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Sporotrichosis—The main differential diagnosis with tegumentary leishmaniosis in dogs from Rio de Janeiro, Brazil

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Abstract

Seventy-four dogs from the State of Rio de Janeiro with ulcerated cutaneous lesions were submitted to clinical, dermatological, parasitological, mycological, histopathological and cytopathological exams, a leishmanin skin test, an indirect immunofluorescence (IIF) test for leishmaniosis, and nonspecific laboratory tests such as blood count and serum biochemistry. *Sporothrix schenckii* was isolated from 41 dogs and *Leishmania* (*Viannia*) *braziliensis* was isolated from 33 animals. Most dogs with sporotrichosis were from the municipality of Rio de Janeiro (53.7%) and presented ulcerated cutaneous lesions on the head (68.3%). Laboratory alterations in these animals included anemia (58.5%), hypoalbuminemia (83%) and hyperglobulinemia (75.6%). Histopathology revealed the predominance of a chronic granulomatous inflammatory infiltrate (70.7%), and yeast-like structures were detected in 17% of the histopathological exams and in 32% of the cytological exams. Three of 41 dogs with sporotrichosis were seropositive by IIF for leishmaniosis and 2 of 20 animals tested within this group had a positive leishmanin skin test. Similarly, most of the 33 dogs with leishmaniosis were from the municipality of Rio de Janeiro (69.7%) and had ulcerated cutaneous lesions on the head (84.8%). Laboratory alterations in these animals included anemia (66.7%), hypoalbuminemia (100%) and hyperglobulinemia (91%). Histopathology showed the predominance of a chronic granulomatous inflammatory infiltrate (63.6%) and amastigote forms were detected in 30.3% of the histopathological exams and in 31.8% of the 22 cytological exams performed. About 72.7% of the dogs were seropositive by IIF and five of seven animals had a positive skin test. Due to the clinical similarities, histopathological and nonspecific laboratory results similarities, the serological and skin tests for leishmaniosis positive in dogs with sporotrichosis, and the overlapping endemic areas in Rio de Janeiro, the differential diagnosis between the two diseases requires the demonstration of their respective etiological agents.
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Keywords: Sporotrichosis; Tegumentary leishmaniosis; Differential diagnosis; Dog

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1. Introduction

Sporotrichosis is a mycosis caused by the dimorphic fungus *Sporothrix schenckii*, which is widely distributed in nature especially in temperate and tropical climates (Rippon, 1988). Infection occurs through transcutaneous inoculation of the fungus and is limited to the skin and subcutaneous tissue. Pulmonary infection due to the inhalation of conidia is rare (Know-Chung and Bennet, 1992). Canine sporotrichosis has been occasionally described and is characterized by ulcerated cutaneous lesions on the nose, auricle and limbs (Farias et al., 1997; Schubach et al., 2006).

American tegumentary leishmaniosis (ATL) in Rio de Janeiro is a zoonosis caused by *Leishmania* (*Viannia*) *braziliensis*, a parasite widely found in Brazil. In the state of Rio de Janeiro, a pattern of domiciliary and peridomiciliary transmission predominates as a result of the adaptation of the vector, *Lutzomyia intermedia*, to the environment modified by man (Oliveira-Neto et al., 1988; Marzochi and Marzochi, 1994). ATL can affect the skin and mucosae separately or in combination. In dogs, ATL is characterized by the presence of ulcerated cutaneous lesions on the ears, nose, scrotal bag and limbs (Padilla et al., 2002).

Since 1998, the Evandro Chagas Clinical Research Institute (IPEC)-Fiocruz has been monitoring an epidemic of sporotrichosis affecting dogs, cats and humans (Barros et al., 2001, 2004; Schubach et al., 2004, 2006), whose main differential diagnosis has been ATL. In the present study, we compare the clinical and laboratory characteristics of 41 dogs with sporotrichosis with those of 33 dogs with ATL.

2. Materials and methods

Seventy-four dogs with ulcerated cutaneous lesions were seen at the outpatient clinic of the Zoonosis Service of IPEC. Twenty-two of these dogs have been previously described by Schubach et al. (2006).

