fitted with placebo collars containing triphenyl phosphate but no deltamethrin. These collars gave no protection from bites and the anti-feeding effect is, therefore, attributed to the pyrethroid, not the excipient.

High proportions of sand flies were killed in three of the four laboratory trials. The exception, the trial in Iran, is explained by the timing of the test after the collars were fitted to the dogs. This was at eight days by which time the deltamethrin has not spread fully over the body of dogs. It seems probable that, although there was sufficient insecticide in the skin of the dogs to inhibit feeding by the eighth day, the level was insufficient to kill.

Scalibor is the only product on the market for which protection from sand fly bites can be officially claimed. Four laboratory trials with four different species of sand flies and various breeds of dogs have shown that the dogs were protected from over 90% of sand fly bites. In the second year of the Italian field trial when the force of transmission was judged to be high, it was





estimated that the protection from leishmaniasis was 86%. These combined field and laboratory observations show that, when dogs are exposed to different species of sand flies, deltamethrin-impregnated collars give a high degree of protection irrespective of their coat. If fitted to the majority of dogs in a focus of canine leishmaniasis, it is hoped that Scalibor will interrupt the circulation of the parasite to such a degree that the risk to the human population will be markedly reduced. The field trials under way in Brazil will test this assumption.

#### **ACKNOWLEDGEMENTS**

I thank Prof. Michele Maroli and Prof. John David for permission to refer to their findings in papers submitted for publication.

#### **References**

<sup>1</sup> Killick-Kendrick, R., Killick-Kendrick, M., Focheux, C., Dereure, J., Puech, M. & Cadiergues, M.C. (1997). Protection of dogs from the bites of phlebotomine sandflies by deltamethrin collars for the control of canine leishmaniasis. *Medical and Veterinary Entomology*, 11: 105-111.

<sup>2</sup> Lucientes, J. (1999). Laboratory observations on the protection of dogs from the bites of *Phlebotomus perniciosus* with Scalibor® ProtectorBands: preliminary results. In: *Canine Leishmaniasis: an update* (ed. R. Killick-Kendrick). *Proceedings of a Canine Leishmaniasis Forum, Barcelona (Sitges), 28-31 January, 1999*, Wiesbaden: Hoechst Roussel Vet, 92-94.

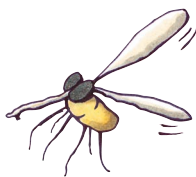
<sup>3</sup> Halbig *et al.* (2000). Further evidence that deltamethrin-impregnated collars protect dogs from sandfly bites. *Med. vet. Ent.*, 14 (2), 223 - 226.

<sup>4</sup> David, J.R., Stamm, L.M., Bezarra, H.S., Nonato, Souza, R., Killick-Kendrick, R & Oliveira Lima, J.W. (2001). Deltamethrin-impregnated plastic dog collars have a potent anti-feeding effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*. (Submitted for publication.)

<sup>5</sup> Maroli, M., Gradoni, L., Mizzoni, V. & Siragusa, C. (1999). Deltamethrin-impregnated dog collars to control canine leishmaniasis: a pilot field study in an endemic focus of southern Italy. In: *Canine Leishmaniasis: an update* (ed. R. Killick-Kendrick). *Proceedings of a Canine Leishmaniasis Forum, Barcelona (Sitges), 28-31 January, 1999*, Wiesbaden: Hoechst Roussel Vet, 96-98.

<sup>6</sup> Maroli, M., Mizzoni, V., Siragusa, C., D'Orazi A. & Gradoni, L. (2001). The impact of deltamethrin-impregnated dog collars on the incidence of canine leishmaniasis: a pilot field study in an endemic area of southern Italy. (Submitted for publication.)

<sup>7</sup> Killick-Kendrick, R. (1999) Anti-feeding effects of synthetic pyrethroids against phlebotomine sand flies and mosquitoes, and the prospects of controlling canine leishmaniasis with deltamethrin-impregnated ProtectorBands (Scalibor®). In: *Canine Leishmaniasis: an update* (ed. R. Killick-Kendrick). *Proceedings of a Canine Leishmaniasis Forum, Barcelona (Sitges), 28-31 January, 1999*, Wiesbaden: Hoechst Roussel Vet, 82-88.





## Immune responses in canine leishmaniasis

E.J. Ruitenber<sup>1,2</sup>, L. Solano-Gallego<sup>3</sup>, J. Monen<sup>1</sup>, E. Pinelli<sup>1,4</sup> and V.P.M.G. Rutten<sup>1</sup>

- 1) Department of Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
- 2) CLB, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands
- 3) Departament de Farmacologia i Terapèutica, Facultat de Veterinària, Universitat Autònoma de Barcelona, Spain
- 4) National Institute of Public Health and Environment, Bilthoven, The Netherlands

### Introduction

Infection of humans and dogs by *Leishmania infantum* may result in visceral leishmaniasis, which is characterised by impaired T-cell mediated immune responses to parasite antigens.

Dogs are the main reservoir hosts for this intracellular pathogen. Most of the clinical signs in dogs, including irregular fever, hypergammaglobulinemia, immune suppression, hepatosplenomegaly and anemia (Abranches et al, 1991; Bettini and Gradoni, 1986) are similar to those in man.

