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Original Research

Seroprevalence of *Sarcocystis neurona* and Its Association With Neurologic Disorders in Argentinean Horses

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ABSTRACT

Equine protozoal myeloencephalitis (EPM) is generally caused by *Sarcocystis neurona* and can produce substantial economic losses on equine production in America. The aims of the present study were to evaluate the seroprevalence of *S. neurona* in the main horse-production area of Argentina and associate it with the occurrence of neurologic disorders. Serum samples were collected from 640 horses in nine Argentinean provinces. Most of the samples correspond to animals ≥1.5-year-old from different breeds (n = 628); 12 samples were from younger horses. Further seroprevalence comparison was conducted from the older animals grouped with (n = 148) or without neurologic signs (n = 480). Immunoblot: proteins from 2×10^7 *S. neurona* merozoites were used as antigen on each membrane. Reactivity to antigens with relative mobility of 7, 10, and 16 kDa was considered specific for antibodies against *S. neurona*; reactivity at 30 kDa was recorded separately. The overall seroprevalence for *S. neurona* was 26.1% (167/640), and all the provinces had positive horses. Seroprevalence of animals with neurologic signs was greater ($P < .001$) than what was observed in normal horses (39.2% vs. 22.1%), with an odds ratio of 2.27. Reactivity at 30 kDa was detected in 71% of all samples. This study identified a wide distribution of *S. neurona*–positive animals in Argentina and horses with neurologic signs having a greater seroprevalence than normal horses. *Sarcocystis neurona* infection should be considered for early differential diagnosis and treatment of animals with neurologic disorders to decrease the economic impact of EPM in Argentina.

1. Introduction

Equine protozoal myeloencephalitis (EPM) is generally caused by *Sarcocystis neurona* and could produce substantial economical losses on equine production in the Western Hemisphere [1]. Geographical distribution of this apicomplexan parasite is related to the distribution of their definitive hosts, the opossums *Didelphis albiventris* and *Didelphis virginiana* [2,3]. The South American opossum (*D. albiventris*) is frequently observed in farms and suburban areas of Argentina. However, all the attempts to isolate *S. neurona* from Argentinean opossums have thus far resulted in identification of other *Sarcocystis* species [4,5].

The principal signs of EPM are diverse neurologic disorders, mainly ataxia and focal muscle atrophy, which

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occur generally in adult horses [1]. Subclinical infections are common, similar to what occurs with other *Sarcocystis* spp. infections [1,6,7]. Serologic tests such as immunofluorescence antibody test, immunoblot, and Enzyme-Linked ImmunoSorbent Assay with recombinant proteins are the most important tools for demonstrating *S. neurona* infection [1,8–10]. However, the simple detection of antibodies against *S. neurona* is only indicative of parasite exposure and not always related to the development of clinical signs [1,11]. Traditionally, most of the serologic studies have been performed in the United States with the immunoblot and Enzyme-Linked ImmunoSorbent Assay as reference tests [1,12–14]. Regarding interpretation of immunoblot results, the *S. neurona* antigens that migrate at around 30 kDa are considered immunodominant, but their diagnostic value is uncertain [1,14–16].

In Argentina, information regarding *S. neurona* infection has been restricted to a study demonstrating seropositivity in 27 of 76 horses (35.5%) without neurologic signs from Chaco, a Northern Province [17]. The distribution of *S. neurona* infections and its relation with the detection of clinical signs in Argentinean horses remain unknown. Other serologic studies performed in the United States, Mexico, Costa Rica, and Brazil using large number of samples ($n \geq 315$) demonstrated seroprevalences ranging from 33.6% to 89.2%, with greater values for older animals [1,13–15,18–20].

The aims of the present study were to evaluate the seroprevalence of *S. neurona* in the main horse-production area of Argentina and associate the *S. neurona* serologic status with the occurrence of neurologic signs.

