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Pulmonary sarcocystosis in a puppy with canine distemper in Costa Rica

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Abstract. Canine distemper and pulmonary sarcocystosis were diagnosed in a 10-week-old Rottweiler with 4-day history of diarrhea, vomiting, and weakness. Microscopic examination of the lung revealed bronchointerstitial pneumonia typical of morbillivirus infection. Also, numerous apicomplexan parasites were scattered in the alveolar walls. This protozoan infection was first thought to be toxoplasmosis but immunoperoxidase staining revealed large numbers of *Sarcocystis canis*. This is the first case of canine sarcocystosis reported from Latin America that further emphasizes the importance of immunohistochemistry in the differential diagnoses of apicomplexan infections in dogs.

Sarcocystis spp. are 2-host (prey–predator) apicomplexan parasites that frequently encyst in the skeletal and cardiac musculature of healthy ruminants and to a lesser extent in the same musculature of birds, reptiles, and humans.[1] Although the cystic stages in musculature are generally asymptomatic, the immature stages or schizonts parasitize the vascular endothelium and may cause clinical signs and sporadic instances of death in susceptible animals.[1,8] Clinical diseases caused by *Sarcocystis* spp. are collectively referred to as sarcocystosis or sarcosporidiosis and are prevalent in farm animals, particularly in fetal and neonatal ruminants.[8] In contrast, there are only a few documented cases of clinical sarcocystosis in dogs caused by the immature forms of *Sarcocystis* spp.[3,12,15]

Eight puppies (5 females and 3 males), born to a primiparous 3-year-old Rottweiler bitch, were taken to a local veterinarian in Guanacuaste, Costa Rica, at 4 weeks of age for routine examination and vaccination. All puppies were found to be healthy on physical examination and were vaccinated for canine parvovirus. Four of these puppies were sold by the owner, and the remaining 4 were taken back to the veterinarian 10 weeks later for a parvovirus-booster as well as for the first vaccination for distemper, hepatitis, and leptospirosis. One day after this vaccination, a male puppy developed diarrhea that was empirically treated by the owner with an unknown oral solution. On the following day, the puppy continued to have diarrhea, became weak, and started vomiting. The puppy was taken to the veterinarian who administered 250 mg tetracycline intramuscularly 3 times a day. In spite of medical intervention, the puppy died 4 days later and was submitted for postmortem examination to the Facultad de Medicina Veterinaria, Heredia, Costa Rica.

Two other littermates developed diarrhea, salivation, and convulsions and died 1 and 2 weeks, respectively, after the initial presentation. On the basis of clinical signs, the veterinarian tentatively diagnosed canine distemper. Also, the owner was notified that during this time 1 of the sold littermates had died with neurological signs. The owner elected not to have postmortem examinations done on these 3 puppies.

The puppy submitted for necropsy was in fair body condition and severely dehydrated. The lungs failed to collapse when the thorax was opened and had a distinctive mottled red color with focal areas of consolidation. On palpation, the pulmonary parenchyma was elastic, and on cut surfaces, the trachea and bronchi contained abundant frothy fluid. The intestine contained a mucous material, but the mucosa was grossly normal. No other significant lesions were grossly observed. Samples of lung, heart, liver, kidney, spleen, pancreas, and intestine were fixed in 10% neutral-buffered formalin and routinely processed for histopathological examination.

Significant microscopic changes were limited to the lung, spleen, and intestines. The spleen had necrosis of germinal centers, and numerous mononuclear cells had intranuclear eosinophilic inclusion bodies. The tips of the intestinal villi were necrotic and hemorrhagic. The lungs were diffusely congested, and many alveoli were filled with protein-rich fluid admixed with fibrin, macrophages, and neutrophils. There was profuse intra-alveolar hemorrhages and edematous distention of the bronchovascular interstitium. The lumen of some bronchioles contained serocellular exudates, and the lining mucosa had focal areas of necrosis, leaving basement membranes partially denuded. The cytoplasm of numerous bronchial and bronchiolar cells contained large and conspicuous eosinophilic inclusion bodies (Fig. 1a).