Although the dog is an important reservoir host of *Leishmania* parasites and may also serve as a model for human visceral leishmaniasis, there are relatively few studies on the immune responses in canine leishmaniasis. These have been mainly concerned with responses in naturally infected dogs. Brandonisio et al (1988) reported that both the number of T cells and their function in chronically infected dogs were reduced compared to normal non-infected dogs. Parasite-specific cellular immunity and detection of anti-leishmania antibodies in asymptomatic and symptomatic naturally infected dogs have been reported (Cabral et al, 1992; Solano-Gallego et al, 2001). Studies on the immune response of dogs experimentally infected with leishmania parasites are notably limited.

### Experimental infection in dogs

Experimental infection of dogs have encountered several difficulties. An important new approach has been introduced by Killick-Kendrick et al (1994). These authors reported the preparation of infective doses of *L.infantum* from experimentally infected sand flies.

These included an extract of sand fly salivary glands which, improves parasite infectivity (Titus and Ribeiro, 1988). In this study, dogs were infected by the intradermal inoculation of  $5-8 \times 10^3$  metacyclic promastigotes harvested

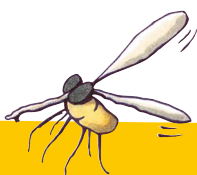
from the midguts of sand flies that were experimentally infected by allowing them to feed on a dog with clinical leishmaniasis. Evidence that the dogs became infected after inoculation of the metacyclic promastigotes was obtained by the isolation of parasites from these animals.

### **Immune responses to *Leishmania infantum* in dogs**

Our studies were done to gain an improved understanding of the immune responses in dogs infected with *L. infantum*, including the identification and characterisation of factors and mechanisms involved in protective immunity. Based on the experimental infection procedure described above we have studied the humoral and cellular responses associated with either resistance or susceptibility to experimental and natural infections with *L. infantum* in dogs (Pinelli et al, 1994b). Resistance to infection was found to be associated with a strong parasite-specific cellular immune response and the production of cytokines such as IL-2 and TNF. The role of T cells in protective immunity was also studied. We have described the generation of Leishmania specific T cell lines, that following **in vitro** restimulation are capable of producing IFN- $\gamma$  and to lyse infected macrophages in a MHC-restricted manner (Pinelli et al, 1994a, Pinelli et al, 1995). These T cell lines could be generated from asymptomatic but not from symptomatic infected dogs. Also we found that peripheral blood mononuclear cells from asymptomatic infected dogs produce IFN- $\gamma$  upon parasite-specific stimulation, whereas lymphocytes from symptomatic dogs do not. These findings suggest a possible role for both the production of Th1-derived cytokines and the destruction of parasited host cells by Leishmania-specific cytotoxic T cells in protective immunity of dogs harbouring a chronic leishmania infection. Furthermore, we examined how infection with *L. infantum* affects canine macrophages and the consequences of these changes for T cell activation **in vitro**. Proliferation of T cell lines to specific antigen decreases to back-ground levels when infected autologous macrophages are used as antigen presenting cells. In addition, we observed a decreased expression of costimulatory B7 molecules on infected macrophages. The results indicated that for the activation of parasite-specific T cells producing IFN- $\gamma$ , sufficient expression of B7 molecules on infected macrophages is required (Pinelli et al, 1999a) .

### **Development of T helper cell subsets**

Although several factors influence the development of T helper cell subsets, the most important may be their early exposure to cytokines. In studies on the immunopathogenesis of canine leishmaniasis we were interested in measuring cytokines and their role in disease and protective immunity. These studies have been limited due to the lack of assays to measure canine cytokines. Recently we have been able to develop new tools to measure sever-





al canine cytokines including IL-2, IFN-g, TNF-a, and IL-4. The gene for IL-4 (Van der Kaay et al, 1999) was cloned and sequenced, and tools were developed to measure the canine cytokines mentioned above by using a quantitative competitive PCR (Pinelli et al, 1999b).

Further studies including the analysis of these cytokines will help to elucidate the role of Th1 and Th2 type cells and other effector cells in disease or protective immunity in *Leishmania* - infected dogs.

Thus, we performed *in vivo* DTH-tests in leishmania-infected dogs, followed by an analysis of cytokine expression patterns in tissue biopsies. We observed a tendency to express a mixture of a type 1 and type 2-like cytokine pattern in the case of a positive DTH reaction. Negative DTH reactions showed a tendency to express a type 2-like cytokine pattern. On the basis of these findings at the site of a DTH reaction it is important to determine cytokine patterns in lesional and non-lesional skin of *Leishmania*-infected dogs. These studies are currently underway.

Further elucidation of the role of Th1 and Th2 type cells and other effector cells in disease or protective immunity in *Leishmania*-infected dogs will provide information required for the development of a vaccine or immunotherapy of (canine) leishmaniasis.

### Summary

*Leishmania infantum* may cause fatal infections in both man and dogs. Experimentally infected dogs may serve as models for developing immunotherapy and vaccines both for human and canine visceral leishmaniasis.

Our group has focused on the identification and characterisation of the factors and mechanisms involved in protective immunity in dogs infected with *Leishmania infantum*.

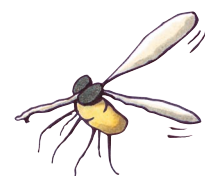
Analysis of the cytokine secretion pattern of peripheral blood mononuclear cells (PBMC) from asymptomatic experimentally infected dogs showed that protective immunity to *L.infantum* is associated with a Th-1 like response. This conclusion is based on results indicating that stimulation of PBMC from asymptomatic animals with parasite antigens or mitogens results in a higher production of IL-2, TNF-a and IFN-g compared to symptomatic dogs. Further studies revealed the role of **in vitro** restimulation, being capable of producing IFN-g and lysing infected macrophages in a MHC-restricted manner. Next, it was observed that for the activation of parasite-specific T cells producing IFN-g sufficient expression of B7 molecules on infected macrophages is required.