2. Material and Methods

2.1. Samples

A total of 640 horses' serum samples from nine Argentinean Provinces were collected and preserved at −20°C until serologic analysis was performed. Most of the samples correspond to animals ≥1.5-year-old (adults) from different breeds (n = 628), with 12 samples from younger horses exhibiting neurologic signs (ranging between 2-days-old and 14-months-old). To compare seroprevalences for *S. neurona* and associate this with the occurrence of neurologic disorders, the animals ≥1.5-year-old were divided into two groups: (1) horses with neurologic clinical signs (n = 148) collected from 2006 until 2011 and (2) horses without neurologic signs (n = 480) collected during 2010. Samples from animals with neurologic signs were collected during clinical evaluation performed by some of the authors. A few of these horses were sampled more than once, but the consecutive samples and serologic follow-ups were not included in the present study. Samples from animals showing no clinical signs were collected in the field by veterinary practitioners participating in a viral diseases surveillance program. Most horses were sampled in Buenos Aires Province (n = 531) followed by Cordoba (n = 51), Santa Fe (n = 11), La Pampa (n = 8), Corrientes (n = 5), Santiago del Estero (n = 4), San Juan (n = 4), Neuquen (n = 1), Entre Rios (n = 1), and origin not declared (n = 24). The sampled area represents the main horse-production region of Argentina.

2.2. Immunoblot

Proteins from 2×10^7 cell culture–derived merozoites of *S. neurona* SN3 strain were separated in 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels in the presence of 2-mercaptoethanol. A low-range molecular marker was used (Bio-Rad). Proteins were transferred to a polyvinylidene difluoride membrane, which was cut into around 40 strips. Blocking solution was 5% nonfat dried milk in phosphate-buffered saline (PBS)–Tween 20 (0.2%); serum samples were diluted 1/10 and control reference sera 1/300 in blocking solution. Antihorse immunoglobulin G peroxidase conjugate (1/500 in blocking solution) was used as the secondary antibody. Blocking and serum and conjugate incubation steps were performed at room temperature for 1 hour each in a rotational shaker. After the incubations, three washings of 3 minutes each were performed with PBS–Tween 20 (0.05%) solution. The last washing step was performed with PBS. Reactions were revealed with chloronafthol–H_2O_2 in PBS–ethanol solution. Reactivity to antigens with relative mobility of 7–10 and 16 kDa was considered specific for antibodies against *S. neurona* [1,14]; reactivity at 30 kDa was recorded separately.

2.3. Statistical Analysis

The differences in *S. neurona* seroprevalence between symptomatic and asymptomatic horses were analyzed with chi square for proportions and calculating the odds ratio using the Win Episcope 2.0 software. The difference between seropositivity in Buenos Aires province versus the other sampled regions was analyzed by chi square for proportions (significant values $P < .05$).

3. Results

The overall seroprevalence for *S. neurona* was 26.1% (167/640), and all Provinces with more than four samples showed at least two positive horses. Samples from animals ≥1.5-years-old showed a seropositivity of 26.15% (164/628), whereas 25% (3/12) of young horses tested positive (one of 1-month-old and two of 6-months-old). There was no significant difference in the proportion of seropositive animals from Buenos Aires province versus other regions ($P \geq .05$).

Comparison of animals ≥1.5-years-old revealed that the group with neurologic signs had a seroprevalence of 39.2% (58/148), significantly greater ($P < .001$) than the 22.1% (106/480) observed in the normal horse group. Parasite exposure in all horses based on positive serology to *S. neurona* and the occurrence of clinical signs had an odds ratio value of 2.27 (95% confidence interval, 1.53–3.37).

Reactivity to 30 kDa protein band was detected in 71% of all samples (455/640), with 79% (117/148) and 69% (332/480) testing positive in horses with or without neurologic disorders, respectively. Reactivity at 30 kDa was observed in 50% (6/12) of the young animals. Of the samples considered positives to *S. neurona*, 98% (164/167) reacted at 30 kDa.