Intra-uterine exposure of horses to Sarcocystis spp. antigens

[Exposição intrauterina ao Sarcocystis spp. em equinos]

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ABSTRACT

The aim of this study was to examine the intra-uterine exposure to Sarcocystis spp. antigens, determining the number of foals with detectable concentrations of antibodies against these agents in the serum, before colostrum ingestion and collect data about exposure of horses to the parasite. Serum samples were collected from 195 thoroughbred mares and their newborns in two farms from southern Brazil. Parasite specific antibody responses to Sarcocystis antigens were detected using the indirect immunofluorescent antibody test (IFAT) and immunoblot analysis. In 84.1% (159/189) of the pregnant mares and in 7.4% (14/189) of foals we detected antibodies anti-Sarcocystis spp. by IFAT. All samples seropositive from foals were also positive in their respective mares. Serum samples of seropositive foals by IFAT, showed no reactivity on the immunoblot, having as antigens S. neurona merozoites. In conclusion, the intra-uterine exposure to Sarcocystis spp. antigens in horses was demonstrated, with occurrence not only in mares, but also in their foals, before colostrum ingestion these occurrences were reduced.

Keywords: equine protozoal mieloencephalitis, transplacental infection, seroprevalence, indirect fluorescent antibody test, immunoblot

INTRODUCTION

The genus Sarcocystis has more than 100 species that infect various animal species, requiring an intermediate and a definitive host to complete the life cycle and the sarcocystosis is one of the most prevalent parasitic diseases in animals with worldwide distribution (Tenter, 1995). Canids and opossum (Didelphis sp.) are definitive hosts of the Sarcocystis cruzi (Dubey and Lindsay, 2006) and the Sarcocystis neurona (Fenger et al.,

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1995; Dubey et al., 2001) respectively, and horses can be their intermediate hosts (Dubey et al., 2001). Transplacental transmission by Apicomplexa protozoa belonging to species of Sarcocystis, Neospora and Toxoplasma are related in animals of different species (Dubey, 1994; Tenter, 1995; Dubey, 2003). In horses, Neospora spp. and T. gondii have been detected in fetuses, newborn and foals, indicating that vertical transmission of the parasites may occur (Turner and Savva, 1990; Lindsay et al., 1996).

The S. neurona is described as the main agent of equine protozoal myeloencephalitis (EPM), a progressively debilitating neurological disease, which is often fatal (Dubey et al., 2001). The N. hughesi is also associated with EPM, causing similar clinical signs and microscopic lesions as S. neurona (Marsh et al., 1996). The diagnosis of EPM is usually based on results of clinical examination and by detection of the presence of Sarcocystis spp. specific antibodies in serum and cerebrospinal fluid (CSF) evaluation by immunoblot test (Granstrom et al., 1993).

The S. neurona is phylogenetically closely related to other cyst-forming protozoa that showed vertical transmission and some studies suggest its endogenous pathway transmission in horses. Evaluation of results of serological tests in young horses for the presence of Sarcocystis spp. antibodies may be complicated by the possibility of in utero exposure and the passive transfer of specific maternal antibodies (Cook et al., 2001). Detection of antibodies against S. neurona in pre-colostrum newborn serum indicates that the foal was exposed to parasite antigens in intra-uterine life, by the transplacental route (Perryman et al., 1980; Abd-Elnaeim et al., 2006). Therefore, the aim of this study was to examine the intra-uterine exposure to Sarcocystis spp. antigens, determining the number of foals that have detectable antibody dilution against these agents in the serum, before colostrum ingestion, and collect data about exposure of horses to the parasite in Brazil.

MATERIAL AND METHODS

Serum samples were collected from 195 thoroughbred mares and their newborns in two farms from Southern Brazil. The animals were routinely monitored by a veterinarian and all deliveries were attended. Blood samples were collected immediately after delivery from mares and before colostrum ingestion from newborns. After collection, whole blood was centrifuged at 250xg for 10 minutes at room temperature to obtain the serum, which was stored at -20°C until testing.

The survey of immunoglobulin G (IgG) anti-S. neurona was performed using indirect fluorescent antibody test (IFAT). S. neurona merozoites of SN-37R strain (Sofaly et al., 2002) were used as antigen. To carry on IFAT with the mares’ serum samples, we used a cutoff of 1:50 for screening, whereas the foals were considered positive at a dilution of 1:25. Anti-equine IgG (Goat Anti-Equine IgG FITC®, 160A, Southern Biotech, Oxmoor Blvd, Birmingham, USA) antibody conjugated to fluorescein was used as the secondary antibody. Serum samples known to be positive (from a mare experimentally infected, that was seronegative for Neospora spp.) or negative (from a seronegative newborn) for the presence of antibodies against S. neurona were used as positive and negative controls, respectively, on each slide. Samples were considered positive if there was fluorescence on the entire S. neurona merozoites surface, and as negative when the fluorescence was either apical or absent (Duarte et al., 2003). After obtaining the positive and negative results from IFAT, samples from positive mares were titrated. Titers were determined by the maximum dilution at which the fluorescence was observed.