Further studies in disease progression are now possible since we recently cloned and sequenced the gene for canine IL-4. Thus, in **in vivo** DTH tests in *Leishmania*-infected dogs we observed a tendency to express a mixture of a type 1 and type 2 like cytokine pattern, whereas in the case of a negative DTH reaction a tendency to express a type 2-like cytokine pattern was seen.

Now it is possible to further explore the role of Th1 and Th2 type cells in disease progression and protective immunity.

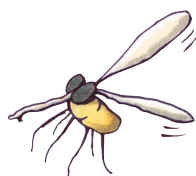
## References

- \* Abranches, P., Santos-Gomes, G., Rachamin, N., Campino, L., Schnur, L. and Jaffe, C.L.  
An experimental model for canine visceral leishmaniasis.  
*Parasite Immunology* **13**, 537-550 (1991)
- \* Bettini, S. and Gradoni, L.  
Canine leishmaniasis in the Mediterranean area and implications for human leishmaniasis.  
*Insect Science and its Application*, **7**, 241-245 (1986)
- \* Bradonisio, O., Altamura, M., Carelli, G., Ceci, L., Antonaci, S. and Jirillo, E.  
Lymphocyte functions in *Leishmania infantum* infected dogs.  
*J. of Immunology and Immunopharmacology* **9**, 37-40 (1988)
- \* Cabral, M., O'Grady, J. and Alexander, J.  
Demonstration of *Leishmania* specific cell mediated and humoral immunity in asymptomatic dogs.  
*Parasite Immunology* **14**, 531-539 (1992)
- \* Killick-Kendrick, R., Killick-Kendrick, M., Pinelli, E., Del Real, G., Molina, R., Vitutia, M.M., Cănavete, M.C. and Nieto, X.  
A laboratory model of canine leishmaniasis: the inoculation of dogs with *Leishmania infantum* promastigotes from midguts of experimentally infected phlebotomine sand flies. *Parasite* **1**, 311-318 (1994)
- \* Pinelli, E., Boog, C.J.P., Rutten, V.P.M.G., Van Dijk, B., Bernadina, W. and Ruitenbergh, E.J.  
A canine CD8+ cytotoxic T-cell line specific for *Leishmania infantum* infected macrophages.  
*Tissue Antigens* **43**, 189-192 (1994a)
- \* Pinelli, E., Gonzalo, R.M., Boog, C.J.P., Rutten, V.P.M.G., Gebhard, D., Del Real, G. and Ruitenbergh, E.J.  
*Leishmania infantum*-specific T cell lines derived from asymptomatic dogs that lyse infected macrophages in a major histocompatibility complex restricted manner.  
*European Journal of Immunology*, **25**, 1594-1600 (1995)





- \* Pinelli, E., Killick-Kendrick, R., Wagenaar, J., Bernadina, W., Del Real, G., and Ruitenber, E.J.  
Cellular and humoral immune responses in dogs experimentally and naturally infected with *Leishmania infantum*.  
*Infection and Immunity* **62**, 229-335 (1994b)
- \* Pinelli, E., Rutten, V.P.M.G., Bruysters, M., Moore, P.F., and Ruitenber, E.J.  
Compensation for decreased expression of B7 molecules on *Leishmania infantum* infected canine macrophages results in restoration of parasite-specific T cell proliferation and gamma interferon production.  
*Infection and Immunity* **67**, 237-243 (1999a)
- \* Pinelli, E., Van der Kaaij, S.Y., Broeren, C.P.M., Schetters, T.P.M., Haghparast, A., Ruitenber, E.J., and Rutten, V.P.M.G.  
Measurement of canine cytokines by reverse transcription quantitative polymerase chain reaction.  
*Immunogenetics* **49**, 696-699 (1999b)
- \* Solano-Gallego, L., Riera, C., Roura, X., Iniesta, L., Gallego, M., Valladares, J.E., Fisa, R., Castillejo, S., Alberola, J., Ferrer, L., Arboix, M. and Portus, M.  
*Leishmania infantum*-specific IgG, IgG1 and IgG2 antibody responses in healthy and ill dogs from endemic areas. Evolution in the course of infection and treatment.  
*Veterinary Parasitology* **2028**, 1-12 (2001)
- \* Titus, R.G. and Ribeiro, J.M.  
Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity.  
*Science* **239**, 1306-1308 (1988)
- \* Van der Kaaij, S.Y., Pinelli, E., Broeren, C.P.M., Schetters, T.P.M., Haghparast, A., Ruitenber, E.J. and Rutten, V.P.M.G.  
Molecular cloning and sequencing of the cDNA for dog interleukin-4.  
*Immunogenetics* **49**, 142-143 (1999)



## Chemotherapy of canine leishmaniasis

*Gad Baneth, School of Veterinary Medicine, Hebrew University,  
P.O.Box 12, Rehovot 76100, Israel. E-mail: baneth@agri.huji.ac.il*

### **Preface**

Therapy of canine leishmaniasis has been an important issue since the first descriptions of the causative agent of this disease in humans, followed by the early reports of canine leishmaniasis, and the subsequent realization that the infection in people and canines can be caused by the same agents, as occurs in the Mediterranean, throughout much of Asia, Africa and south and central America.

