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CASE REPORT

Isolation and Biological and Molecular Characterization of *Toxoplasma gondii* from Canine Cutaneous Toxoplasmosis in Brazil

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Cutaneous toxoplasmosis is a rare manifestation. This study represents a case report of an immunosuppressed dog that developed nodular dermal lesions caused by *Toxoplasma gondii*. The isolate (TgDgBr20) was characterized as mouse virulent and was genotyped as type BrI (ToxoDB genotype 6) using PCR-restriction fragment length polymorphism (RFLP) and as Africa 1 through microsatellite analysis.

CASE REPORT

A male, mixed-breed, approximately 2-year-old dog was found on the street and adopted. The dog was taken to a private veterinary clinic in the city of São Paulo, São Paulo State, Brazil, 2 months after adoption and was diagnosed with severe erythroid and myeloid aplasia, megakaryocytic aplasia, myelonecrosis with lymphoplasmocytic infiltration, and grade II fibrosis. The animal weighed 18 kg and had good body condition (score of 7 to 8/9, according to the Laflamme body condition scoring system for dogs).

Immunosuppressive therapy was initiated with prednisone (2 mg/kg of body weight twice a day [BID]), which was gradually replaced with cyclosporine (CsA) 20 days after the beginning of treatment because the animal developed side effects from corticosteroid therapy. Combined drug therapy at doses of 2 mg/kg BID of prednisone and 10 mg/kg BID of CsA was started. The prednisone dose was gradually reduced by 25% per week, and the CsA dose of 10 mg/kg BID was maintained.

Two and a half months after the start of the immunosuppressive therapy, when only CsA was being used, the dog was noticed to have dermal lesions. These lesions were initially small, hard, and slightly erythematous epidermal nodules approximately 1 cm in diameter that rapidly evolved to large hard nodules with diameters of between 2 and 6 cm that were erythematous and ulcerated with drainage of purulent material. These lesions were found dispersed all over the body of the animal (see Fig. S1 in the supplemental material). The material collected from the skin lesions was sent for fungal and bacterial cultures, with negative results.

Direct smears obtained from the nodular material were stained with Giemsa stain, and the animal was diagnosed with sporotrichosis by a private laboratory. The Giemsa-stained slides and material collected by fine-needle aspiration biopsy were then sent to the Laboratory of Parasitic Diseases, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil. Structures that were particularly extracellular and morphologically compatible with tachyzoites were found on the Giemsa-stained smears as individuals or groups of organisms, as well as tissue cysts (Fig. 1).

The dog serum was tested for antibodies to *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* (*Leishmania*) *infantum chagasi*, and *Leishmania amazonensis* IgG antibodies using the indirect fluorescent antibody test (IFAT) with cutoffs of 1:16, 1:50, and 1:40, respectively, for *T. gondii*, *N. caninum*, and the *Leishmania* species (1, 2, 3); only *T. gondii* antibodies were detected, and the titer was high (1:65,536).

Based on these results, treatment with trimethoprim-sulfamethoxazole (Bactrim) at a dose of 30 mg/kg BID was initiated; the dog had a positive response (see Fig. S2 in the supplemental material), and the lesions disappeared by 28 days after the initiation of treatment. The *T. gondii* antibody titer also declined, to 1:2,048.

DNA was extracted from the material scraped from the Giemsa-stained slides using a phenol-chloroform method (4). The DNA was examined by nested PCR for the detection of a 155-bp fragment of the B1 gene of *T. gondii* (5) and by seminested PCR for the detection of a 227-bp fragment of the NC-5 gene of *N. caninum* (6), thereby confirming the diagnosis as *T. gondii*.

Material obtained by needle aspiration from the nodules was used to attempt the isolation of protozoa. Two mice and three gerbils subcutaneously inoculated developed acute toxoplasmosis and died 9 to 11 days postinoculation (dpi); tachyzoites were observed in direct smears from the lungs and peritoneal exudate. Tachyzoites were also observed 23 dpi in cell culture using the CV-1 cell line (*Cercopithecus aethiops* monkey kidney cell line).