All the animals were submitted to clinical and dermatological examination, cutaneous lesion biopsy, and venous blood collection for blood count, serum biochemistry and serological tests for leishmaniosis, and 27 were submitted to leishmanin skin test. The serological tests were performed according to the instructions of the indirect immunofluorescence (IIF) kit produced by Biomanguinhos-Fiocruz. Samples showing fluorescence at a dilution of 1:40 or higher were considered to be positive. Positive and negative controls were tested on each slide. The skin test was carried out using *L.* (*V.*) *braziliensis* antigen (MHO/BR/86/DCB-02) produced by Biomanguinhos-Fiocruz, containing 2 mg protein/ml physiological saline stored in 0.4% phenol. An intradermal injection of 0.1 ml antigen was applied to the internal side of the thigh of the animals and the largest diameter of induration was measured 48 h later (Sokal, 1975).

Incisional biopsies were obtained from the borders of active cutaneous lesions with a 3–4 mm punch after asepsis and local anesthesia with 2% lidocaine. Each specimen was divided into three fragments: one fragment was fixed in 10% buffered formalin and embedded in paraffin for histopathological analysis after staining with hematoxylin–eosin, periodic acid Schiff (PAS) and Grocott's silver stain. Before fixation, a smear was obtained by apposition of the fragment on a glass slide, followed by fixation of the slide in methanol and staining with Giemsa (Luna, 1968), for cytopathological exam. The second fragment was seeded onto biphasic medium (NNN plus Schneider's medium supplemented with 10% fetal bovine serum) for the detection of the promastigote forms of *Leishmania* sp. (Chang and Hendricks, 1985). The isolates were identified by isoenzyme electrophoresis (Cupolillo et al., 1994). The third fragment was seeded onto Mycobiotic medium (Difco) and incubated at 25 °C, for mycological culture. Isolates of *S. schenckii* were replated on BHI agar (Difco) and incubated at 37 °C for observation of conversion into the yeast form (Rippon, 1988; Werner and Werner, 1994).

3. Results

3.1. Mycological and parasitological culture

Sporothrix schenckii was isolated from biopsies of cutaneous ulcers of 41 dogs, which had parasitological culture negative. *L.* (*V.*) *braziliensis* was isolated and identified in ulcerated cutaneous lesions of 33 (44.6%) dogs, which had mycological culture negative.

3.2. Dogs with sporotrichosis

Twenty-two (53.7%) animals were from the city of Rio de Janeiro and 19 (46.3%) were from neighboring municipalities. There was a predominance of male dogs ($n = 23$, 56.1%) and 17 (41.4%) dogs were of mixed breed. The median age was 4 years (range, 6 months to 9 years), 37 (90.2%) dogs were in good general health, and 30 (73.2%) lived together with cats with sporotrichosis.

The predominant cutaneous lesions were round ulcers with elevated borders and a granular bottom, sometimes covered with secretion and crusts. Most

lesions were located on the head ($n = 28$), mainly the nose ($n = 20$, 48.8%) and ears ($n = 6$, 14.6%). The number of lesions ranged from 1 to 10 (median = 3), with most animals presenting two lesions (31.7%). Twenty-five (61%) dogs had lesions at a single site, 6 (14.6%) at two sites, and 10 (24.4%) at multiple noncontiguous sites. Nineteen (46.3%) dogs presented localized lymphadenitis and nine (22%) had generalized lymphadenitis. Respiratory signs such as sneezing, nasal secretion and dyspnea were observed in 23 (56.1%) animals.

The most frequent hematological and biochemical alterations were anemia in 24 (58.5%) cases, hypoalbuminemia in 34 (83%) and hyperglobulinemia in 31 (75.6%).

Histopathological analysis revealed the predominance of a chronic inflammatory infiltrate with granuloma formation ($n = 29$, 70.7%) and the presence of yeast-like structures in seven (17%) cases. Cytopathological exams performed on 28 dogs identified yeast-like structures in 9 (23%) smears.

The skin test was performed on 20 animals. Two (10%) of these animals tested positive, with the diameter of induration ranging from 6 to 7 mm. Other three (15%) dogs were seroreactive in the leishmaniosis IIF test (Table 1).

3.3. Dogs with leishmaniosis

Twenty-three (69.7%) dogs were from the municipality of Rio de Janeiro, four (12.1%) from Maricá, and six (18.2%) were from other towns. Age ranged from 3 months to 8 years (median = 36 months). All dogs were of mixed breed, 27 (81.8%) were in good general health, and 23 (69.7%) were males.

The lesions were round ulcers with elevated borders and a granular bottom, sometimes covered with secretion and crusts. In most animals the lesions were located on the head ($n = 28$, 84.8%), mainly the ears (54.5%) and nose (36.4%). The number of lesions ranged from 1 to 5 (median = 2), with most animals presenting two lesions (36.4%). Lesions were found at a single site in 21 (63.6%) animals, at two sites in 11 (33.3%), and at multiple sites in 1 (3%). Thirteen (39.4%) animals had localized lymphadenitis and seven (21.2%) had generalized lymphadenitis. Signs such as apathy and weight loss were observed in 18.2% ($n = 6$) of the animals.

