Rapid communication

Rhinitis and disseminated disease in a ferret (Mustela putorius furo) naturally infected with Sarcocystis neurona

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1. Introduction

Equine protozoal myeloencephalitis (EPM) associated with the apicomplexan, Sarcocystis neurona, is a major cause of neurological disease in horses (Dubey et al., 2001a). S. neurona is transmitted via the fecal oral route from opossums, the only known definitive host, to an unusually wide array of intermediate hosts including raccoons, armadillos, cats, marine mammals, skunks and brown-headed cowbirds (Forest et al., 2000; Dubey et al., 2006; Mansfield et al., 2008). S. neurona infection in association with EPM-like disease has been reported in a diverse range of other wild and domestic mammals, including Pacific harbor seals, sea otters, a Canada lynx, mink, domestic cats and dogs, raccoons, striped skunks, and a fisher (reviewed in Dubey et al., 2006).

Naturally acquired S. neurona infection is typically localized to the central nervous system, reflecting an affinity of the organism for neural tissue (Dubey et al., 2001a). Disseminated disease, involving the brain and numerous other organs, has not been reported in naturally infected animals, although organisms were observed in the heart of a naturally infected raccoon (Hamir and Dubey, 2001). Experimentally, disseminated disease can be induced by oral inoculation of immunosuppressed mice with S. neurona sporocysts (Dubey, 2001) or by feeding large numbers of sporocysts to immunocompetent raccoons (Dubey et al., 2001b; Stanek et al., 2002).

In this paper, we describe disseminated disease including rhinitis in a ferret (Mustela putorius furo) naturally infected with S. neurona. To our knowledge, this is the first report of naturally acquired S. neurona rhinitis and disseminated disease in any species.

2. Materials and methods

2.1. Naturally infected ferret

A male, juvenile domestic ferret weighing 374 g was presented to the Animal Health Centre (AHC), Abbotsford,
British Columbia, Canada on October 1, 2008 for necropsy. The ferret had been purchased from a pet store in Nanaimo, British Columbia. One dose of modified live virus (MLV) canine distemper virus (CDV) vaccine had been administered to the ferret prior to arrival at the pet store. Within one week of purchase, the ferret developed a nasal discharge, respiratory disease, dehydration and hind end paresis. Due to the poor prognosis, the ferret was euthanized.

2.2. Immunohistochemical examination

Tissue samples were fixed in 10% buffered formalin, routinely processed, sectioned at 3–5 μm and stained with hematoxylin and eosin for histopathology. Immunohistochemistry (IHC) for CDV was conducted at AHC on paraffin-embedded tissues using the labelled streptavidin–biotin alkaline phosphastase method and the Ventana Nexes automated stainer (Tucson, AZ, USA) as previously described (Haines and Chelak, 1991) employing a polyclonal rabbit anti-measles virus primary antibody.

Immunohistochemistry for *S. neurona*, *Toxoplasma gondii*, and *Neospora caninum* was performed at the Animal Parasitic Diseases Laboratory (APDL), U.S. Department of Agriculture, Beltsville, MD, using reagents and methods described previously (Lindsay and Dubey, 1989; Dubey et al., 1999; Dubey and Hamir, 2000).

2.3. Molecular examination

Unfixed, tissue samples from brain, lung, liver, kidney and spleen were pooled and analyzed by RT-PCR for the CDV phosphoprotein (P) gene. External primers 5'-GAAGATGCTGACAGTCTCGTG-3' and 5'-CAACTATCCC-CATTCCATGTG-3' identifying a 364 bp product were employed in the first round. Nested primers 5'-TCTGGCGAGATTATTCCG-3' and 5'-TCCCTACATTCTGCTTGTC-3' were employed in the second round, yielding a 142 bp product.

