Prevalence of Antibodies to *Sarcocystis neurona* in Dogs With and Without Neurologic Disease

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ABSTRACT

This study compared the prevalence of *Sarcocystis neurona* canine serum antibodies from dogs with and without a presumptive diagnosis of neurologic disease, and sera from dogs with no accompanying history. Generally, *Sarcocystis* have a limited host range. However, *Sarcocystis neurona* causes neurologic disease in many species, including dogs. Major surface antigens displayed by strains of *S. neurona* may be important in host susceptibility. The results of this study indicate that while *S. neurona* is an uncommon infection in dogs and SnSAG 1 strains may be more prevalent than SnSAG 5 or SnSAG6 strains, all are implicated in canine protozoal encephalitis due to *S. neurona*.

INTRODUCTION

The apicomplexan parasite *Sarcocystis neurona* uses the opossum as a definitive host, and has an unusually extensive intermediate host range that includes horses, cats, raccoons, sea otters, skunks, and dogs. In 2005, a *S. neurona*-like species WEREfound in a dog with myositis.[1] Several cases of *sarcocystis* infection found in dogs with *Sarcocystis canis* [2] were actually *S. neurona* [3] and in 2007, *S. neurona* was identified in a dog with encephalitis.[4] Additional disease associated apicomplexan parasites recognized in dogs are *Toxoplasma gondii* and *Neospora caninum*. Immunohistochemistry or molecular tests run on post-mortem tissues can distinguish between *Sarcocystis, Toxoplasma*, and *Neospora*.

The presence of immunodominant *S. neurona* specific antibodies in the serum of diseased animals can be used pre-mortem to assist in diagnosis. There are strain differences in the expression of surface antigen (SAG) genes that distinguish phenotypes of *S. neurona*. [5-8] The majority of clinical disease due to *S. neurona* has been associated with SAG's 1 and 5. The SAG 6 strain of *S. neurona* was identified in diseased southern sea otters. The SnSAG 6 gene is nearly identical to the SfSAG 6 gene expressed by some strains of *S. falcatula* Therefore, antibody would not be sensitive enough to discern *S. neurona* from *S. falcatula* strains

Table 1. *Distribution of dog sera (%) SAG1 antibody titers in groups 1, 2, and 3.*

	Group		
	1	**2**	**3**
< 1:2	8 (80)	10 (55,6)	115 (95)
1:2			2 (1,7)
1:4			1 (0,8)
1:8	1 (10)	3 (16,7)	1 (0,8)
1:16		1 (5,6)	1 (0,8)
1:20		3 (16,7)	1 (0,8)
1:40	1 (10)	1 (5,6)	
total	**10 (100)**	**18 (100)**	**121 (100)**

that display SAG 6 (data not shown). The opossum serves as a definitive host for both *S. neurona* and *S. falcatula*, and is a source of infective oocysts for dogs.

The prevalence of *S. neurona* infections in dogs is unknown. Canine sera submitted to a diagnostic laboratory, sera from dogs with neurologic disease unrelated to parasitic protozoa, and dogs with neurologic disease of unknown etiology, were used to detect *S. neurona* antibody by indirect ELISA employing recombinant SAG 1, 5, and 6 (rSAG 1, 5, 6).

MATERIALS AND METHODS

DNA and Recombinant Proteins

The DNA sequences were obtained for SnSAG 1 and SnSAG 5 as previously described [9-11]. The DNA for the production of rSAG 6 in this study was obtained from the bioassay of oocysts in horses and birds, a gift from Ellis Greiner (University of Florida, Gainesville, Fl).∞ The recombinant proteins were commercially produced (GenScript Piscataway, NJ) based on nucleotide sequence. Recombinant proteins were titrated for the ELISA using monospecific polyclonal antibodies that were produced in horses by vaccination of a horse with the respective recombinant protein.

Dog Sera

Dog sera obtained by Bush Veterinary Neurological Service (Richmond, VA) were divided into two groups: those with a neurological disease possibly caused by parasitic protozoa Toxoplasma gondii, *Neospora caninum*, *Sarcocystis neurona* (n=23), and those that did not (n=18) have parasitic protozoa as a differential diagnosis. Additional dog sera were obtained from Antech Diagnostic Laboratory (Fishers, IN) with no accompanying history (n=100). These sera samples were grouped as: 1) dogs with neurologic disease, protozoan suspected (group 1), 2) sera were from dogs with neurologic disease, protozoan not suspected (group 2), and 3) sera from dogs with no accompanying history (group 3).