Anemia was detected in 22 (66.7%) cases. The most frequent biochemical alterations were hypoalbuminemia in 100% ($n = 33$) and hyperglobulinemia in 91% ($n = 30$) of cases.

Table 1
Indirect immunofluorescence (IIF) titers of reactive dogs

	Number of dogs	IIF titer
Sporotrichosis ($n = 3$)	3	1:80
Leishmaniasis ($n = 24$)	3	1:40
	9	1:80
	8	1:160
	3	1:320
	1	1:640

Histopathological analysis showed the predominance of a chronic inflammatory infiltrate with granuloma formation ($n = 21$, 63.6%) and the presence of amastigote forms in 30.3% ($n = 10$) of cases. Twenty-two dogs were submitted to cytopathological examination and seven of them (31.8%) were positive for amastigote forms.

The skin test was performed on seven dogs and five (71.4%) were reactive. The induration diameter ranged from 6 to 10 mm (median = 7). In the serological tests, 72.7% ($n = 24$) of the dogs were seroreactive in the IIF test (Table 1).

4. Discussion

Due to the clinical and laboratory similarities between the two diseases and overlapping endemic areas, sporotrichosis has become the main differential diagnosis for ATL in dogs from Rio de Janeiro. These similarities may lead to false diagnoses when the etiological agents are not identified.

Seventy-four dogs with ulcerated cutaneous lesions were studied over a period of 2 years, with *S. schenckii* being isolated from 55.4% of these cases and *L.* (*V.*) *braziliensis* from 44.6%.

Canine sporotrichosis is considered to be a rare disease, with isolated cases being reported in the literature (Freitas et al., 1965; Kier et al., 1979; Gonzalez cabo et al., 1989). However, since 1998 the Zoonosis Service of IPEC-Fiocruz has been observing a growing number of canine cases in Rio de Janeiro, suggesting the occurrence of this disease at a certain frequency in the region (Barros et al., 2001, 2004; Schubach et al., 2004, 2006). In parallel to the growing number of canine sporotrichosis cases, an increase in the number dogs with ATL seen at this service has been noted (Schubach et al., 2006).

Canine ATL is frequent in Rio de Janeiro and overlaps with visceral leishmaniosis in the region of Gericinó and Pedra Branca massifs (Madeira et al., 2004). The disease is difficult to treat because the drugs used are highly toxic

and can cause severe side effects, clinical cure occurs without parasitological cure, and recurrences after treatment are frequent (Noli and Auxilia, 2005). The control measures applied are distinct for each disease. In the case of ATL, insecticides are sprayed inside and outside the dwelling in endemic areas and dogs are fitted with deltamethrin-impregnated collars (Killick-Kendrick et al., 1997; Noli, 1999). The importance of dogs in the transmission of ATL remains unknown, but, in areas where the disease overlaps with visceral leishmaniosis, euthanasia of seroreactive animals with IIF titers of at least 1:40 is practiced (Ministério da Saúde, 2003), although the serological tests may show false-positive results due to cross-reactions (Reed et al., 1990; Reithinger and Davies, 1999).

Canine sporotrichosis is easy to treat and shows a good prognosis. Dogs do not seem to be directly involved in the transmission of the disease in view of the scarcity of yeast-like structures in cutaneous lesions, the absence of *S. schenckii* in the oral cavity, and the lack of human cases associated with the transmission through dogs in the current epidemic in Rio de Janeiro (Schubach et al., 2006). Since cats have been indicated as the most important vector in the transmission of the disease (Schubach and Schubach, 2000; Schubach et al., 2004), no control measures for canine sporotrichosis are available.

These factors support the importance of the demonstration of the etiological agent for the differential diagnosis between the two diseases, since false diagnoses of leishmaniosis may lead to the unnecessary elimination of dogs. Generally, the differential diagnosis between sporotrichosis and ATL is made based on epidemiological evidence, clinical aspect of the lesion and serology for leishmaniosis. However, the present study demonstrated that these criteria are not sufficient to differentiate the two diseases.