Domestic dogs and humans are treated for leishmaniasis, often with the same drugs or with related compounds. Gaspar Vianna was the first doctor to use a relatively effective drug against leishmaniasis in people. In 1912, he adapted the use of tartar emetic (antimony potassium tartrate) which was found earlier to be effective in the therapy of African trypanomiasis, to the treatment of *Leishmania braziliensis* infection in South America (Manson Bahr, 1996). Tartar emetic, a trivalent antimonial, rapidly became widely used in other regions against the agents of visceral and cutaneous leishmaniasis. Phenylstibonic acid consisting of a less toxic pentavalent antimonial linked to a benzene ring was synthesized in 1920, and in 1937, sodium stibogluconate (Pentostam®), a more stable and less toxic pentavalent antimonial was synthesized by Schmidt in Germany. The antimonial compounds that have been used now for nearly 90 years, are still the main drugs used for therapy of both human and canine leishmaniasis today. The antimonials limitations include: the formation of parasitic resistance, toxicity to the host, financial restrictions due to the high cost of the drug, and the lack of complete cure in many dogs. These limitations have driven clinicians and scientists to seeking other effective and affordable drugs.

Although anti-leishmanial treatment with the currently used drugs often achieves temporary clinical improvement in dogs with leishmaniasis, it is frequently not associated with the elimination of the parasite carriership, the cessation of infectivity to sand fly vectors feeding on the canine reservoir, and the prevention of clinical disease relapse. The definition of a "successful treatment" of canine leishmaniasis may therefore have different meanings. To the dog owner and to his attending veterinarian, success in treatment often equals remission from the clinical signs of disease which caused the worried owner to bring the dog for veterinary care in the first place. This approach to treatment success may be valid in areas where the disease can not be transmitted, such as in the case of infected dogs brought from endemic areas to northern Europe where disease vectors are not present. It can not be accept-



able in areas where the disease is endemic and transmission may occur to people or other dogs. To the parasitologist, cure would be complete elimination of the parasite from the host, while public health officials, epidemiologists and entomologists may suffice with cessation of the infectivity of the dog to sandfly vectors, therefore stopping further transmission of potentially drug-resistant parasites from the canine patient.

Many studies have been published on the therapy of naturally-occurring canine leishmaniasis with various anti-protozoal drugs. These publications have frequently addressed one or two aspects of the response to treatment such as the changes in the clinical status of animals before and after treatment, or the serologic response and immunological changes. Other important aspects such as the parasitological status of the animals and their infectivity to sand flies were less often assessed. Experimental infection of dogs which induces a symptomatic disease and mimics the natural course of infection is difficult to establish. Therefore, controlled experiments on the response to therapy in artificial-infections are few and often not done on dogs that have developed a clinical disease that is similar to the symptomatic naturally-occurring disease.

When evaluating different studies on the chemotherapy of canine leishmaniasis, it must be taken into account that a great variability exists in the experimental methods used and in the evaluation of the results. The factors that need to be assessed include: the doses and frequencies of drugs administered, the route of administration, the length of treatment, and the period during which the dogs were under observation. Drugs may be used as single therapeutic agents or in combination with other drugs, therefore altering the results expected from monotherapy. Additional factors that need to be evaluated are the genetic composition of the individual dogs used which are often associated with the dog's breed. Inherited traits may reflect upon the nature of the immune response that is mounted during the infection, and possibly also on the dogs' ability to recover from infection following therapy. The virulence of the infecting parasite may also play an important part in the response to therapy and the dog's recovery.

#### **Pentavalent antimonials**

The pentavalent antimony compounds meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) are widely used for treatment of canine leishmaniasis in Europe and other parts of the world. Pentavalent antimonials selectively inhibit leishmanial enzymes that are required for glycolytic and fatty acid oxidation. The pharmacokinetics of meglumine antimoniate in the dog were established (Tassi et al., 1994, Valladares et al., 1996) with more than 80% of the antimony excreted in the urine within 9 hours from administration (Valladares et al., 1996). Intramuscular injections of pen-

tavalent antimonials may cause severe side effects including muscular fibrosis and abscess formation. Other side effects of antimonials include gastrointestinal disturbances, delayed muscular pain and joint stiffness.

The most common protocol for meglumine antimoniate therapy is at 100 mg/kg body weight/day for 3-4 weeks administered subcutaneously (SC) or intravenously (IV). In a study of 41 naturally-infected dogs treated with meglumine antimoniate (Slappendel and Teske, 1997), 35 dogs (85.4%) reached partial or complete remission in clinical signs and a negative cytological examination for parasites after 3 to 6 weeks of treatment. Twenty six (74.3%) of the dogs that reached remission experienced a relapse of the disease within 1 year.

The response to pentavalent antimonials was also tested by an experimental infection of 6 beagle dogs which were inoculated by IV injection of *L. infantum* promastigotes (Riera et al., 1999). The beagles were treated with meglumine antimoniate at 40.8 mg/kg/day SC after some indications of disease were evident. One dog died, and the remaining five continued to be parasitologically positive after the end of treatment. In addition to that, the anti-leishmanial antibody levels increased starting 3 to 5 months after treatment indicating a progression of antigenic stimulation.

In another study (Steuber et al., 1999), 13 naturally-infected dogs were treated with two cycles of meglumine antimoniate starting at 50 mg/kg/day for 2 days and then 100 mg/kg/day for an additional 8 days. Twelve of 13 (92.3%) dogs remained positive for *Leishmania* DNA by PCR following therapy.