The immunoblotting was performed according to Granstrom et al. (1993), with modifications in foals’ positive serum samples for Sarcocystis spp. Immunoblots were prepared with S. neurona antigens by using a concentration of 10^7 parasites/mL sonicated four times, 15s each at 40MHz. Buffer was added and samples were heated at 95°C for 5min and the proteins were separated electrophoretically on a 15% sodium dodecyl sulfate-polyacrylamida gel electrophoresis (SDS-PAGE) at 120V and 10mA by 120min. Pre stained SDS-PAGE molecular weight markers (High-and low-range SDS-PAGE molecular weight markers, Bio-Rad Laboratories) were included with each gel. After separation by electrophoresis, antigens were then transferred to Nitrocellulose Supported Transfer Membrane (Gibco®). Before applying the primary antibody, membranes were blocked with 5% (w/v) dried milk in tris-buffered saline plus.
tween (TBS-t) solution. Membranes were allocated in mini blottter, with each lane probed with equine serum samples at dilution 1:10 (Vardeleon et al., 2001). Serum from a horse infected with *S. neurona* was included as positive control for immunoblot, and a negative control horse were included as well. These were incubated overnight at 4°C and membranes were then washed three times for 10min each with TBS-t and incubated with a secondary antibody, peroxidase labeled rabbit anti-equine IgG (Anti-Horse IgG – Peroxidase, Sigma-Aldrich, St. Louis, MO) for 1h at room temperature followed by washing and development using 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO).

The serological prevalence of mares and precolostral foals, as well as the frequency of vertical transmission were compared using chi-square contingency tables. P values to assess the statistical difference were calculated by Fisher's test (GraphPad Prism 5®).

All procedures of animal handling and experimentation were performed according to recommendations from the Brazilian Committee and Experimentation (Cobea; law # 6.638 of May 8, 1979) and were approved by the Animal Use Ethics Committee (CEUA) of Universidade Federal de Santa Maria, registration number 081/2009.

**RESULTS AND DISCUSSION**

The genus *Sarcocystis* host range is large, making it difficult to control in the herds. To investigate the occurrence of transplacental transmission, the presence of immunoglobulins in mare serum during parturition and in their newborns before colostrum ingestion was evaluated. To determine the vertical transmission occurrence by genus *Sarcocystis* in horses, antibodies anti-*Sarcocystis* spp. was researched in serum from mares and their foals by IFAT. Screening of pregnant mares revealed that 84.1% (159/189) were seropositive for *Sarcocystis* spp., and 7.4% (14/189) of foals were positive for antibodies to *Sarcocystis* spp. A high occurrence among mares was observed when compared with other studies (Dubey et al., 1999; Hoane et al., 2006), considering that the prevalence varies according to the region and group of animals evaluated (Vardeleon et al., 2001). The lower antibodies against *Sarcocystis* spp. detected in foals (7.4% - 14/189) was expected, because the neonatal foals before suckling are a gamma globulinemics, due to the equine placenta being characterized as a difuse, epitheliochorial type which prevents any significant placental transmission of antibodies during gestation (Jeffcott, 1974; Jeffcott, 1975).

No reports were found regarding intrauterine exposure to *Sarcocystis* in spite of its phylogenetic proximity with other protozoa of which transplacental infection has been confirmed as *Neospora* spp. (Locatelli-Dittrich et al., 2006; Pusterla et al., 2010; Antonello et al., 2012) and *T. gondii* (Williams et al., 2005). However, there is evidence that as other apicomplexas, the *Sarcocystis* can also establish endogenous infection. As the report of a foal whose mother was seropositive for *S. neurona* at two days of age, the foal showed signs of EPM and at two months old the cerebrospinal fluid was positive in the westernblot test (Gray et al., 2001). Since the seroconversion occurs at about 30 days after infection (Cutler et al., 2001), it is suggestive that this foal was infected with *S. neurona* in the uterus. Other evidence that supports the hypothesis of vertical transmission by this protozoan is the occurrence of seropositive horses in areas where the definitive host of *S. neurona* is absent (Pitel et al., 2002) found in seropositive animals born in France that never left the country and had no contact with *Didelphis* spp.

To detect vertical transmission, serum samples were collected from newborn foals. To minimize these biases, serum samples from neonatal foals were collected before ingestion of colostrum. In this study, 14 foals had low IFAT titers (antibody level of 25) to *Sarcocystis* spp. and these titers were lower than the cutoffs considered indicative of infection, with all samples positive from foals being also positive in mares. Lower cutoff values were used to enhance test sensitivity for exposure screening to the parasite. Fetal horse is able to form a humoral immune response around 180 days of gestation (Perryman et al., 1980), thus antibodies will be present in precolostral newborn serum if it is exposed to protozoan thereafter. Statistical analysis of these data demonstrate that there was no difference in likelihood of transplacental infection when the evaluated variable is the titer of maternal
antibodies. This way, it can be stated that the antibody detected in newborns’ serum without passive immunity ingestion by colostrum could be the intrauterine infection occurrence and the development of adaptive immunity for infants. Therefore, in this study the foals that were seropositive and that were born of seropositive mares may indicate that vertical transmission may be occurring among the studied horses.

Serological tests can generate false results due to cross-reaction with associated parasites, but this risk was decreased by the methodology used. No positive foal samples by IFAT were positive for immunoblotting, indicating that animals were not infected with S. neurona. The IFAT has specificity and sensibility similar to immunoblot, the gold standard test for S. neurona. Moreover, despite genera belonging to the same phylum sharing some surface antigens, a result is positive only if the entire Sarcocystis merozoites surface was fluorescent (Duarte et al., 2003). The immunoblot test detects the presence of S. neurona specific antibodies in serum and cerebrospinal fluid, with sensitivity and specificity to nearly 100% based on the elimination of cross-reactivity with S. cruzi (Granstrom et al., 1993; Rossano et al., 2000).

CONCLUSION

This study demonstrated the intra-uterine exposure to Sarcocystis antigens in horses, showing an occurrence of antibodies against Sarcocystis spp. not only in mares, but in their foals. Before colostrum ingestion these occurrences were reduced.

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REFERENCES