DNA extraction was also performed using lungs and peritoneal

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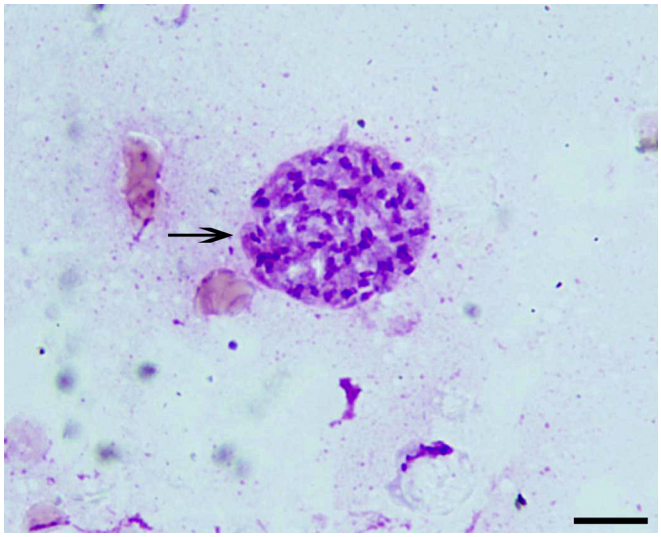

FIG 1 Fine-needle-aspiration sample of a skin nodule in a dog. Giemsa-stained *Toxoplasma gondii* cyst is shown. Bar = 10 μm.

exudate from rodents and tachyzoites from cell culture for *T. gondii* genotyping studies. The genotypic characterization of the isolate, referred to as TgDgBr20, was performed with PCR-restriction fragment length polymorphism (RFLP) using 11 markers. The primers, reaction solutions, and PCR conditions have been previously described (7, 8). Genotyping was also performed, using 15 microsatellite markers and following published protocols (9). The atypical genotype (ToxoDB PCR-RFLP genotype 6), which corresponds to type BrI (8), was obtained by PCR-RFLP; when using microsatellite analysis, the isolate corresponded to the atypical genotype Africa 1 (10).

To assess the virulence of the *T. gondii* isolate, BALB/c mice (6 animals/group) were intraperitoneally infected with 1×10^4 or 1×10^2 tachyzoites of the TgDgBr20 isolate or of the RH strain (clonal type I) or with 50 and 20 tissue cysts of the Me-49 (clonal type II) strain of *T. gondii*. The differences in survival rates between groups were compared using the log rank and χ^2 tests. Differences were considered statistically significant for P values of <0.05.

According to the survival curves (see Fig. S3 in the supplemental material), the animals infected with isolate TgDgBr20 had a survival rate that was very similar to that of the mice infected with the RH strain, with no surviving mice in either group at 7 or 8 dpi, whereas 100% of the animals infected with the Me-49 strain remained alive at 30 dpi.

The Scientific Committees of Universidade de São Paulo and Universidade Federal de Uberlândia authorized the laboratory animal proceedings performed in this study.

Cutaneous manifestations of toxoplasmosis are rare in animals and humans, with only three reported cases in dogs (11, 12), and the animals were also receiving immunosuppressive therapy. Whether the strain of *T. gondii* (TgDrBr20) contributed to the severity of the disease in the present dog is unknown. *T. gondii* isolates of type BrI, as in this case report, have been obtained from asymptomatic animals in Brazil and have also been reported in clinical human cases of toxoplasmosis (13). The microsatellite characterization of TgDrBr20 revealed that it has a unique genotype.

Neospora caninum, the protozoan most closely related to *T. gondii*, is a common cause of dermatitis in dogs (14). The results of the present study emphasize the need for inclusion of toxoplasmosis in differential diagnosis of protozoal dermatitis in dogs.

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