The potential of infectivity to *Phlebotomus perniciosus* sandflies by dogs that had undergone therapy was assessed by Alvar and colleagues (Alvar et al., 1994). Six dogs with naturally-occurring leishmaniasis were treated with a combination of 40 mg/kg/day meglumine antimoniate intramuscularly (IM) for 20 days and allopurinol at 20 mg/kg/day orally for 30 days. Five dogs were parasitologically positive 10 months post-treatment. Two of these dogs were infective to sandflies 3-6 months post-treatment and the % of sandflies which became infected after feeding on these two dogs increased with time, indicating that the number of tissue parasites was probably increasing during that time.

Therapy of canine leishmaniasis with pentavalent antimonials is expensive and can be cost-prohibitive in the less affluent regions of world. Another disadvantage to the use of antimonials for canine leishmaniasis is the formation of resistant leishmanial strains, which can be very harmful to humans and to other dogs.



### **Amphotericin B**

Amphotericin B is a polyene macrolide agent produced by the actinomycete *Streptomyces nodosus*. It is primarily used as an anti-fungal drug, but also has activity against some protozoa species including *Leishmania spp.* and *Naegleria spp.*. Amphotericin B acts by binding to sterols, primarily ergosterol, in the cell membrane and altering the permeability of the membrane allowing intracellular potassium and other cellular constituents to leak out. Amphotericin B binds less strongly to cholesterol, the main sterol in mammalian cells, therefore it is less harmful to the host cells than it is to certain microorganisms. Amphotericin B has a toxic effect on the canine kidney, which is exerted via renal vasoconstriction causing reduction of the glomerular filtration rate, and possibly also by direct action on renal epithelial cells. Amphotericin B can be administered to dogs with leishmaniasis in its highly nephrotoxic free form, in a liposomal formulation (AmBisome®) which reduces its toxic effects and directs the drug to macrophages, or in a lipid emulsion (Lamothe, 1999).

In a study on 13 dogs naturally infected with *L. infantum* that were treated with different doses of liposomal amphotericin B and followed for 8 months (Oliva et al., 1995), most dogs achieved clinical improvement, but all were parasitologically positive and experienced a clinical relapse 4-6 months after treatment, except for one animal.

In a report published on the use of the free form of Amphotericin B which was administered rapidly IV at a dose of 0.5 to 0.8 mg/kg 2-3 times a week until an accumulated dose of 15 mg/kg was reached, clinical improvement was obtained in 28 of 30 naturally infected dogs (Lamothe, 1997). Twenty seven of the 28 dogs were reported not to have clinically relapsed after 12 months. This form of treatment required a close monitoring of the kidney functions during treatment, and the temporary withdrawal of therapy when creatinine levels were elevated above 2.5 mg/dl.

### **Aminosidine**

Aminosidine (paromomycin) is an aminoglycoside antibiotic produced by *Streptomyces rimosus* that has been used for the treatment of visceral leishmaniasis in humans in Africa and Europe (Chunge et al., 1990; Scott et al., 1992).

A study on the treatment of naturally occurring canine leishmaniasis with aminosidine IM at 3 different dose regimens indicated that a lower dose of 20 mg/kg/day for 15 days induced temporary clinical improvement that was followed by a clinical and parasitological relapse within 50-100 days after the initiation of therapy (Vexenat et al., 1998). Higher doses of 40 mg/kg/day for

30 days and 80 mg/kg/day for 20 days achieved a low prevalence of parasitological cure, and were associated with mortality and adverse effects including blindness and ototoxicity leading to deafness.

Another study on the treatment of naturally occurring canine leishmaniasis with aminosidine included 12 dogs that were treated with 10 mg/kg/day SC for 4 weeks and followed for 60 days after the beginning of therapy (Poli et al., 1997). Clinical improvement was observed in 11 of the dogs treated within 30 days, and adverse effects of renal impairment and deafness were noted in one dog that had a pre-existing renal disease. Despite the clinical improvement after treatment, 4 dogs that were subjected to lymph node culture before aminosidine treatment remained parasitologically positive after therapy.

### **Pentamidine**

Pentamidine (Lomidine®) is an aromatic diamidine derivative used also for pneumocystosis, babesiosis and trypanomiasis. It causes muscular irritation at the site of injection and may also induce hypotension, tachycardia and vomiting. Most dogs treated with pentamidine improve clinically and relapse several months after treatment (Baneth, unpublished data). In a study on the effect of dimethasulfonate pentamidine treatment on the immune response in dogs with *L. infantum* infection (Rhalem et al., 1999), 3 naturally infected and 5 experimentally infected dogs were treated by 2 courses of pentamidine therapy at an interval of 3 weeks. Each course consisted of 8 IM injections of pentamidine at 4 mg/kg with a 3 days interval between injections. The dogs improved clinically after therapy, had a decrease in antibody titer and were able to mount a specific immune cellular response to leishmanial antigen. A parasitological examination by culture and cytology was performed post-treatment only on 2 of the experimentally infected dogs, which were negative 6 months after the cessation of therapy.

### **Allopurinol**

Allopurinol is an oral purine analogue that is metabolized by *Leishmania* parasites to nucleotides and incorporated in RNA causing breakdown of the nucleic acid and subsequently an interruption in protein synthesis. Although very few side effects caused by allopurinol in the dog are known, prolonged use of this drug may result in xanthine urolith formation (Ling et al, 1991).

Allopurinol is widely used for canine leishmaniasis as monotherapy or in combination with pentavalent antimonials. The relative non-toxicity of allopurinol to the host, its efficiency in improving the patients clinical status, its low cost and the possibility of oral administration which can be performed by the dog's owner, have made allopurinol a popular choice for the treatment of canine leishmaniasis. It is often recommended as daily therapy for an indefinite period of time.



