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Clinical *Sarcocystis neurona*, *Sarcocystis canis*, *Toxoplasma gondii*, and *Neospora caninum* infections in dogs

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Abstract

Sarcocystis neurona, *Sarcocystis canis*, *Toxoplasma gondii*, and *Neospora caninum* are related apicomplexans that can cause systemic illness in many species of animals, including dogs. We investigated one breeder's 25 Basset Hounds for these infections. In addition, tissues from dogs and other non-canine hosts previously reported as *S. canis* infections were studied retrospectively. Schizonts resembling those of *S. neurona*, and recognized by polyclonal rabbit anti-*S. neurona* antibodies, were found in six of eight retrospective cases, as well as in two additional dogs (one Basset Hound, one Springer Spaniel) not previously reported. *S. neurona* schizonts were found in several tissues including the central nervous system, lungs, and kidneys. Fatal toxoplasmosis was diagnosed in an adult dog, and neosporosis was diagnosed in an adult and a pup related to the one diagnosed with *S. neurona*. No serological reactivity to *S. neurona* antibodies occurred when *S. canis*-like liver schizonts were retrospectively assayed from two dogs, a dolphin, a sea lion, a horse, a chinchilla, a black or either of two polar bears. Sequencing conserved (18S) and variable (ITS-1) portions of nuclear ribosomal DNA isolated from the schizont-laden liver of a polar bear distinguished it from all previously characterized species of *Sarcocystis*. We take this genetic signature as provisionally representative of *S. canis*, an assumption that should be tested with future sequencing of similar liver infections in other mammalian hosts. These findings further extend the uncharacteristically broad intermediate host range for *S. neurona*, which also causes a neurologic disease in cats, mink, raccoons, skunks, Pacific harbor seals, ponies, zebras, lynxes, and sea otters. Further work is necessary to delineate the causative agent(s) of other cases of canine sarcocystosis, and in particular to specify the attributes of *S. canis*, which corresponds morphologically to infections reported from wide range of terrestrial and marine mammals.
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Keywords: *Sarcocystis neurona*; Dogs; Schizonts; Immunoreactivity; Electron microscopy; *Sarcocystis canis*; *Toxoplasma gondii*; *Neospora caninum*

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1. Introduction

Sarcocystis neurona, *Sarcocystis canis*, *Toxoplasma gondii*, and *Neospora caninum* are related apicomplexans that can cause systemic illness in many species of animals, including dogs (Dubey and Beattie, 1988; Dubey and Speer, 1991; Dubey et al., 2001b; Dubey, 2003). Opossums serve as the definitive host for *S. neurona* increasingly notable for its unusually broad distribution of intermediate or aberrant hosts including horses, raccoons, armadillos, cats, sea otters and skunks. In intermediate hosts, only asexual stages are found and they are confined primarily to the central nervous system (CNS). *S. neurona* causes a fatal neurologic disease in horses (equine protozoal myeloencephalitis, EPM) and EPM-like infections have been reported in raccoons, domestic cats, a Canadian lynx, mink, skunks, a pony, a zebra, a fisher, Pacific harbor seals, and sea otters (Dubey et al., 2001b, 2002, 2003a; Forest et al., 2001; Gerhold et al., 2005). *S. neurona*-like infection was reported in a monkey (Klumpp et al., 1994) but the identification was not confirmed by *S. neurona* immunohistochemistry (Dubey and Hamir, 2000). Clinical EPM infections in animals have been reported from USA, Canada, Brazil, and Panama.

Canine sarcocystosis has previously been attributed to *S. canis*, a taxon currently defined only by morphological criteria, whose schizonts have also been diagnosed in the livers of a sea lion (Mense et al., 1992), a horse (Davis et al., 1999), two polar bears (Garner et al., 1997), a black bear (Zeman et al., 1993), a chinchilla (Rakich et al., 1992), a dolphin (Resendes et al., 2002), and a Hawaiian monk seal (Yantis et al., 2003). The schizont structure reported for *S. canis* resembles that of *S. neurona* (Dubey and Speer, 1991), and its life cycle has not been completed. In non-canine hosts, only the schizont stage has been identified, occurring only in the liver. All but two reports of *S. canis* infections in animals were from USA (listed in the present study), one report was from a dog from Costa Rica (Berrocal and Lopez, 2003), and one was in a dolphin from Spain (Resendes et al., 2002).

Clinical, immunohistochemical, ultrastructural and genetic data have recently implicated *S. neurona* infection in a dog suffering from myositis (Vashisht et al., 2005). We were therefore curious to use immunohistochemical criteria to determine whether *S. neurona* occurred in any of two new, or eight retrospective cases of canine sarcocystosis presumed to have been caused by *S. canis* (Table 1). We characterized and compared a portion of the 18S ribosomal DNA and the entire ITS-1 of a polar bear isolate of *S. canis* in order to evaluate its phylogenetic position with respect to *S. neurona* and other congeners. Additionally, we confirmed that parasites reported by Vashisht et al. (2005) correspond, according to these genetic criteria, to *S. neurona*. Finally, we documented primary toxoplasmosis and neosporosis in a group of Basset Hounds.

2. Materials and methods

2.1. Dogs in the present study

2.1.1. Dog no. 1

A 9-year-old intact male Springer Spaniel presented with a 2-week history of left rear limb

Table 1
Sarcocystis neurona and *S. canis*-like infections in dogs

Dog no., location	Tissues	*S. neurona* staining	Reference	
1, Maryland	Brain	Positive	Present study	
2, Maryland	Brain, spinal cord	Positive	Present study	
Maryland	Liver	Negative	Dubey et al. (1991b)	
Illinois	Skin, others	Positive	Dubey et al. (1991a)	
Illinois	Brain	Positive	Dubey and Slife (1990)	
Lousiana	Lung, kidney, spleen, liver	Positive	Dubey et al. (1992)	
Kansas	Liver	Negative	Trasti et al. (1999)	Dog no. 1
Illinois	Lung	Positive		Dog no. 2
Oklahoma	Brain	Positive		Dog no. 3
Arkasas	Brain	Positive		Dog no. 4