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Detection of Antibodies Against *Sarcocystis neurona*, *Neospora* spp., and *Toxoplasma gondii* in Horses From Costa Rica

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ABSTRACT: Serum samples from 315 horses from Costa Rica, Central America, were examined for the presence of antibodies against *Sarcocystis neurona*, *Neospora* spp., and *Toxoplasma gondii* by using the surface antigen (SAG) SnSAG2 enzyme-linked immunosorbent assay (ELISA), the NhSAG1 ELISA, and the modified agglutination test, respectively. Anti-*S. neurona* antibodies were found in 42.2% of the horses by using the SnSAG2 ELISA. Anti-*Neospora* spp. antibodies were found in only 3.5% of the horses by using the NhSAG1 ELISA, and only 1 of these horses was confirmed seropositive by Western blot. Antibodies to *T. gondii* were found in 34.0% of the horses tested, which is higher than in previous reports from North and South America. The finding of anti-*S. neurona* antibodies in horses from geographical areas where *Didelphis marsupialis* has wide distribution suggests that *D. marsupialis* is a potential definitive host for this parasite and a source of infection for these horses.

Sarcocystis neurona is an apicomplexan recognized as the primary cause of equine protozoan myeloencephalitis (EPM). *Neospora hughesi* and *Toxoplasma gondii* are closely related to *S. neurona* and are also known to infect equids. *Neospora hughesi* is an infrequent etiologic agent of EPM, but there is no evidence of *T. gondii* causing neurologic disease in horses. *Sarcocystis neurona* has been isolated from the spinal cord of a clinical case of EPM from Panama (Granstrom et al., 1992), but most seroprevalence studies for these parasites have been performed in horses from only the North or South American countries. Overall, high prevalences of anti-*S. neurona* antibodies in the horse populations in United States (30–50%), Brazil (35–69.6%), and Argentina (35.5%) have been demonstrated previously (MacKay, 1997; Dubey, Venturini et al., 1999; Hoane et al., 2006). Lower seroprevalences of 3.5% in the United States and 2.5% in Brazil have been observed for *Neospora* spp. when using an enzyme-linked immunosorbent assay (ELISA) based on a recombinant surface antigen (SAG), NhSAG1 (Hoane, Yeargan et al., 2005; Hoane et al., 2006). A very low seroprevalence for *T. gondii* was reported in the United States (Dubey, Thulliez et al., 1999; Dubey et al., 2003), but data from South American countries show that antibodies to *T. gondii* are somewhat common (Brazil, 15.8% and Argentina, 13.1%) (Dubey, Kerber, and Granstrom, 1999; Dubey, Venturini et al., 1999).

Sporocysts or oocysts of these protozoans are shed by their respective definitive hosts, i.e., opossums for *S. neurona* and felids for *T. gondii*. The definitive host for *N. hughesi* has not been demonstrated but may be canids. Three species of opossums, *Didelphis virginiana*, *Didelphis marsupialis*, and *Didelphis albiventris*, are present in the Americas. *Didelphis virginiana* is a well-known definitive host for *S. neurona* in the United States (Fenger et al., 1995), whereas the South American opossum *D. albiventris* also has been shown to be a competent definitive host for *S. neurona* (Dubey, Lindsay, Kerber et al., 2001). The capability of *D. marsupialis* to serve as a definitive host for *S. neurona* has not been demonstrated.

The present study reports the seroprevalence of *S. neurona*, *N. hughesi*, and *T. gondii* in horses from Costa Rica. The results of the study imply that horses in this Central American country are commonly exposed to *S. neurona* and *T. gondii*, whereas infection with *Neospora* spp. is uncommon. The results further suggest that *D. marsupialis* might be a definitive host capable of disseminating *S. neurona* sporocysts because *D. virginiana* only occurs in the northern part of the country (Fig. 1).

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Sera were collected from 315 horses in total (195 females, 153 males) from 7 provinces of Costa Rica: Alajuela (n = 132), Cartago (n = 12), Guanacaste (n = 6), Heredia (n = 82), Limón (n = 8), Puntarenas (n = 6), and San José (n = 69). Among the 315 horses, 161 were in the age group of 1–6 yr, 132 of them were >6-yr-old, and 22 were <1 yr old. Most of these horses were housed indoors. History relating to opossum activity in these geographical areas also was collected.

