

SHORT COMMUNICATION

Comparison between Splenic and Lymph Node Aspirations as Sampling Methods for the Parasitological Detection of *Leishmania chagasi* Infection in Dogs

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The sensitivities of spleen and lymph node cultures for the diagnosis of canine visceral leishmaniasis were compared in 64 anti-Leishmania antibody positive dogs from an endemic area in Brazil. The sensitivity of spleen cultures for Leishmania detection was 97.9%; in lymph node cultures it was 25%. Positive spleen culture was more frequent ($p = 0.048$, Fisher's exact probability test) in symptomatic (28 out of 33 animals) than in asymptomatic animals (19 out of 31 animals). These results support the use of spleen instead of lymph node aspiration as the choice method for the parasitological diagnosis of the infection.

Key words: *Leishmania chagasi* - dog - diagnosis - Brazil

Zoonotic visceral leishmaniasis is caused by *Leishmania chagasi/Leishmania infantum* (Herwaldt 1999). The disease is endemic in American, European, and Asiatic countries (Pearson & Sousa 1996, Herwaldt 1999) with new areas of infection dissemination being identified (Enserink 2000, Silva et al. 2001). The fact that dogs are considered the most important urban reservoir of *L. chagasi* makes canine visceral leishmaniasis (CVL) not only a major veterinary concern, but also a so far unsolved public health problem. Accurate diagnosis of canine infection by *Leishmania* is essential in veterinary practice, since the treatment of infected dogs includes 15-20 daily injections of potentially toxic drugs, and in some countries, killing of infected animals is one of the measures applied in order to control the disease dissemination (Costa & Vieira 2001, Moreno & Alvar 2002). Dogs with symptomatic disease have fever, anemia, skin lesions, emaciation, hypergammaglobulinemia, hepato-splenomegaly, and lymphadenomegaly, among other signs of the disease (Feitosa et al. 2000, Moreno & Alvar 2002). The main pathological substrate of visceral leishmaniasis is amastigote multiplication in the mononuclear phagocytic

cells of internal organs such as spleen, liver, and bone marrow (Oliveira et al. 1993). In dogs, parasites are also found in peripheral lymph nodes and skin. The direct examination or cultivation of aspirates from spleen or bone marrow are the mainstay techniques used for the diagnosis of visceral leishmaniasis in both human beings and dogs. In fact, high sensitivity has been attributed to the parasitological diagnosis based on bone marrow or spleen examination in canine (Ashford et al. 1995) and human (Pearson & Sousa 1996, Pintado et al. 2001) visceral leishmaniasis. Some authors, however, have used popliteal lymph node aspiration for parasite demonstration in CVL (Mathis & Deplazes 1995, Ozensoy et al. 1998, Santos-Gomes et al. 2002). The reasons for using lymph node instead of spleen or bone marrow for diagnosis of CVL is the more peripheral location of lymph nodes (such as popliteal lymph node usually used in the procedure) and concerns about safety of the procedures (i.e. a possible risk of internal hemorrhage following spleen or liver puncture). However, no studies, to the best of our knowledge, have comparatively assessed the advantages and limitations of lymph node over internal organ examinations for the diagnosis of CVL.

In order to perform aspirative biopsies from lymph node or spleen, slight sedation must be induced in the animal, using pre-anesthetic drugs. Both procedures require technical training that do not involve different levels of difficulty. The main risks that can be anticipated for these procedures are spleen hemorrhage and popliteal neurovascular plexus lesion. In our group's practice, however, after more than 300 spleen puncture and more than 100 lymph node puncture, none of these interferences was observed, and only slight signs of discomfort were observed during lymph node puncture under sedation.

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In this study, the sensitivities of splenic and popliteal lymph node cultures for diagnosis of CVL were compared. We examined 64 dogs from Monte Gordo, an endemic area for visceral leishmaniasis in the state of Bahia, Brazil. These animals varied in age (from 4 months to 9 years), sex (38 male, 26 female) and breed. All dogs had positive serological tests (ELISA or IF) for anti-*Leishmania* antibodies. Thirty one animals were asymptomatic, 26 had a few clinical signs of disease (oligosymptomatic), and 7 had multiple signs of visceral leishmaniasis (polysymptomatic), including emaciation, anemia, skin lesions around the nose, eyes, and on limbs, increase in size of lymph node and spleen and onicophrosis (Table).