To diagnose the parasitic agent, pooled, frozen, unfixed tissues of the ferret were processed at APDL. A 982 bp portion of parasite 18S rDNA was amplified using primers 18S1F and 18S11R (Rosenthal et al., 2008) and the complete first internal transcribed ribosomal spacer (ITS-1) was amplified using primers situated in the flanking 18S and 5.8S rDNA: 18S-14F 5'-GTTGGTTTCTAGGACTGA-3' and 5.8S-2R 5'-TTCGCTGCGTTCTTCATCGAGAGCCAAGA-3'. BLAST analysis was used to identify similar sequences in

![Fig. 1. Nasal mucosa. Schizonts of *S. neurona* are present within epithelial cells (arrowheads) lining the nasal mucosal surface. H&E.](image-url)
GenBank, and MUSCLE (Edgar, 2004) was used to construct a multiple sequence alignment prior to phylogenetic reconstruction under the criterion of minimum evolution using the maximum composite likelihood method as implemented by MEGA4 after complete deletion of gapped positions (Tamura et al., 2007).

3. Results

3.1. Lesions and immunohistochemistry

Gross findings were restricted to the respiratory tract. The ferret exhibited mucopurulent exudate around the nares and over the mucosal surface of the turbinates. The lungs were diffusely firm, dark red to purple in color with pinpoint, pale tan foci randomly scattered over the pleural surface.

Microscopically, moderate, multifocal, mixed inflammatory cell infiltrates were observed within the nasal lamina propria with a suppurative exudate along the mucosal surface. Mucosal epithelium was disrupted by cell swelling and infiltration of inflammatory cells. Schizonts could be observed within both mucosal epithelium and lamina propria in H&E stained sections (Fig. 1).

The organism reacted positively to antibodies with affinity for *S. neurona* but not to antibodies specific to either *T. gondii* or *N. caninum*. With the aid of IHC, numerous *S. neurona* schizonts and merozoites were demonstrated in the lamina propria, most intensely congregated around the turbinate bones (Fig. 2). Both *S. neurona* schizonts and zoites were observed in the mucosal epithelium, often associated with thickened areas compatible with olfactory epithelium, and occasionally within glandular epithelium (Fig. 2, inset). Throughout the remainder of the tissues, IHC revealed *S. neurona* merozoites, and to a lesser extent schizonts, in lung, brain, heart, skeletal muscle, adrenal gland, liver, spleen, lymph nodes, kidney, skin and intravascular monocytes. Within the lung, multifocal to coalescing areas of alveolar consolidation, characterized by macrophages and lymphocytes, were associated with moderate numbers of schizonts and merozoites. Moderate numbers of schizonts and merozoites were also observed in the brain, with multifocal, perivascular mixed inflammation at all levels.

![Fig. 2. Nasal mucosa. Large numbers of schizonts and zoites can be observed within the lamina propria and epithelium as a result of positive immunohistochemical staining for S. neurona. Note the marked intensity of organisms around turbinate bone (t), zoites within glandular epithelium (g) and the inflammatory exudate on the mucosal surface (*). Inset: Higher power of adjacent mucosal epithelium demonstrating intraepithelial zoites (arrowheads) and schizonts (arrow).](image-url)
and moderate multifocal gliosis largely in grey matter. Lesser numbers of organisms were associated with mild to moderate mixed inflammatory cell infiltrates in the heart, skeletal muscle, adrenal gland, liver and skin. Few schizonts and zoites were observed in the gastrointestinal epithelium. Marked thymic and moderate nodal and splenic lymphoid depletion was observed. Mid-shaft femoral bone marrow was highly cellular with all cell lines represented and an increase in the myeloid to erythroid ratio.

3.2. CDV examination

Polymerase chain reaction assay for CDV was positive on pooled tissues. Immunohistochemistry for CDV was negative on lung, urinary bladder, thymus, brain and kidney. No epithelial inclusion bodies of CDV were observed in any tissues.

3.3. Molecular systematics

The parasite PCR product was 1097 bp. BLAST analyses identified several sequences closely related to those amplified from the present case, all derived either from *S. neurona* or from parasites which may or may not have been accurately distinguished from *S. neurona* on the basis of host associations or morphological attributes. Phylogenetic analyses of the more discriminatory ITS-1 sequences consistently differentiated the present isolate from representatives of the closely related taxon *S. falcatus*, instead placing them in an invariant clade comprised only of several isolates of *S. neurona* and to certain parasites, derived from nine-banded armadillos, attributed to *S. dasypi* (Fig. 3). In spite of the nomenclatural uncertainty arising from the lack of genetic distinction evident among several parasite lineages that are currently recognized as distinct taxa, the available data are entirely consistent with the diagnosis of *S. neurona* in this case.