Enzyme-Linked Immunosorbent Assay

The indirect ELISA was performed as previously described.[10] Serum samples were screened at a 1:2 dilution against each rSAG, and end point titers were preformed on positive sera. All incubations were performed at 37 °C and anti-dog-IgG alkaline phosphatase conjugate (Sigma-Aldrich, St. Louis, A0793) in 0.01 % bovine serum albumin was used as a secondary antibody. The plates were incubated with 100 µl 0.1 % 4-Nitrophenyl phosphate disodium salt

Table 2*. Distribution of dog sera (%) SAG5 antibody titers in groups 1, 2, and 3.*

	Group		
	1	**2**	**3**
< 1:2	10 (90,9)	14 (77,8)	118 (97,5)
1:2			1 (0,8)
1:4		1 (5,6)	
1:8	1 (9,1)		2 (1,7)
1:16		3 (16,7)	
1:20			
1:40			
total	**11(100)**	**18 (100)**	**121 (100)**

hexahydrate (pNPP; Sigma-Aldrich, St. Louis, N2765) per well for 15 minutes. Extinction was measured at 405 nm with an Emax microplate reader (Molecular Devices, LL). In order to diminish inter-plate variation of ODs, the percent positivity (PP) was calculated for each OD (PP (%) = [$OD_{(sample)}$ - $OD_{(negative\ control)}$] / [$OD_{(positive\ sample)}$ - $OD_{(negative\ control)}$] * 100).[12] The serum titer was recorded as the reciprocal of the last dilution with a positive reaction. Sera with a titer > 16 were examined in a serial two-fold dilution beginning with 1:10 ending with 1:80.

***Table 3**. Distribution of dog sera (%) SAG6 antibody titers in groups 1, 2, and 3.*

	Group		
	1	**2**	**3**
< 1:2	10 (90,9)	14 (77,8)	118 (97,5)
1:2			1 (0,8)
1:4		1 (5,6)	
1:8	1 (9,1)		2 (1,7)
1:16		3 (16,7)	
1:20			
1:40			
total	**11(100)**	**18 (100)**	**121 (100)**

RESULTS

Screening of sera at a 1:2 Dilution

Comparing OD values, a mean OD value of 0.298 was measured for rSAG 1, 0.352 for rSAG 5, and 0.310 for rSAG 6 for negative sera. As a cut-off for possible positive samples, an OD of 0.500 was chosen being twice the average OD of negative controls (mean $OD_{(negative\ control)}$ = 0.249).

rSAG 1 screening 1:2

Twenty-two of 140 sera (15.7%) had an OD > 0.500 when evaluated against rSAG 1. Two of these sera belonged to dogs with neurologic disease, protozoan suspected (group 1). Eleven sera were from dogs with neurologic disease, protozoan not suspected (group 2), and nine sera from dogs with no accompanying history (group 3).

rSAG 5 and rSAG 6 screening 1:2

Sixteen of 141 sera (11.3%) had an OD >0.500. With both antigens, 3 sera (30%) were from dogs of group 1, 6 sera (33.3%) from dogs of group 2, and 7 sera (5.8%) of group 3. Nineteen sera reacted to one antigen at an OD > 0.500, 14 reacted to two antigens and 3 sera reacted to all three antigens.

Titration of Sera

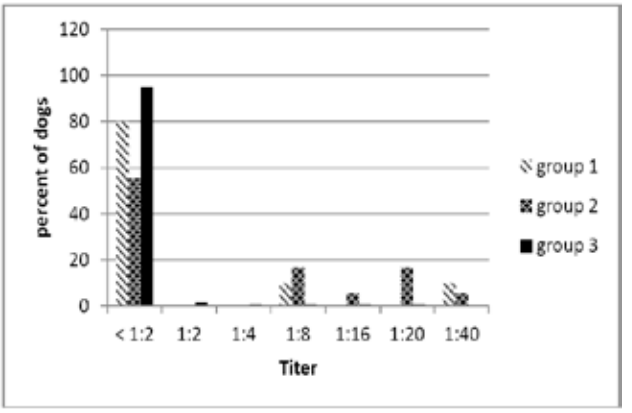
The distribution of the titers between groups can be seen in Tables 1-3, and the relative distribution is shown in Figures 1-3. Comparing the different antigens, 13 sera (9.3%) had a titer of 8 or higher against rSAG 1, 6 sera (4.3%) against rSAG 5, and 3 sera (2.1%) against rSAG 6, indicating a higher prevalence of antibodies against rSAG 1 than against rSAG 5 and rSAG 6 in dog sera. The highest titer against rSAG 1 was 40, while 16 was the highest titer against rSAG 5 and rSAG 6. In group 1, two dogs (20%) had antibody titers equal or higher than 8. In group 2, 10 dogs (55.6%) were positive at a screening dilution of 1:2 and 5 of those (27.8%) had a titer of 8 or greater. Eleven dogs (9.1%) in group 3 were positive on screening at 1:2 but only 7 dogs (5.8%) had a titer of 8 or greater.

For better comparison of the values, the PP was calculated for every plate. Due to the lack of a serum of a dog with putative positive diagnosis of *S. neurona* infection, the sample with the highest OD values was chosen as positive sample. A relatively high cut-off of 25% was considered being least likely to produce false positive results.

DISCUSSION

Sarcocystis neurona was described in a dog with severe myositis1 and in a dog with encephalitis.4 Immunohistopathology and molecular tests were used to suggest that in some cases *S. neurona* infections may have been misidentified as *S. canis*.[3] The life-cycle of *S. canis* and the role of *S. neurona* in dogs are not known. The incidence of *S. neurona* infections in dogs is unknown.