The recombinant SnSAG2 and NhSAG1 ELISAs were performed as described previously (Hoane, Morrow et al., 2005; Hoane, Yeargan et al., 2005). All horse serum samples were tested at a 1:500 dilution. Positive and negative control serum samples were from confirmed EPM horses and a seronegative foal, respectively, as described by Hoane, Morrow et al. (2005) and Hoane, Yeargan et al. (2005). All test samples and standards (positive and negative sera) were run in duplicate. To account for inter-plate variation, the optical density$_{450}$ of each test sample was expressed as a percent positivity (PP) value, as described previously (Wright et al., 1993).

Western blot analysis using *N. hughesi* tachyzoite antigen was performed on serum samples that tested positive on rNhSAG2 ELISA. *Neospora hughesi* tachyzoite proteins were solubilized in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer (without 2-mercaptoethanol) containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, Missouri), and antigen from 1×10^6 parasites was resolved in 12% polyacrylamide gels. For Western blot, proteins were transferred to a 0.45-μm nitrocellulose membrane by using a semi-dry transfer cell (Bio-Rad Laboratories, Hercules, California) and probed with 1:500 diluted sera. Samples were considered seropositive for *Neospora* spp. when both the 29-kDa (NhSAG1) and 35-kDa (NhSRS2) SAGs of the parasite were recognized (Marsh et al., 1999).

To examine for antibodies to *T. gondii*, 2-fold dilutions of the sera were prepared in phosphate-buffered saline and tested in the modified agglutination test that uses the formalin-preserved and 2-mercaptoethanol-treated whole tachyzoites of *T. gondii* as antigen (Dubey and Desmonts, 1987). Horses with titers of ≥1:25 were considered seropositive for *T. gondii* infection.

At a liberal PP cut-off value of 10%, 42.2% (133 of 315) of the horses were found to have antibodies to the SnSAG2 antigen of *S. neurona*, whereas 21.9% (69 of 315) were considered seropositive by using a more conservative PP cut-off of 20% (Table I). In contrast, a much lower *N. hughesi* seroprevalence of only 3.5% (11 of 315) was observed in this sample set by using a PP cut-off value of 20% with the rNhSAG1 ELISA. Because occasional false positive results are known to occur with the rNhSAG1 ELISA, Western blot analysis of the ELISA-positive samples was conducted to prevent overestimation of *N. hughesi* seroprevalence. This analysis of the 11 ELISA-positive samples, along with an additional 2 samples exhibiting borderline PP values of 19.9 and 17.9%, revealed that only the sample with a PP = 19.9% reacted with both the NhSAG1 and NhSRS2 SAGs. Five of the 11 ELISA-positive samples and the additional sample with PP = 17.9 reacted with only the NhSAG1 antigen and were therefore considered as probable positives. Antibodies to *T. gondii* were found in 34.0% (107 of 315) of the horses tested on modified agglutination test (MAT; Table I). However, low titers of 1:25 were observed in most horses that tested positive (57 horses); only 4 horses showed titers >1:3,200. Antibody titers of 1:50 and 1:100 were observed in 23 and 21 horses, respectively, whereas titers of 1:200 and 1:1,600 were found in 1 horse each. Two horses that had reciprocal titers >3,200 on *T. gondii* MAT were also positive for *S. neurona* antibodies.

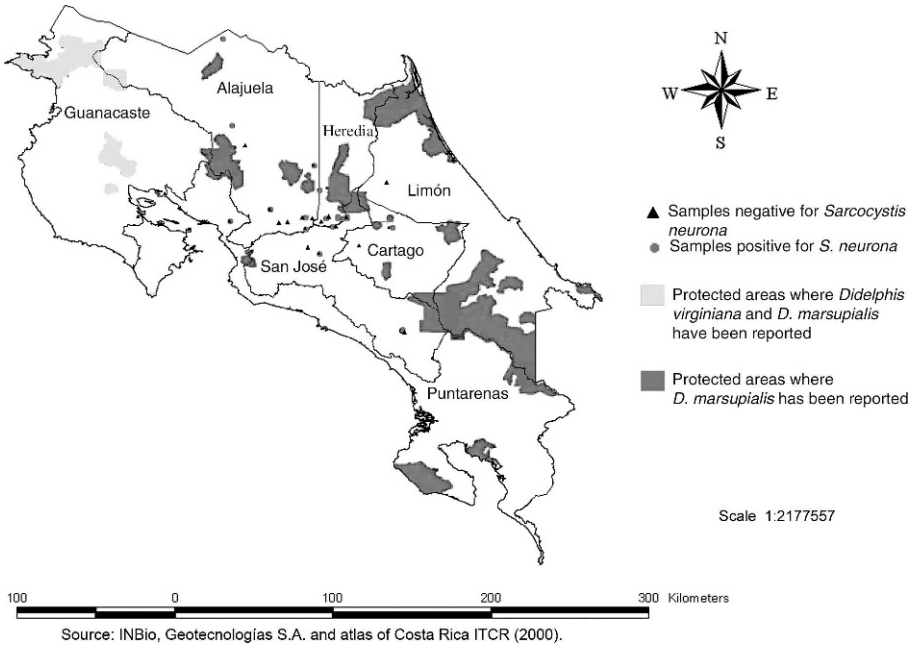

FIGURE 1. Map of Costa Rica showing geographical distribution of *Didelphis virginiana* and *Didelphis marsupialis* and locations of sampled horses.