4. Discussion

Disease associated with *S. neurona* is rarely observed outside the central nervous system in naturally infected animals (Dubey et al., 2001a). This case is exceptional among naturally acquired infections because meningoencephalitis was accompanied by severe disseminated disease, including marked rhinitis. Meningoencephalitis with disseminated disease can be experimentally produced in gamma interferon knockout mice (Dubey, 2001), but not in immunocompetent mice (Marsh et al., 1997) via oral inoculation suggesting that immunosuppression likely facilitates extra-neural dissemination of *S. neurona*. Co-infection with CDV in raccoons suffering from *S. neurona* meningoencephalitis has been reported (Stoffregen and Dubey, 1991; Thulin et al., 1992) and immunosuppression by CDV was thought to have predisposed these animals to
S. neurona infection. Canine distemper virus infection causes severe lymphopenia (Kauffman et al., 1982; Von Messling et al., 2004) coincident with lymphoid depletion in the thymus, spleen and lymph nodes (Dungworth, 1993).

Ferrets are exquisitely susceptible to CDV infection, which is highly immunosuppressive and 100% fatal in this species (Fox et al., 1998; Williams et al., 1988). The ferret in this study did not have CDV disease. However, the ferret had been vaccinated with a modified live CDV vaccine and exhibited significant depletion of lymphocytes in the thymus, spleen and lymph nodes, changes associated with CDV immunosuppression (Dungworth, 1993). Lymphopenia similar to natural infection can be produced by MLV CDV vaccines (Kauffman et al., 1982) and ferrets are reported to be susceptible to secondary infections during the post-vaccinal period of lymphoid depletion (Williams et al., 1996). Immunosuppression due to MLV CDV vaccination can also predispose dogs to toxoplasmosis (Dubey et al., 2003). Thus, it is probable that post-vaccinal immunosuppression predisposed the ferret to S. neurona infection.

Rhinitis with intense schizogony was observed in the ferret. Rhinitis has not been reported previously in association with natural or experimental S. neurona infection in any host. The marked schizogony in the nasal mucosa was unparalleled in any other organ, suggesting that nasal mucosa may be a predilection site for S. neurona. Unlike most other species of Sarcocystis, asexual reproduction of S. neurona is not commonly observed in endothelium; instead, schizonts are more often observed within neurons and monocytes, a finding compatible with the affinity of S. neurona for the central nervous system (Dubey et al., 2001a; Dubey, 2001). Olfaction is the primary function of the nasal cavity in carnivores, such as the ferret. Consequently, large areas of the nasal mucosal surface are devoted to olfactory epithelium, a specialized, thickened structure comprised of olfactory neurons continuous with nerve bundles in the lamina propria. These run through the cribriform plate to the olfactory bulb of the brain (Reznik, 1990). The intense population of schizonts and merozoites associated with the nasal mucosa in the ferret may thus reflect an affinity of S. neurona for olfactory neurons.

An olfactory nerve pathway route of infection is well established for a number of pathogens which cause encephalitis, such as CDV (Rudd et al., 2006) and the free living amoebae, Naegleria fowleri and Balamuthia mandrillaris (Jarolim et al., 2000; Kiderlen and Laube, 2004). While CDV is distributed to the olfactory nerves hematogenously (Rudd et al., 2006), N. fowleri and B. mandrillaris infect the olfactory neurons directly after nasal mucosal invasion. It is unclear whether S. neurona entered the brain via the olfactory nerve pathway in the ferret. Large numbers of merozoites were present in the nasal lamina propria, but whether these were invading olfactory nerve bundles was difficult to determine due the small size of the bundles and the marked degree of inflammation.

S. neurona schizonts and merozoites were observed in the nasal epithelium of the ferret. Entry of S. neurona via the nasal mucosal epithelium would afford direct access to a large number of olfactory nerves leading directly to the brain. On the basis of the rhinitis with intense schizogony observed in this ferret, intranasal inoculation studies and further investigation of both a nasal epithelial portal of entry and an olfactory nerve pathway route of infection for S. neurona is warranted.

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References