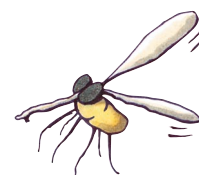
Allopurinol at the dose of 10 mg/kg/day PO was used to treat 10 dogs with naturally occurring canine leishmaniasis (Cavaliero et al., 1999). Nine dogs showed clinical recovery within 2-6 months of therapy and no relapses were observed during treatment of up to 20 months. However, 3 of 4 dogs relapsed after treatment was discontinued. These dogs improved again after therapy was resumed. Parasitological cure was not attained in 8 of 9 dogs tested by culture or PCR of lymph node aspirates.

Allopurinol treatment at 5 mg/kg PO every 8 hours was administered to 11 dogs. All of the dogs were free of clinical signs within approximately 2 months (Vercammen et al., 1995). A decrease in the anti leishmanial antibody titers was detected in 9 dogs, although most dogs remained seropositive.

The effectiveness of allopurinol at 20 mg/kg/day for one week each month in the maintenance of clinical remission, which was achieved earlier by an induction combination treatment of 100 mg/kg/day meglumine antimoniate and 30 mg/kg/day allopurinol, was described in 15 naturally infected dogs (Ginel et al., 1998). The results from this group of dogs was compared to a control group that received only the induction combination treatment of meglumine antimoniate with allopurinol, without maintenance allopurinol. Relapses occurred in 86% of the control dogs within 14 months of discontinuing treatment, whereas the intermittent allopurinol maintenance group maintained its clinical remission during a follow up period of 10-44 months.

Another study compared 45 dogs that were treated initially with a combination of 100 mg/kg/day of meglumine antimoniate with 15 mg/kg every 12 hours of allopurinol, and then allopurinol alone at the same dose for 8 months, to a second group of 40 dogs treated with antimony alone, and a third group of 11 dogs that received allopurinol alone (Denerolle and Bourdoiseau, 1999). The study concludes that antimony in combination with allopurinol therapy produces a higher rate of clinical cures than antimony or allopurinol alone.

A study that assessed the treatment canine leishmaniasis in Holland indicated that dogs treated with 20 mg/kg/day allopurinol have a 78% chance of survival for more than 4 years provided that severe renal failure was not present when therapy was started (Slappendel and Teske, 1999). This finding was similar to the survival of Dutch dogs treated with meglumine antimoniate (Slappendel and Teske, 1997), and therefore allopurinol was recommended for therapy by the authors.



### **Alkylphosphocholines**

Hexadecylphosphocholine (miltefosine) is a membrane-active alkylphosphocholine which accumulates in macrophages and is active against *Leishmania* parasites. It is effective in the oral treatment of leishmanial infections in mice and people (Kuhlencord et al., 1992; Sundbar et al., 1999). This particular drug induces severe side effects in dogs, and a structurally close alkylphosphocholine molecule (liposomal oleic-phosphocholine), which appears to be well tolerated, is currently under evaluation in dogs.

### **Metronidazole, ketoconazole, fluconazole and miscellaneous drugs**

Several antimicrobial and anti-fungal drugs have been investigated for the therapy of leishmaniasis in humans, dogs or in rodent experimental models. Metronidazole, ketoconazole, fluconazole, itraconazole and terbinafine were found to be less effective than meglumine antimoniate in reducing the hepatic parasite load in a murine model of *L. infantum* infection (Gangneux et al., 1999). Ketoconazole did demonstrate a marked activity against splenic parasites in the same study. Buparvaquone (Butalex®) which is used for therapy of theileriosis has not been shown to be ineffective in the treatment of canine leishmaniasis (Vexenat et al., 1998).

### **Conclusions**

Canine leishmaniasis in the early 21<sup>st</sup> century is still a disease that does not have an ultimately effective therapy. It is desirable that leishmaniasis in humans and in dogs should be treated by different drugs that act by separate mechanisms in order to minimize the danger of generating resistant parasite strains. New drugs, delivery systems and treatment strategies are needed for the improvement in the therapy of canine leishmaniasis and the accomplishment of a parasitological cure.

### **References:**

Alvar, J. Molina, R., San Andres, M., Tesouro, M., Nieto, J., Vitutia, M., Gonzalez, F., San Andres, M.D., Boggio, J., Rodriguez, F., Sainz, A., Escacena, C. Canine leishmaniasis: clinical, parasitological and entomological follow-up after chemotherapy. *Annals of Tropical Medicine and Parasitology*. 1994. 88: 371-378.

Cavaliero, T., Arnold, P., Mathis, A., Glaus, T., Hofmann-Lehmann, R., Deplazes, P. Clinical, serologic, and parasitologic follow-up after long-term allopurinol therapy of dogs naturally infected with *Leishmania infantum*. *Journal of Veterinary Internal Medicine*. 1999. 13: 330-334.





Chunge, C.N., Owate, J., Pamba, H.O., Donno, L. Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1990. 84; 221-225.

Denerolle, P., Boirdoiseau, G. Combination allopurinol and antimony treatment versus antimony alone and allopurinol alone in the treatment of canine leishmaniasis (96 cases). *Journal of Veterinary Internal Medicine*. 1999. 13: 413-415.

Gangneux, J.P., Dullin, M., Sulahain, A., Garin, Y.J., Derouin, F. Experimental evaluation of second-line oral treatments of visceral leishmaniasis caused by *Leishmania infantum*. *Antimicrob Agents Chemother*. 1999. 43: 172-174.

