Short communication

Dermatitis in a dog associated with an unidentified Toxoplasma gondii-like parasite

J.P. Dubey a,*, A.L. Pimenta b, L.C.S. Abboud b, R.R. Ravasani b, M. Mense c

a Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agriculture, BARC-East, Building 1001, Beltsville, MD 20705-2350, USA
b Instituto Municipal de Medicina Veterinária Jorge Vaitzman, Avenue Bartolomeu de Gusmao, 1120 Rio de Janeiro, Brazil
c Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000, USA

Received 10 March 2003; received in revised form 18 June 2003; accepted 20 June 2003

Abstract

Protozoal dermatitis was diagnosed in a 6-year-old female Great Dane dog from Rio de Janeiro, Brazil. The dog died because of a chronic illness with an Ehrlichia-like organism. Numerous apicomplexan parasites were identified histologically in the section of dermal lesions. The protozoan reacted with Toxoplasma gondii polyclonal rabbit serum but not with Neospora caninum or Sarcocystis neurona antibodies. Ultrastructurally, the protozoa was not T. gondii because it had schizont-like structures with merozoites arranged around a prominent residual body, and the merozoites had several rhoptries with electron-dense contents; rhoptries in T. gondii tachyzoites are electron-lucent and a residual body is not found in groups of tachyzoites. This is the first report of unidentified T. gondii-like protozoa in the skin of a dog.

Keywords: Protozoa; Apicomplexa; Dog; Toxoplasma gondii; Neospora caninum; Skin; Dermatitis

1. Introduction

Protozoal dermatitis in dogs has been associated with Caryospora spp. (Dubey et al., 1990), Sarcocystis canis (Dubey and Speer, 1991), Leishmania spp. (Levine, 1973), and Neospora caninum (Dubey et al., 1988; Lindsay and Dubey, 2000; Dubey, 2003). We report protozoal dermatitis in a dog associated with an unidentified Toxoplasma gondii-like organism.
2. Materials and methods

A 6-year-old female Great Dane developed weakness, anemia, thrombocytopenia, jaundice and ulcerative bleeding skin nodules. The dog died after a 3-month illness and a necropsy examination was performed. Samples of bone marrow, kidney, urinary bladder, heart, lung, lymph nodes (auxiliary, inguinal, mediastinal, and pancreatic), pancreas, spleen, liver, and three skin nodules were fixed in 10% buffered neutral formalin. Paraffin-embedded sections were cut at 5 μm thickness and examined after staining with hematoxylin and eosin (H&E). One paraffin block (block A) containing a piece of skin nodule, lymph node, and liver was sent to the Animal Parasitic Diseases Laboratory, USDA, Beltsville, MD for diagnosis of suspected protozoal infection. Tissue sections from this block were examined after staining with H&E and/or periodic acid Schiff (PAS) reaction.

Deparaffinized sections from block A were immunohistochemically reacted with polyclonal rabbit antibodies to *T. gondii*, *N. caninum*, *Sarcocystis neurona*, and BAG-1 antibodies using sera described previously (McAllister et al., 1996; Dubey and Hamir, 2000; Dubey et al., 2001a,b). Tissues from experimentally infected animals containing tachyzoites and tissue cysts of *T. gondii* and *N. caninum*, schizonts of *S. neurona*, and the skin of a dog naturally infected with *Caryospora* sp. (Dubey et al., 1990), were included in the immunohistochemical tests as controls.

Retrospectively, a small piece of skin from block A was deparaffinized, post-fixed in osmium, and processed for transmission electron microscopy.

3. Results

The dog was considered to have acute pneumonia, acute hepatitis with focal necrosis, and plasmacytosis in the kidney, urinary bladder, heart, pancreas, spleen and lymph node. *Ehrlichia*-like organisms were seen in smears of spleen and liver. One ear nodule had a mastocytoma type G II. These observations were made in the laboratory in Brazil; the tissue sections were not available for further examination.

A section of skin, liver and two lymph nodes (of unknown origin) were examined by light microscopy. In the section of skin, there was an acute, focally extensive, severe dermatitis (Figs. 1–3). Overlying areas of inflammation, the epidermis was hyperkeratotic, necrotic and contained numerous viable and degenerate inflammatory cells. The dermis contained higher numbers of primarily neutrophils (often degenerate) forming microabscesses, lesser lymphocytes, plasma cells, macrophages, edema, hemorrhage and numerous apicomplexans (Figs. 1 and 2). This inflammation extended deep into the subdermal adipose and fibrous connective tissue. There was marked folliculitis, furunculosis, and apocrine gland ectasia. Frequently, the organisms were located within the cytoplasm of macrophages, epithelial cells lining the sebaceous glands (Fig. 2B) and unidentified cells, individually and in groups (Figs. 2 and 3). Occasionally, merozoites were arranged around a residual body (Figs. 2 and 3). These organisms were approximately 2–3 μm long in light microscopic sections but few structural details were visible even under the oil immersion lens. The protozoa were PAS-negative.
Fig. 1. Severe dermatitis involving dermal and subdermal areas in the dog (H&E stain). (A) Low power: an abscess (arrow). (B) High power: area of inflammation and hemorrhage (arrow). Arrowheads point to protozoal groups.
Fig. 2. Protozoal-associated dermatitis in the dog (H&E stain). (A) Necrosis and groups of protozoa. Note an individual (arrowhead), and a group without a residual body (small arrow). One group with a large residual body (large arrow). (B) Protozoa in an epithelial cell (arrow) of a sebaceous gland. Arrowheads point to debris in the glandular lumen. (C) A group of protozoa. Note the central nucleus in a tachyzoite. (D) Protozoa around a central residual body (arrow). (E) A tissue cyst-like group of organisms (arrow). Bar in B–E = 10 μm.
Fig. 3. Protozoa in a tissue section of skin of the naturally infected dog. Bar in A = 100 μm and bar in B–F = 20 μm. A–D, H&E stain; E and F, immunohistochemical stain with *T. gondii* antibodies. (A) Edema, necrosis, and infiltration with mixed leukocytes in the dermis. (B and C) Three groups of protozoa (arrows). (D) Merozoites arranged around a central residual body (arrow). (E and F) Individual (arrowheads) and groups of organisms (arrows) stained with *T. gondii* antibody.
Immunohistochemically, the protozoa did not react with *N. caninum*, *S. neurona*, or BAG-1 antibodies. Organisms stained positively with polyclonal *T. gondii* antibodies (Fig. 3E and F). The *Caryospora* in the skin of the naturally infected dog did not react with *T. gondii* polyclonal serum, confirming earlier results (Dubey et al., 1990). A search for *T. gondii* DNA in the paraffin block was not performed because the technique is not always reproducible.