TABLE

Clinical categories and presence of parasite in culture lymph node and spleen aspirates of dogs with natural infection with *Leishmania chagasi*

Clinical category	Spleen	Spleen and lymph node		None	Total
		Lymph node	lymph node		
Asymptomatic	16	-	3	12	31
Oligosymptomatic	17	-	5	4	26
Polysymptomatic	3	1	3	-	7
Total	36	1	11	16	64

The animals were tranquilized with an intravenous injection of 0.5 mg/kg of acepromazine and laid down on the right lateral side. Hairs were removed from the skin over the sites of puncture, and asepsis was done with a 2% alcohol-iodine solution. Splenic puncture was done in the left flank, near the edges of the last two ribs, using a 40 x 12 mm needle and a 10 ml syringe. The lymph node aspirates were done in the left popliteal lymph node using a 25 x 8 mm needle and a 10 ml syringe. The spleen and lymph node samples (approximately 100-200 µl) were cultured in a biphasic medium containing 1.5 ml of blood-agar solid medium and 2 ml of Schneider's medium supplemented with 20% of fetal calf serum. Cultures were maintained at 23°C and examined weekly under light microscopy during one month.

As shown in the Table, 16 out of the 64 dogs studied had negative spleen and lymph node cultures for *Leishmania*, 11 (17.2%) had both spleen and lymph node positive cultures, 36 (56.2%) had only spleen culture positive, and 1 (1.6%) had only lymph node positive cultures. Therefore, sensitivity of spleen culture for diagnostic of *L. chagasi* was 73.4% (47/64), and of lymph node culture was 18.8% (12/64), both using serology as reference. Considering only the animals with infection confirmed in culture, the sensitivity of spleen culture for diagnostic of *L. chagasi* was 97.9%, and of lymph node culture was 25%. Positive spleen culture was more frequent ($p = 0.048$, Fisher's exact probability test) in symptomatic (28 out of 33 animals, = 84.8%) than in asymptomatic animals (19 out of 31 animals, = 61.3%). There was no difference in the presence of positive lymph node culture between symptomatic and asymptomatic animals.

The results presented herein support the use of spleen instead of lymph node aspiration as the choice method for parasitological diagnosis of CVL caused by *L. chagasi*. Also, spleen puncture appeared to be better tolerated than the lymph node puncture, even in the most clinically affected dogs, exhibiting severe anemia and caquexia. The anatomic site where the popliteal lymph node is located is rich in nerves and nervous fibers, originated from the lombossacral plexus, which are susceptible to accidental traction and physical trauma during lymph node puncture. This fact may explain the transitory signs of pain demonstrated by most animals.

An interesting aspect of this work is the discrepancy of our findings on the sensitivity of lymph node aspirates with the experience reported by other authors in the literature (Mathis & Deplazes 1995, Reale et al. 1999). Popliteal lymph node culture has been considered as a sensitive method for diagnosis (Mathis & Deplazes 1995) and follow-up (Riera et al. 1999) of dogs with CVL. Reale et al. (1999) and Ozbel et al. (2000), on the other hand, found direct examination more effective than culture for the diagnosis of CVL, using total blood or lymph node aspirate. The low sensitivity of lymph node culture in the diagnosis of CVL observed in the study described herein cannot be attributed to poor parasite growth in culture due to culture conditions, since the sensitivity of spleen cultures performed under similar conditions and using specimens from the same animals was high. Contamination by microorganisms such as fungus or bacteria does not explain also the difference between spleen and lymph node culture sensitivities reported herein, since these cultures remained free of visible contamination for at least three weeks, a sufficient time for promastigote identification even under conditions of low inocula (Buffet et al. 1995). Another factor, which affects the ability to detect *Leishmania* in different tissues, is the host immunological status. *Leishmania* dissemination to unusual sites and high parasite burden are observed in hosts with severe impairment of the immunological response (Alvar et al. 1997). Generally, animals with clinical forms of CVL are in a more advanced stage of the infection and have depression of the cellular immune response to *Leishmania* (Rhalem et al. 1999). No differences in the frequency of positive lymph node culture between polysymptomatic animals and those with less severe disease, however, were observed in the present work. Finally, actual difference may exist in the tissue distribution of *L. chagasi* to the spleen and lymph node. Some studies reporting high sensitivity of lymph node examination for diagnosis of CVL were produced in Europe, where *L. infantum* is the infecting agent (Mathis & Deplazes 1995, Reale et al. 1999). Evidences exist that *L. chagasi* and *L. infantum* are the same *Leishmania* species (Mauricio et al. 2002), probably introduced into the American Continent during European colonization. Hence, one of the possible explanations for the discrepancy between our data and that presented by those authors reporting high sensitivity of lymph node aspiration is the existence of variations in the *Leishmania* strains causing CVL in the Old and in the New World. In fact, the ability for disseminating and causing lesions at different sites such as skin and internal organs varies

with the zymodeme expressed by different strains of *L. infantum* (Alvar et al. 1997, Sulahian et al. 1997). Similarly, and not yet well understood, differences in parasite distribution are also observed with strains of *L. chagasi* (Noyes et al. 1997). Further studies are necessary in order to identify the factors responsible for the putative differences in the ability of *L. chagasi/L. infantum* strains to disseminate to lymph nodes and also the molecular mechanisms involved in *Leishmania* tropism to different tissues.

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