Figure 1. *The relative distribution of the dog sera against rSAG 1. Antibody titers are shown by group. In group 1 and 2 (neurologic diseased dogs), more dog sera have titers of 8 or greater compared to dog sera from group 3 (unknown history).*



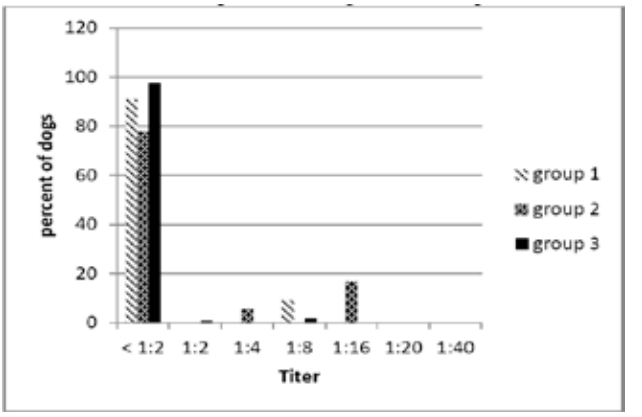
It was suggested that the prevalence of various *S. neurona* strains lacking SnSAG 1 predominate in the mid-Atlantic region.[13] However, this was based on a positive cut-off value when testing equine serum against rSAG 1 that was unusually high (1:32). Paired sera from diseased horses that were used in immunodiagnostic tests demonstrated that SAG 1, 5, and 6 phenotype antibodies are present in all regions of the United States.10 Although SnSAG 1 and 5 phenotypes are responsible for the majority of diseased animals the SnSAG 6 phenotype was responsible for a protozoal-associated epizootic impacting marine wildlife,[8, 14] and must be a consideration when conducting seroprevalence studies for *S. neurona*.

The present study examined dog sera for antibodies against all known pathogenic phenotypes of *S. neurona* using indirect ELISA employing rSAG 1, rSAG5, and rSAG 6. A definitive control positive sera and a defined disease model in dogs was not available. Therefore, based on our results we suggest a titer of 8 as a cut-off for a positive sample. We identified a prevalence of antibodies against rSAG 1, rSAG 5, and/or rSAG 6 was 5.8% in a dog population with unknown histories (group 3). This is comparable to the prevalence of antibodies reported in domestic cats,[15, 16] but much lower than the average prevalence of 45-89 % in horses[17-20] or from feral cats residing on horse farms.

The antigen used for seroprevalence testing in cats was an SnSAG 5 strain derived from a sea otter (David Lindsay, personal communication). The results from the current study may indicate that *S. neurona* infections in dogs are rare. However, in dogs that had a history of neurologic disease, we observed a higher percentage of positive samples. Twenty percent of the dogs suspected for protozoan disease (group 1) and 27.8% of dogs with a neurologic disease, presumed unrelated to protozoan parasites (group2), were positive for antibodies against rSAG1, 5 and/or rSAG6.

It cannot be determined what role *S. neurona* played as a pathogen in these dogs. But a generally higher prevalence of antibodies against *S. neurona* surface antigens suggests that *S. neurona* is correlated to neu-

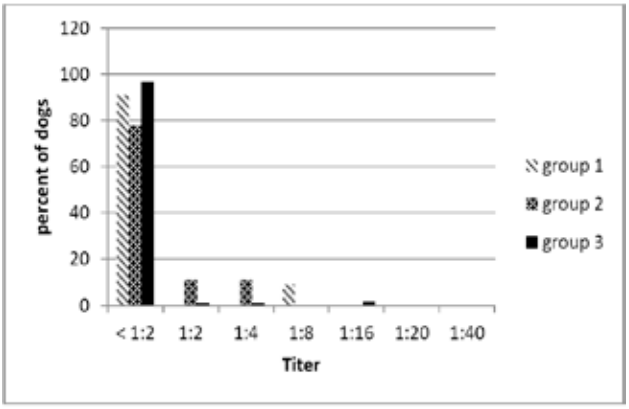
Figure 2. *The relative distribution of dog sera against rSAG 5. Antibody titers are shown by group. The distribution is comparable to Figure 1 although there are less sera with higher titers.*



rologic disease in dogs in groups 1 and 2. These data suggest that antibodies against *S. neurona* surface antigens are unusual in a normal dog population. In dogs with neurologic signs, *S. neurona* should be considered as a possible cause of disease. If antibodies are detected in dogs with neurological deficits, an antiprotozoal treatment should be integrated in the therapy plan. We detected antibodies against rSAG1, five and six in dogs, and propose that a serologic test for detection of antibodies against *S. neurona* in dog serum should include these three surface antigens. Further studies are necessary to determine the role *S. neurona* plays in canine infections.

∞ DNA for the SfSAG 6 gene was obtained from the bioassay of opossum oocysts in birds by Dr. Tim Cutler as was a gift of Dr. Ellis Greiner (University of Florida, Gainesville, Florida). Sequence data for SnSAG 6 and SfSAG 6 are available at www.pathogenes.com/technical

Figure 3. *The relative distribution of dog sera to rSAG 6. Antibody titers are shown by group. The distribution is comparable to Figure 1 although there are less sera with higher titers.*



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