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Seroepidemiology of *Sarcocystis neurona* and *Neospora hughesi* infections in domestic donkeys (*Equus asinus*) in Durango, Mexico

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**Abstract** — There is currently no information regarding *Sarcocystis neurona* and *Neospora hughesi* infections in donkeys in Mexico. Here, we determined the presence of antibodies against *S. neurona* and *N. hughesi* in donkeys in the northern Mexican state of Durango. Serum samples of 239 domestic donkeys (*Equus asinus*) were assayed for *S. neurona* and *N. hughesi* antibodies using home-made enzyme-linked immunoassays; six (2.5%) of the 239 donkeys tested seropositive for *S. neurona*. The seroprevalence of *S. neurona* infection was comparable among donkeys regardless of their origin, health status, or sex. Multivariate analysis showed that seropositivity to *S. neurona* was associated with increased age (OR = 2.95; 95% CI: 1.11–7.82; *p* = 0.02). Antibodies to *N. hughesi* were found in two (0.8%) of the 239 donkeys. Both exposed donkeys were healthy, 3- and 6-year-old females. This is the first evidence of *S. neurona* and *N. hughesi* infections in donkeys in Mexico.

**Key words:** *Sarcocystis neurona*, *Neospora hughesi*, Seroprevalence, Donkeys, Cross-sectional study, Mexico.

**Résumé** — Séroépidémiologie des infections par *Sarcocystis neurona* et *Neospora hughesi* chez les ânes domestiques (*Equus asinus*) à Durango, au Mexique. Il n’y a actuellement aucune information concernant les infections par *Sarcocystis neurona* et *Neospora hughesi* chez les ânes au Mexique. Dans cet article, nous avons déterminé la présence d’anticorps contre *S. neurona* et *N. hughesi* chez les ânes dans l’État de Durango au nord du Mexique. Des échantillons de sérum de 239 ânes domestiques (*Equus asinus*) ont été testés pour les anticorps de *S. neurona* et *N. hughesi* à l’aide d’immunoessais enzymatiques faits maison; six (2.5 %) des 239 ânes ont été testés séropositifs pour *S. neurona*. La séroprévalence de l’infection par *S. neurona* était comparable chez les ânes, quels que soient leur origine, leur état de santé ou leur sexe. L’analyse multivariée a montré que la séropositivité à *S. neurona* était associée à l’âge (OR = 2.95; IC à 95 % : 1.11–7.82 ; *p* = 0.02). Les anticorps contre *N. hughesi* ont été trouvés chez 2 (0.8 %) des 239 ânes. Les deux ânes positifs étaient des femelles de 3 à 6 ans en bonne santé. Ceci est la première preuve d’infections à *S. neurona* et *N. hughesi* chez les ânes au Mexique.

**Introduction**

The apicomplexan protozoa *Sarcocystis neurona* (*S. neurona*) and *Neospora hughesi* (*N. hughesi*) are the etiological agents of equine protozoal myeloencephalitis, which is an important neurological disease of horses in the Americas [3, 4]. Additionally, *Neospora* spp. can cause abortion in horses. Although *S. neurona* and *Neospora* spp. can cause disease in equids other than horses, there are no reports of clinical disease in donkeys. There is scarce information regarding *S. neurona* and *N. hughesi* infections outside of the United States [3, 4]. We recently reported the first seroprevalence of these infections in horses in Durango, Mexico [16]. However, we are not aware of any study of these infections in donkeys.

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(Equus asinus) in Mexico. Here we determined the seroprevalences of S. neurona and N. hughesi infections in donkeys in the northern Mexican state of Durango because there are no reports of these infections in donkeys.

Materials and methods

Study design, donkeys surveyed, and serological examination

We performed a cross-sectional study using serum samples from a previous Toxoplasma gondii serosurvey of 239 domestic donkeys in Durango, Mexico [1]. As a strategy to enroll donkeys in the study, we obtained permission to sample donkeys in four equal gathering premises (trade centers) in the municipality of Durango, Mexico. These premises trade donkeys for slaughter in abattoirs outside Durango State. All donkeys were mixed breed. A veterinarian obtained the clinical data for the donkeys. Of the 239 donkeys examined clinically, 193 were healthy, one was malnourished, one had an abdominal mass, and 44 had dermal sores. Donkeys were 0.2–12 years old, and included 170 (71.1%) females and 69 (28.9%) males. According to the donkeys’ owners, all donkeys were kept on pasture.