The alveolar walls were thickened and hypercellular, and the epithelial and endothelial cells contained small punctiform cysts (10–20 μm). These cysts were filled with basophilic bodies that were interpreted as merozoites (Fig. 1c). Areas with severe alveolar inflammation had 2–3 cysts per high-power field. An occasional cyst had the merozoites arranged in rosette-like pattern.

On the basis of microscopic findings in the lung, the diagnosis of acute interstitial pneumonia with necrotizing bronchiolitis and intralesional protozoa was made. The bronchointerstitial pneumonia with intracytoplasmic inclusion bodies was considered typical of canine distemper.[13] The intralesional cysts were initially regarded as *Toxoplasma gondii*. To confirm these 2 etiological diagnoses, blocks of paraffin-embedded lungs were submitted for immuno-

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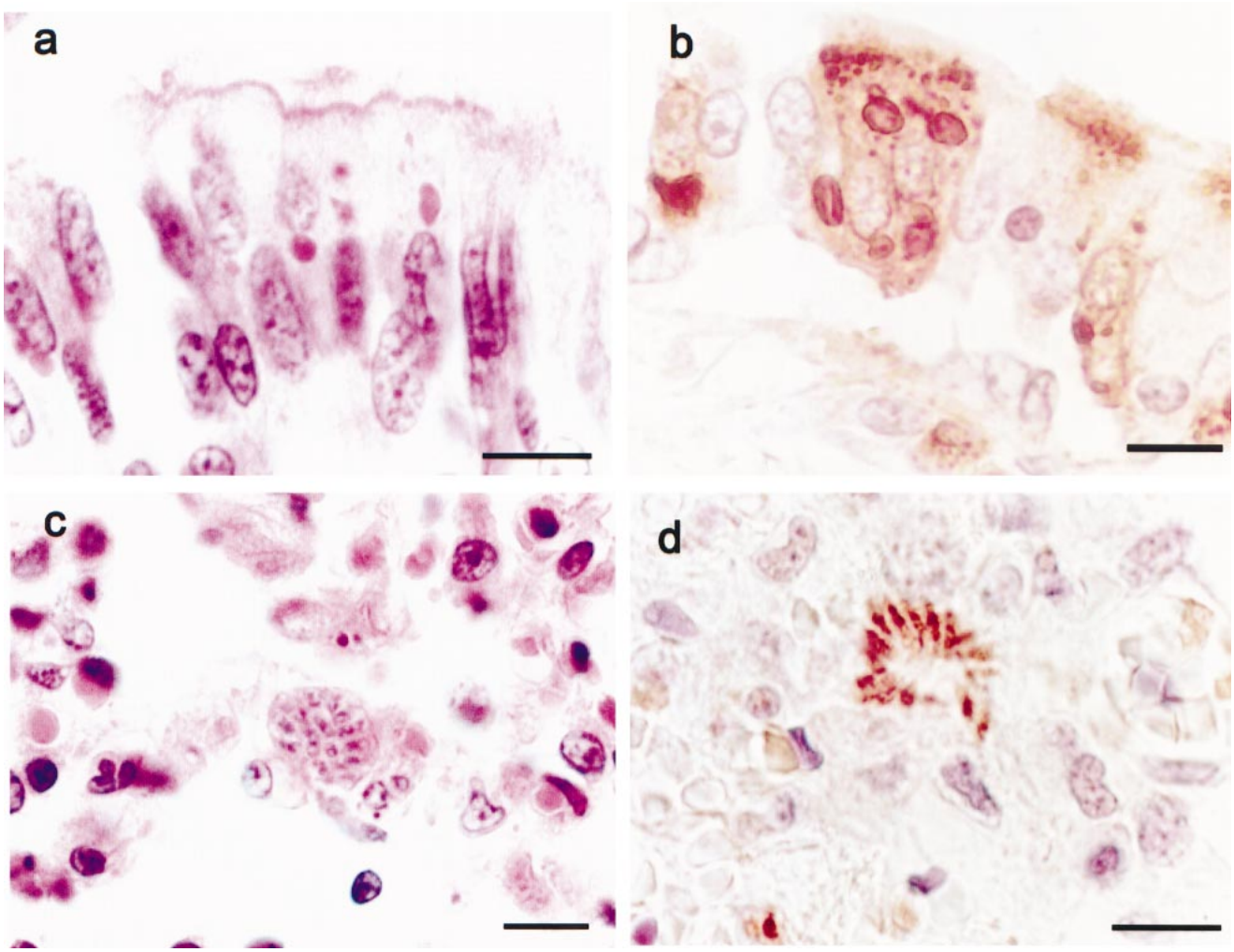