Ginel, P.J., Lucena, R., Lopez, R., Molleda, M. Use of allopurinol for maintenance of remission in dogs with leishmaniasis. *Journal of Small Animal Practice*. 1998. 39: 271-274.

Kuhlencord, A., Maniera, T., Eibl, H., Unger, C. Hexadecylphosphocholine: oral treatment of visceral leishmaniasis in mice. *Antimicrob Agents Chemother*. 1992. 36: 1630-1634.

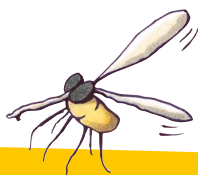
Lamothe, J. Essai de traitement de la leishmaniose canine par l'amphotericine B. 39 cas. *Pratique Medicale et Chirurgicale de l'Animal de Compagnie*. 1997. 32: 133-141.

Lamothe, J. Treatment of canine leishmaniasis. In: Killick-Kendrick, R (ed.) *Canine leishmaniasis: an update. Proceedings of the International Canine Leishmaniasis Forum, Barcelona, Spain*. 1999. Hoechst Roussel Vet. Germany. pp. 12-17.

Ling, G.V., Ruby, A.L., Harrold, D.R., Johnson, D.L. Xanthine-containing urinary calculi in dogs given allopurinol, *Journal of the American Veterinary Medical Association*. 1991. 198: 1935-1940.

Manson Bahr, P.E.C. Old World Leishmaniasis. In: Cox, F.E.G. (ed.) *Illustrated history of tropical diseases*. The Wellcome Trust. London. 1996. pp. 207-217.

Oliva, G., Gradoni, L., Ciaramella, P., De Luna, R., Cortese, L., Orsini, S., Davidson, R.N., Persechino, A. Activity of liposomal amphotericin B (AmBisome) in dogs naturally infected with *Leishmania infantum*. *Journal of Antimicrobial Chemotherapy*. 1995. 36: 1013-1019.



Poli, A., Sozzi, S., Guidi, G., Bandinelli, P., Mancianti F. Comparison of aminosidine (paromomycin) and sodium stibogluconate for treatment of canine leishmaniasis. *Veterinary Parasitology*. 1997. 71: 263-271.

Rhalem, A., Sahibi, H., Lasri, S., Jaffe, C.L. Analysis of immune responses in dogs with canine visceral leishmaniasis before, and after, drug treatment. *Veterinary Immunology and Immunopathology*. 1999. 71: 69-76.

Riera, C., Valladares, J.E., Gallego, M., Alisa, M.J., Catillejo, S., Fisa, R., Ribas, N., Carrio, J., Alberola, J., Arboix, M. Serological and parasitological follow-up in dogs experimentally infected with *Leishmania infantum* and treated with meglumine antimoniate. *Veterinary Parasitology*. 1999. 84: 33-47.

Scott, J.A.G., Davidson, R.N., Moody, A.H., Grant, H.R., Felminghad, D., Scott, G.M.S., Olliaro, P., Bryceson, A.D.M. Aminosidine (paromomycin) in the treatment of leishmaniasis imported into the United Kingdom. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1992. 86: 617-619.

Slappendel, R.J. and Teske, E. The effect of intravenous or subcutaneous administration of meglumine antimoniate (Glucantime®) in dogs with leishmaniasis. *The Veterinary Quarterly*. 1997. 19: 10-13.

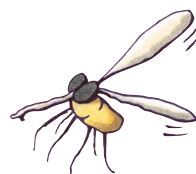
Slappendel, R.J. and Teske, E. A review of canine leishmaniasis presenting outside endemic areas. In: Killick-Kendrick, R (ed.) *Canine leishmaniasis: an update*. Proceedings of the International Canine Leishmaniasis Forum, Barcelona, Spain. 1999. Hoechst Roussel Vet. Germany. pp. 54-59.

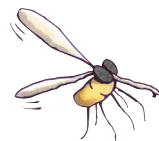
Steuber, S., Mortiz, A., Schirrmann, I., Greiner, M. PCR follow-up examination after treatment of canine leishmaniosis (CaL). *Tokai Journal of experimental and clinical medicine*. 1999. 23: 285-292.

Sundbar, S., Gupta, L.B., Makharia, M.K. Singh, M.K., Voss, A., Rosenkaimer, F., Engel, J., Murray, H.W. Oral treatment with miltefosine for visceral leishmaniasis. *Annals of Tropical Medicine and Parasitology*. 1999. 93: 589-597.

Tassi, P., Ormas, P., Madonna, M., Carli, S., Belloli, C., De Natale, G., Ceci, L., Marcotrigiano, G.O. Pharmacokinetics of N-methylglucamine antimoniate after intravenous, intramuscular and subcutaneous administration in the dog. *Research in Veterinary Science*. 1994. 56: 144-150.

Valladares, J.E., Alberola, J., Esteban, M., Arboix, M. Disposition of antimony after the administration of N-methylglucamine antimoniate to dogs. *Veterinary Record*. 1996. 138: 181-183.

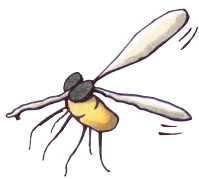


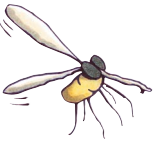


Vercammen, F., De Dekken, R., Kageruka, P. First evaluation of the use of allopurinol as a single drug for the treatment of canine leishmaniosis. *Vlaams Diergeneeskd Tijdschr.* 1995. 64: 208-214.