Serum samples were evaluated for antibodies against S. neurona and N. hughesi using the rSnSAG2/4/3 [15] and the rNhSAG1 [10] enzyme-linked immunosorbent assays (ELISAs), respectively. Antibodies were detected using goat anti-horse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) diluted 1:10,000. The positive control serum for S. neurona was from a horse with equine protozoal myeloencephalitis that was confirmed historically [9]. The positive control serum for N. hughesi was from a mare with confirmed N. hughesi transplacental passage to the foal (kindly provided by Dr. Nicola Pusterla, University of California-Davis, USA). The negative control serum for both ELISAs was a pre-infection sample collected from a weanling used in a prior infection trial. Percent positivity (PP) values were calculated using the optical densities obtained from the test sample and the positive and negative control samples, as described previously [9]. PP cut-off values of 10% and 20% were used for the S. neurona and N. hughesi ELISAs, respectively. Western blot analysis using whole-parasite antigen was conducted with all ELISA-positive samples to confirm the presence of antibodies.

Statistical analysis

For statistical analysis, SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) and the Fisher exact test were used for comparison of the frequencies among groups. The association between the donkeys’ characteristics and S. neurona and N. hughesi seropositivities was analyzed by stepwise regression analysis using the backwards elimination method. The dependent variable was seropositivity to S. neurona. Independent variables included in the regression analysis were only those with p < 0.35 obtained in the bivariate analysis: age, sex, and health status. We calculated the odds ratio (OR) and 95% confidence interval (CI), and a p value of < 0.05 was considered statistically significant. The Hosmer-Lemeshow goodness of fit test to assess the fitness of the regression model was used.

Results

Antibodies to S. neurona were found in 6 (2.5%) of the 239 donkeys. A correlation between seropositivity to S. neurona and donkeys’ characteristics is shown in Table 1. Seroprevalence of S. neurona infection was comparable (p = 0.65) in donkeys from the valleys region (3.2%) and in those from the mountainous region (2.3%) (Table 1). Seropositive donkeys were found in only one (25%) of the four gathering premises studied, and they came from two (66.7%) of the 3 municipalities.

The seroprevalence of S. neurona infection was comparable among donkeys regardless of their health status or sex (Table 1). Seropositivity to S. neurona was observed only in donkeys aged 6–12 years old. Thus, the seroprevalence of S. neurona was significantly higher in donkeys > 5 years old than in younger donkeys (p = 0.008). The variables age, sex, and health status showed p values lower than 0.35 in the bivariate analysis and were included in the regression analysis. Multivariate analysis showed that seropositivity to S. neurona was associated with increased age (OR = 2.95; 95% CI: 1.11–7.82; p = 0.02). However, the other two characteristics of donkeys (sex and health status) were not associated with S. neurona seropositivity by multivariate analysis. The result of the Hosmer-Lemeshow test was 5 (p = 0.08), indicating an acceptable fit of our regression model.

Table 1. General data for the 239 donkeys studied and seroprevalence of Sarcocystis neurona infection.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Donkeys tested</th>
<th>Seroprevalence of S. neurona infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>1–5</td>
<td>135</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>93</td>
<td>6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>170</td>
<td>6</td>
</tr>
<tr>
<td>Health status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ill</td>
<td>46</td>
<td>2</td>
</tr>
<tr>
<td>Healthy</td>
<td>193</td>
<td>4</td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mountains</td>
<td>177</td>
<td>4</td>
</tr>
<tr>
<td>Valleys</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Municipality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durango</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>Mezquital</td>
<td>172</td>
<td>4</td>
</tr>
<tr>
<td>San Dimas</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Trade center</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>170</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

Antibodies to N. hughesi were found in 2 (0.8%) of the 239 donkeys. One seropositive case was from the valleys region and the second from the mountainous region. Donkeys seropositive to N. hughesi were found in only 1 (25%) of the 4 gathering premises, and they came from the municipalities of Durango and Mezquital. Both donkeys seropositive for N. hughesi were healthy females, 3 and 6 years old.