Figure 1. **a**, Bronchial epithelium with numerous intracytoplasmic inclusion bodies HE. Bar = 10 μm. **b**, Bronchial epithelium stained for canine morbillivirus antigen. Note positive stain in inclusion bodies and cytoplasm of columnar ciliated cell. Avidin–biotin complex immunoperoxidase, diaminobenzidine chromogen, hematoxylin counterstain. Bar = 10 μm. **c**, Alveolar wall with *S. canis* schizont containing numerous merozoites HE. Bar = 10 μm. **d**, Lung tissue stained for *Sarcocystis* spp with a rosette formed by merozoites. Avidin–biotin complex immunoperoxidase, diaminobenzidine chromogen, hematoxylin counterstain.

histochemistry. The lung tested positive for canine morbillivirus using avidin–biotin complex immunoperoxidase.[11] Large amounts of morbilliviral antigen were present in the inclusion bodies, cytoplasm, and apical border of bronchiolar and bronchial epithelial cells (Fig. 1b). Results of lung histochemistry were negative for *T. gondii* and *Neospora caninum. Sarcocystis* was added to the list of possible etiological agents, which was confirmed by avidin–biotin–immunoperoxidase using *Sarcocystis* spp. antiserum.[11] This test revealed a myriad of *Sarcocystis* spp. protozoa scattered everywhere in the alveolar walls (Fig. 1d). The final diagnosis of canine distemper and secondary pulmonary sarcocystosis was made.

Canine distemper virus predisposes dogs and other hosts to a variety of secondary infections, particularly toxoplasmosis.[2,9] The distemper toxoplasmosis synergism is well documented[10] but it has not been until recently that dual distemper–*Sarcocystis canis*infection has been recognized in dogs.[7,15] Infection with *S. canis* generally occurs in dogs with distemper or in dogs with other types of immunosuppressive conditions such as neoplastic disorders involving the bone marrow and lymphoid organs.[11]

The typical combined distemper–toxoplasmosis infection was first suspected in this puppy because of the microscopic similarities between *S. canis* and *T. gondii*.[14] However, immunohistochemistry refuted this diagnosis, as well as that of *N. caninum*, which is in the differential diagnosis of canine apicomplexan pneumonias. Unlike previous cases of disseminated canine sarcocystosis, the infection in this puppy was localized to the lungs.[1,5–7,15]

The lymphoid depletion and intranuclear inclusions in the spleen along with the necrotizing bronchitis and bronchiolitis with intracytoplasmic inclusions were typical of canine distemper.[13] According to the immunoperoxidase results, large amounts of morbilliviral antigen was present in bronchial, bronchiolar, and alveolar cells, as well as in pulmonary and splenic macrophages. The cause of intestinal changes remained undetermined but it was presumed to be associated

to canine distemper because there were no intralesional protozoa in the intestinal mucosa.

An interesting finding, in the lungs of this puppy, was the presence of radially arranged merozoites, which are well documented in *S. canis* but not in *T. gondii*.[4,7] The radial appearance originates when *S. canis* divides by endopolygeny and merozoites become grouped around a central residual body.[3,4] Another interesting observation is that, including this report, half of the cases of canine sarcocystosis reported in the literature have been in Rottweiler dogs.[3,6,7] It is still premature to speculate if there may be a breed predisposition.

All previous cases of canine sarcocystosis have been reported from the United States, and this is the first confirmed report of canine sarcocystosis in Central America. Immunohistochemistry proved invaluable to differentiate *S. canis* from *T. gondii*, raising the inevitable question as to whether some past diagnoses of toxoplasmosis may have been in fact sarcocystosis. This view is particularly intriguing in Latin American countries where canine distemper is highly prevalent and toxoplasmosis is a common diagnosis.[14] Future studies in Latin American laboratories using archived tissues should investigate if cases previously reported as toxoplasmosis may be indeed unsuspected sarcocystosis.

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