Ultrastructurally, although tissue was autolyzed, certain organelles were recognized, including a conoid, subpellicular tubules, electron-dense rhoptries, micronemes, and a centrally located nucleus (Figs. 4 and 5). The residual body was membrane bound.

The lymph nodes had evidence of germinal center depletion with atrophic paracortical areas. One lymph node was markedly enlarged due to medullary cord plasmacytosis, multifocal hemorrhage, and a mild sinus histiocytosis with erythrophagocytosis.
The liver had diffuse moderate panlobular atrophy of cords with sinusoidal dilatation, mild lymphoplasmacytic cuffing in portal areas, and periacinar ischemic degeneration. Multifocally, there were random areas of hepatic necrosis containing a central area of necrotic cellular debris rimmed by primarily degenerate and viable neutrophils. A few *T. gondii*-like parasites were found in hepatocytes. The results of immunohistochemical staining with anti-*T. gondii* serum were not conclusive because of severe pigmentation in the liver.

4. Discussion

The parasite in the present study was not *Leishmania* because a kinetoplast was absent. It was not *Caryospora* because gamonts and caryocysts were not seen and *Caryospora* does not react with *T. gondii* antibodies (Dubey et al., 1990). The organism was not *Sarcocystis* because merozoites had rhoptries (rhoptries are absent in *Sarcocystis* merozoites) (Dubey, 1993). The parasite was not *N. caninum* because it did not stain with *N. caninum* antibody.

The protozoa in this dog reacted with *T. gondii* antibody (and results were reproducible) but it was not *T. gondii* because schizont-like structures have a residual body and tachyzoite-like structures have several electron-dense rhoptries. Rhoptries in *T. gondii* tachyzoites are few in number, and their contents are electron-lucent (Dubey, 1993). *N. caninum* and *T. gondii* multiply in canine tissues by endodyogeny, always forming only two daughter zoites. They do not form schizonts.

Canine ehrlichiosis is typically due to infection by *E. canis*, however, it appears that the agent infecting the horse (*E. equi*) may also cause disease in dogs. Diagnosis is made...
based on the hematological changes and the presence of rickettsial inclusion bodies in the cytoplasm of lymphocytes and monocytes. A consistent finding on clinical examination is emaciation, epistaxis and petechial hemorrhages on gingiva and conjunctiva. Pathologically, there is subcutaneous and interstitial edema, lymphadenopathy, multiorgan hemorrhage, and lymphoplasmacytic perivascular cuffing as in this case. The changes seen by light microscopy in the sections of lymph node and several other organs from this dog are consistent with the clinical diagnosis of canine ehrlichiosis. Dermatitis and hepatic necrosis are not typically regarded as a feature of canine ehrlichiosis. Of particular note in this case were the changes in the liver. Although an anemic periacinar pattern is not unusual in canine ehrlichiosis, we were surprised by the random pattern and severity of hepatocellular necrosis and inflammation overlaid on the diffuse hepatocyte atrophy and degeneration. However, only a few T. gondii-like organisms were observed in sections to explain extensive necrosis.

Mast cell tumors are common tumors in dogs. The disease usually starts in the skin but aggressive types recur after excision and spread locally. The cutaneous mastocytoma reported by the laboratory in Brazil in this animal is most likely an incidental finding as there was no evidence of neoplastic mass cells in the examined sections of skin, lymph nodes and liver.

There are numerous reports of cutaneous neosporosis in dogs and the present dog was initially thought to be another case of Neospora infection. This undiagnosed protozoon should now be considered in the differential diagnosis of Neospora–Toxoplasma-like dermatitis. To our knowledge, there is no well-documented case of cutaneous toxoplasmosis in dogs.

5. Specimens deposited

H&E stained and unstained sections of the skin were deposited in the US National Parasite Collection (USNPC), Beltsville, MD, USA, as USNPC no. 93516, and in Armed Forces Institute of Pathology, Washington, DC, (case no. 2866221).

Acknowledgements

The authors would like to thank John Jenkins for his excellent transmission electron micrographs, and Sean Hahn for technical assistance.

References