Discussion

The seroepidemiology of infection with S. neurona and N. hughesi in equids other than horses has received little attention. The 2.5% seroprevalence of S. neurona infection found in donkeys in Durango was unexpected since a 48.5% seroprevalence of this infection was found in horses in the same Durango State [16]. Opossums of the Didelphis virginiana species, one of the definitive hosts of S. neurona [3], are present widely in Durango State as shown in a previous study of T. gondii infection [5]. It is unclear why donkeys had a lower seroprevalence of S. neurona infection than horses. The same immunoaassay to detect anti-S. neurona antibodies was used in both studies. This assay is based on three parasite surface proteins that are highly immunogenic, and it is likely that the antigens elicit robust antibody responses in donkeys similar to what is observed in all other animals infected or immunized with S. neurona. It is possible that horses were in greater contact with opossums than donkeys. Opossums are present widely in urban Durango. We are not aware of any reports regarding differences in densities of opossums in urban and rural areas. However, it was suggested that urban areas provide more resources and may be beneficial to opossum populations since opossums living within the city limits had a higher seroprevalence of S. neurona than those in rural areas [14]. Interestingly, urban horses had a higher seroprevalence of S. neurona than rural horses [16]. Donkeys in the present study were from rural areas of Durango State. Donkeys studied were pastured on large rural lands and the likelihood of contact with opossum feces was perhaps low. Horses fed with grains and crops had a significantly higher seroprevalence of S. neurona than horses fed with grass [16]. We searched for factors associated with S. neurona in the donkeys studied. Multivariate analysis showed that seropositivity to S. neurona was associated with increased age. Donkeys older than 5 years had the highest seroprevalence of S. neurona infection. This finding is consistent with a previous observation in horses. In a recent study in the United States, age > 5 years in horses was associated with S. neurona seropositivity [11]. As for horses, the likelihood of infection with S. neurona increases with age. There are few reports on the seroprevalence of S. neurona in equids other than horses. We are aware of only two reports of S. neurona prevalence in donkeys. Sera of 18 donkeys from Ohio, USA were analyzed for antibodies to S. neurona using an immunoblot, and 11 (61.1%) of them were positive [13]. Sera of 333 donkeys from four states in Brazil were tested for antibodies to S. neurona by using the indirect fluorescent antibody test (IFAT, cut-off 1:40) and direct agglutination tests (SAT, cut-off 1:50). Ten (3.0%) were seropositive by IFAT and 69 (21.0%) were positive by SAT [7]. However, comparison of these seroprevalences should be interpreted with care since different laboratory methods were used to detect anti-S. neurona antibodies among the studies. In both IFAT and SAT, whole merozoites are used as antigen, whereas in the present study, ELISA based on recombinant antigens was used.

With respect to N. hughesi infection, donkeys had an unexpectedly low (0.8%) seroprevalence of this infection. This seroprevalence is comparable with a 2% seroprevalence of Neospora reported in 333 donkeys from the north-eastern region of Brazil using IFAT (cut-off 1:40) [7]. In another study in Brazil, researchers found antibodies to Neospora in 2 of 500 donkeys studied using IFAT (cut-off 1:100) [6]. Antibodies to Neospora were also reported in 11 (19.7%) of 56 donkeys from Colombia by using Dot-ELISA [2], in 52 (52%) of 100 donkeys from Iran by the Neospora agglutination test (NAT, cut-off 1:80) [8], and 28 (11.8%) of 238 donkeys from Italy by using competitive inhibition ELISA [12]. Again, comparison of seroprevalences among the studies should be interpreted cautiously because of different laboratory methods used to detect anti-Neospora antibodies. Additionally, reports from Brazil, Colombia, Iran, and Italy used antigen of Neospora caninum, whereas in the present study, antigens from N. hughesi were used. Currently, there are two species of Neospora: N. caninum with a wide host range, and dogs (Canis domesticus), coyotes (Canis latrans), and wolves (Canis lupus) as definitive hosts, and N. hughesi with horses as intermediate hosts, and unknown definitive hosts [4].

Conclusions

We conclude that seroprevalences of S. neurona and N. hughesi infections are low in donkeys in Durango, Mexico. This is the first study that provides serological evidence of S. neurona and N. hughesi infections in donkeys in Mexico.

Conflict of interest

The authors declare that they have no conflict of interest.

References