Vexenat, J.A., Olliaro, P.L., Fonesca de Castro, J.A., Cavalcante, R., Furtado Campos, J.H., Tavares, J.P., Miles, M.A. Clinical recovery and limited cure in canine visceral leishmaniasis treated with aminosidine (paromomycin). *American Journal of Tropical Medicine and Hygiene.* 1998. 58: 448-453.

Vexenat, J.A., Croft, S.L., Furtado Campos, J.H., Miles, M.A. Failure of buparvaquone (Butalex) in the treatment of canine visceral leishmaniasis. *Veterinary Parasitology.* 1998. 77: 71-73.

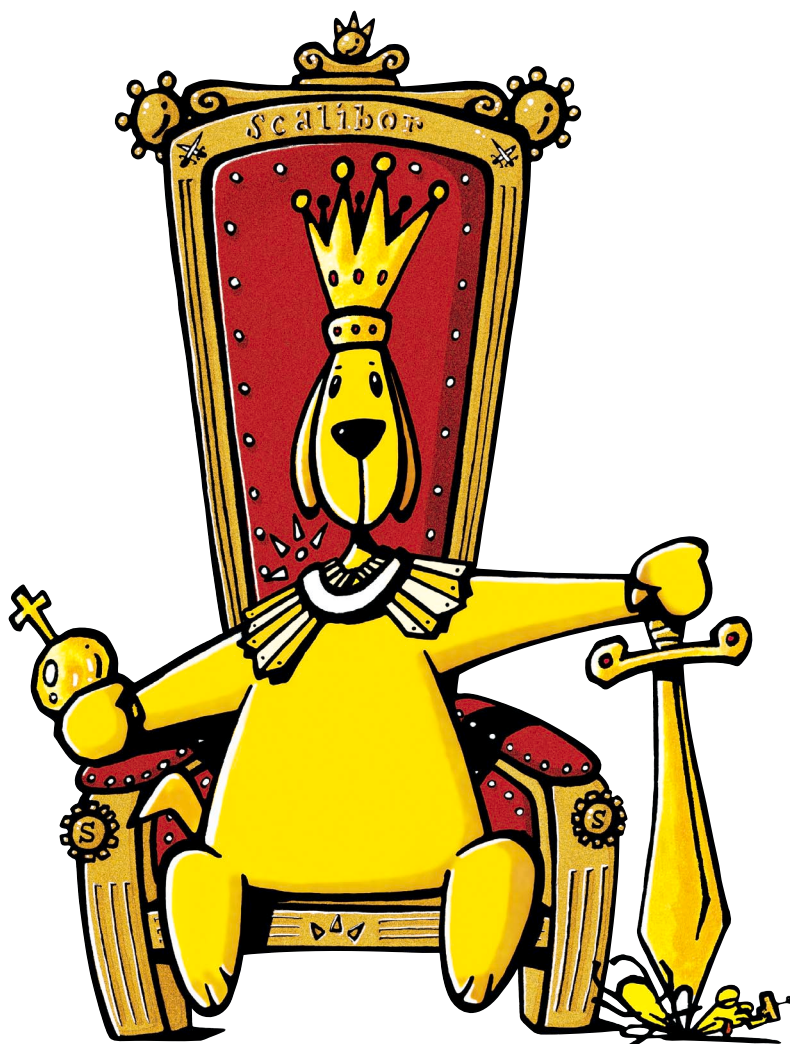






# The new power in parasite protection

## The broad spectrum Scalibor® ProtectorBand.



Protects against ticks,  
fleas & sand flies.

- New technology.
- Easy to use.
- Fast action.
- Protects dog & family all season long.

Scalibor® (active ingredient: Deltamethrin)

Presentation For veterinary use. Scalibor® ProtectorBand Necksize L (65 cm, brown or white): For large dogs. One collar (25g) contains 1 g deltamethrin. Marketing Authorisation No. 675 9370 dated 5.3.97 Scalibor® ProtectorBand Necksize S (48 cm, brown or white): For small and medium-sized dogs. One collar (19 g) contains 0.760 g deltamethrin. Marketing Authorisation No. 675 936.4 dated 5.3.97 Indications Infestations with deltamethrin-sensitive ectoparasites (fleas, sand flies, ticks). Prevention of infestations by ticks and sand flies for 6 months, prevention of infestations by fleas for 4 months. Contra-indications Not for dogs under 7 weeks. Precautions From clinical studies no teratogenic effect is known. As there are no studies on the safety during gestation, the use in pregnant dogs is not recommended. The ProtectorBand can be used during lactation period. Avoid use on animals with extensive cutaneous lesions. Keep out of reach of children. Wash hands carefully with soap and cold water after handling. Dosage and administration For external use only. The ProtectorBand should be adjusted to the dog's neck without pulling it too tight and the excess length be cut off leaving 5 cm up to memory clip. Protection lasts for 4 months (fleas) and for 6 months (ticks and sand flies). \*Optimal efficacy is reached one week after administration; therefore administration is recommended 1 to 2 weeks (ticks and fleas respectively) before the dog is allowed to infested areas. Overdosage In case of accidental ingestion the dog may show following symptoms: uncoordinated movements; tremor; hypersalivation. The effects are reversible within 48 hours. The antidote is diazepam.

\*Note: Efficacy claims may differ from country to country, according to local regulatory variations.

Intervet International bv • P.O. Box 31 • 5830 AA Boxmeer • The Netherlands  
Phone: +31 485 587600 • Fax: +31 485 577333 • E-mail: info@intervet.com • www.intervet.com