Results from ELISAs based on rSnSAG2, rNhSAG1 antigens, and MAT used in this study indicated that an appreciable number of the 315 horses from Costa Rica had been exposed to *S. neurona* or *T. gondii*, whereas only a single horse had definitive evidence of exposure to *Neospora* spp. (Table I). A high percentage of horses (42.2%) had anti-*S. neurona* antibodies by using a liberal PP cut-off value of 10%, whereas a lower proportion was considered positive (21.9%) if a conservative PP cut-off value of 20% was used. Although the initial validation of the rSnSAG2 ELISA defined a PP cut-off of 20% (Hoane, Morrow et al., 2005), results from more recent assays have indicated that a low PP cut-off of 10% provides optimal accuracy with the rSnSAG2 ELISA (Yeargan and Howe, 2011). Therefore, the *S. neurona* seroprevalence data have been presented based on 2 different PP cut-off values, but we believe that the higher seroprevalence (42.2%) based on the 10% cut-off is more accurate. It is important to note that EPM does not develop in all horses exposed to *S. neurona* and that the number of horses showing seroconversion is much greater than actual number of EPM cases (Dubey, Lindsay, Saville et al., 2001). However, it is also important to note that detection of anti-*S. neurona* antibodies in a large proportion of horses suggests a high environmental contamination with *S. neurona* sporocysts. *Didelphis virginiana* and *D. marsupialis* are 2 opossum species native to Costa Rica, each having a different geographic distribution. *Didelphis virginiana* is a definitive host for *S. neurona* (Fenger et al., 1995), but its geographic range is believed to be restricted to northern Costa Rica (Gardner, 1973). Our finding of horses seropositive to *S. neurona* from regions inhabited by *D. marsupialis* alone (Fig. 1) suggests that this opossum species is a potential definitive host for *S. neurona*.

Similar to previous reports (Hoane ,Yeargan et al., 2005; Hoane et al., 2006), antibodies to *Neospora* spp. were detected in only in a small proportion of the horses, as determined by rNhSAG1 ELISA. Furthermore, of the 11 samples that tested positive by ELISA, 5 were suspect positives, and only a single sample was confirmed to be seropositive by Western blot assay against *N. hughesi* whole-tachyzoite antigen. These results imply that there is low risk of *Neospora* spp. infection of horses in Central America.

There are no previous reports on seroprevalence of *T. gondii* in horses from Central America. Relatively low *T. gondii* seroprevalence has been observed in horses from countries in both North and South America, and there is no evidence of *T. gondii*-associated neurologic disease in horses. Seropositivity to *T. gondii* of ≤6.9% in the United States (Dubey, Romand et al., 1999; Dubey et al., 2003), 13.1% in Argentina (Dubey, Venturini et al., 1999), and 15.8% in Brazil (Dubey, Kerber, and Granstrom, 1999) have been reported previously. In the current study, 34.0% seropositivity to *T. gondii* was observed, suggesting that horses are at greater risk of *T. gondii* infection in Central America than in North or South America. In all of the studies cited here, including the current study, the MAT was used to detect anti-*T. gondii* antibodies, and sera were tested by 1 operator. A high *T. gondii* seroprevalence in Costa Rica suggests that future studies to test animal populations from other Central American countries will be useful to determine potentially high environmental contamination with *T. gondii* in Central America.

TABLE I. Seroprevalence of *Sarcocystis neurona*, *Neospora* spp., and *Toxoplasma gondii* in horses in Costa Rica.

		No. of samples positive			
		T. gondii	*S. neurona*		*N. hughesi*
Province of Costa Rica	No. of samples tested	Titers ≥25	10% PP	20% PP	20% PP
Alajuela	132	32	53	28	5
Cartago	12	7	7	5	1
Guanacaste	6	1	None	None	None
Heredia	82	28	36	20	2
Limón	8	4	4	None	1
Puntarenas	6	5	2	2	None
San José	69	30	31	14	2

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