Reliability of Serological Methods for Detection of Leishmaniasis in Portuguese Domestic and Wild Reservoirs


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A direct agglutination test (DAT) and an immunofluorescence technique (IFAT) were compared for detection of Leishmania infantum infection in 43 dogs and five foxes from Alto-Douro and Arrábida, two known endemic areas in Portugal. In four dogs with proved canine leishmaniasis, both DAT and IFAT showed positive readings (titres ≥1:320 and ≥1:128). Of 34 samples collected from apparently healthy dogs, ten were positive by both serological tests and eight were serologically positive by one test or the other. Three foxes out of five captured in this area, scored titres indicative of leishmaniasis in both DAT and IFAT. The concordance between DAT and IFAT in all collected samples (48) was 81.25%. Considering these and previous studies in the adjacent Mediterranean areas, the seroprevalence of L. infantum infection in the canine and vulpine populations appear to be of high magnitude.

Key words: canine - vulpine - leishmaniasis - direct agglutination test - immunofluorescence technique

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ing whole fluorescence detectable to the microscopist. On ground of previous results (Abranches et al. 1984), a titre of 1:128 was considered indicative of Leishmania infection in the canine or vulpine hosts.

Antigen for DAT performance was prepared at the Department of Medical Microbiology, University of Amsterdam, The Netherlands, according to the standard procedures later improved (Harith et al. 1988). The test was performed following the modified version for the canine reservoir (Harith et al. 1989). According to previous studies on evaluation of DAT for the canine reservoir (Harith et al. 1989, De Korte et al. 1990, Semião-Santos et al. 1995), 1:320 titre was taken as a cut-off for L. infantum infection in the canine and vulpine hosts.

Clinical examination of the studied population was carried out whenever possible. Absence of signs or symptoms resembling Leishmania infection were considered clinically negative. Dogs presenting all or some signs or symptoms characteristic of the disease (ex lymph node enlargement, skin involvement, abnormal locomotion) were considered clinically positive.

All dogs showing clinical signs or symptoms referable to Leishmania infection were submitted to parasitological examination; aspirated tissue from popliteal lymph nodes was obtained by injecting a small quantity of sterile Locke’s solution into the nodule. After aspiration the content of the syringe was smeared on to a glass slide, fixed with methanol and stained with Giemsa for microscopic examination according to the technique described by Lanotte et al. (1974) and Abranches et al. (1991). Foxes were subjected to histopathological examination. In one of the foxes, bone-marrow aspiration was performed and aspirate immediately inoculated in NNN in order to observe whether later, Leishmania promastigotes could be demonstrated.

A McNemar’s statistical test (Macnemar 1979) was applied to compare the results of matched sera in the IFAT and DAT tests.

RESULTS

Of the canine (43) and vulpine (5) sera tested, 22 (46%) scored positive results in DAT (≥ 1:320) and IFAT (≥ 1:128) (Fig.). Negative DAT (≤ 1:160) and IFAT (≤ 1:64) titres, were obtained in 17 (35%) canine samples. In 6 of the canine samples tested, weak positive titres were obtained with IFAT (1:128) and negative ones with DAT (≤ 1:160) and in 2 others, the DAT was positive and IFAT negative.

Out of 19 dogs found positive in both tests, 9 had symptoms suggestive of leishmaniasis. In 4 of those 9 dogs Leishmania amastigotes were found in lymph node aspirates. The other 5 were parasitologically negative. The remaining 10, were parasitologically and clinically negative. Of the 5 foxes tested, 3 were positive in both serological techniques and in these Leishmania amastigotes were found despite absence of clinical symptoms. One fox was negative in all tests and another was negative in DAT but positive in IFAT. Histopathological examination of the liver section in this fox showed no amastigote but a granuloma forming.

A Mcnemar’s statistical test (Macnemar 1979) was applied to compare the results of matched sera in the IFAT and DAT tests.

DISCUSSION

Previous results obtained in a Kenyan population including active and treated VL cases and endemic controls, showed good concordance (80%) of DAT and IFAT (Harith et al. 1987). Following further modifications in DAT procedures, sensitivity of DAT was significantly improved to monitor low antibody levels and prepatent infections in the human and the reservoir host (Harith et al. 1988, 1989).

Although the population studied here was rather small, the results obtained indicated a concordance
of 81.25% between DAT and IFAT. Recent studies in Central Tunisia (Ben Saïd et al. 1992) and in the south of Portugal (Semião-Santos et al. 1995) revealed even higher concordance between DAT and IFAT, of respectively 94.04% and 99.62% when evaluated in seroepidemiological studies aimed at local dog populations. In the study reported here, all four parasitologically confirmed dogs with leishmaniasis were positive in both tests. The same holds for the other five dogs with typical clinical symptoms of the disease. The positive readings obtained by both tests in ten apparently healthy dogs may indicate subclinical or prepatent *Leishmania* infection. This phenomenon was earlier pointed out by other scientists (Lanotte et al. 1979, Abranches et al. 1991). A comparatively high prevalence rate has been reported in a dog population in the south of France (De Korte et al. 1990). In another study, 12 out of 38 dogs with negative parasitological findings showed a positive response to anti-*Leishmania* chemotherapy (Harith et al. 1989). IFAT positivity in six of the cases was of a minimum value (titre 1:128) where DAT showed a borderline titre (1:40-1:160) in the vicinity of the cut-off.

The demonstration of *Leishmania* parasites in one of the foxes with negative DAT titres could possibly be due to the use of *L. donovani* as antigen instead of homologous or authochtonous *L. infantum*. A recent study (Harith et al. 1995) showed that by incorporating the reducing agent 2-mercaptoethanol (2-ME) instead of trypsin when processing DAT antigen and when simultaneously combined with the use of an indigenous strain in the process, both sensitivity and specificity levels are increased in human as well as in canine sera. Nevertheless, one should not eliminate the possibility of deterioration of serum or the fact of being in presence of one of the few "false positives" as demonstrated in an earlier study (Zijlstra et al. 1991).

Although presence of granulomas was observed in *Leishmania* infection (Abranches personnal communication) its presence in this study, in one fox, does not exclude infection due to other pathogens. To differentiate between *Leishmania* and other pathogens such as *Hepatozoon canis*, which is also prevalent in domestic and wild canids in this area (Conceição-Silva et al. 1988), more specific techniques are required. As reported in previous studies using DAT and IFAT, significant positive results were obtained in 16 dogs having early or prepatent leishmaniasis (Harith et al. 1989, De Korte et al. 1990, Semião-Santos et al. 1995). This again emphasizes the need to incorporate reliable serological techniques in routine surveillance and control of leishmaniasis in the south of Europe. However, the need for anti-immunoglobulins specific to incriminated reservoirs constitute difficulties in applying IFAT and similar techniques for regular epidemiological surveys.

The importance of the fox as wild reservoir for *Leishmania* in the Mediterranean region has not yet been throughly evaluated. Both the domestic and wild reservoir should be considered for proper control of leishmaniasis in the Mediterranean areas. The steadily growing tourist traffic in this area and the increase in the number of acquired immunodeficiency syndrome (AIDS) cases necessitate a broader approach towards transmission of infections due to *L. infantum*.

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