The predominant findings in dogs with sporotrichosis were cutaneous ulcers located on the head, especially nose and ears, accompanied by localized lymphadenitis, in agreement with other authors (Migliano et al., 1963; Freitas et al., 1965; Schubach and Schubach, 2000). In dogs with leishmaniosis, the lesions were similar to those described above in all aspects (Fig. 1A and B), in agreement with other studies (Pirmez et al., 1988; Padilla et al., 2002), a fact demonstrating the lack of clinical and dermatological differences between ATL and sporotrichosis.

Histopathological analysis revealed the predominance of a chronic inflammatory infiltrate accompanied by a granuloma in both diseases. Similar findings have been reported by other investigators (Freitas et al., 1965; Scoot et al., 1973; Pirmez et al., 1988; Farias et al., 1997). Yeast-like structures of *S. schenckii* and amastigote forms of *Leishmania* sp. were observed in a minority of cases, similar to the findings of Scoot et al. (1973), Farias et al. (1997) and Pirmez et al. (1988). *S. schenckii* yeast-like structures and *Leishmania* sp. amastigote forms have a similar size and can be easily confused upon histopathological examination of hematoxylin/eosin-stained specimens (Schubach et al., 2006). Silver or PAS staining is indicated for the differentiation of these forms. However, isolation in culture is necessary for the identification of the yeast species.

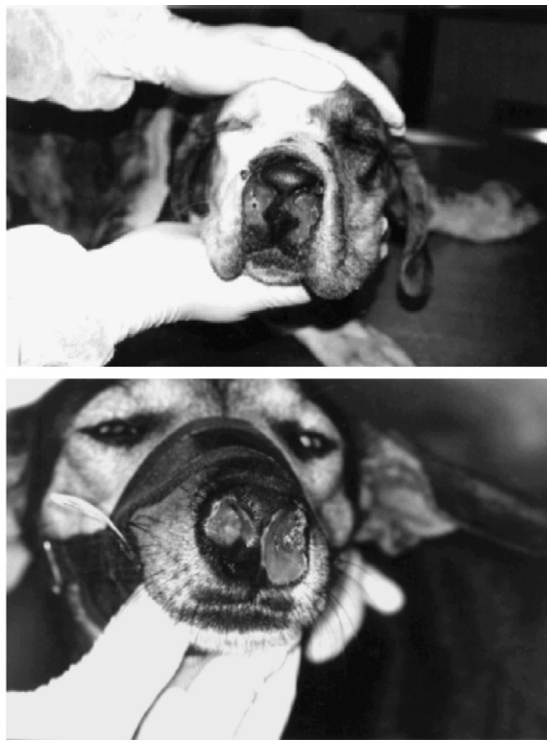

Fig. 1. (A) Dog with cutaneous ulcers located on the nose caused by *Sporothrix schenckii*. (B) Dog with cutaneous ulcers located on the nose caused by *Leishmania* (*Viannia*) *braziliensis*.

The low frequency of positivity and the possibility of confusing amastigote and yeast forms in the cytopathological exam suggest that this method is not adequate for the differential diagnosis between sporotrichosis and ATL in dogs.

Hematological alterations such as anemia and biochemical abnormalities such as hypoalbuminemia and hyperglobulinemia were observed both in dogs with ATL and dogs with sporotrichosis. These findings disagree with Scoot et al. (1973) who did not detect nonspecific laboratory alterations in dogs with sporotrichosis, and with Migliano et al. (1963) who observed leukocytosis in dogs with sporotrichosis.

Regarding the serological tests for *Leishmania* sp., seroreactive dogs with sporotrichosis inhabited areas that were nonendemic for leishmaniosis and not had a history of previous exposure to *Leishmania* sp. These results may have triggered control actions including the unnecessary elimination of these dogs due to an equivocal diagnosis of leishmaniosis. Two dogs with sporotrichosis had a positive skin test, a finding also suggesting a false diagnosis of leishmaniosis due to a possible cross-reaction, allergy to the reagent diluent or co-infection as observed in humans (Barros et al., 2005).

The mycological and parasitological culture has a good sensitivity and specificity to detect *S. schenckii* (Schubach et al., 2006) and *Leishmania* sp. (Faber et al., 2003), respectively. In this study, cultures positive for *S. schenckii* and negative for *Leishmania* sp. were obtained for all dogs with sporotrichosis, and vice versa, demonstrating that the differential diagnosis should be made based on the isolation of the etiological agent.

5. Conclusion

Due to the lack of clinical and laboratory differences, the differential diagnosis between sporotrichosis and ATL in dogs should be made based on the demonstration of the respective etiological agents in material obtained from the cutaneous lesions.

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