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Short communication

Association between the presence of serum antibodies against *Neospora* spp. and fetal loss in equines

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Abstract

A study of the association between the presence of serum antibodies against *Neospora* spp. and fetal loss was performed using serum samples of horses submitted to the laboratory for the detection of antibodies to Equine Herpesvirus-1 and Equine Infectious Anemia Virus. The sera submitted for equine infectious anemia testing were from horses declared healthy and those submitted for the detection of antibodies to Equine Herpesvirus-1 were from mares with late clinical signs of reproductive disorders or males living in close contact with diseased mares. For the detection of *Neospora* spp. infection, the immunofluorescent antibody test was employed, using a cut-off titer of 50 as significant for the presence of antibodies. Among the 483 mares in the diseased group, 15.1% (73/483) was reactant, while 5.8% (19/325) was seropositive in the healthy group. The results show that late clinical signs of reproductive disorders in mares are positively associated ($p < 0.001$) to infection with protozoa belonging to the genus *Neospora* and point to the fact that the participation of this group of coccidia in the genesis of reproductive disorders in equine must be investigated.

Keywords: *Neospora* spp.; Equine; Epidemiology; Immunofluorescent antibody test; Neosporosis; Fetal loss

1. Introduction

The two known species that form the genus *Neospora*, *Neospora caninum* and *Neospora hughesi*, are currently identified as infecting horses (Lindsay, 2001). Equine protozoal myeloencephalitis is a neurological disease of horses that may be caused by either *N. hughesi* or *Sarcocystis neurona* (MacKay et al., 2000; Dubey et al., 2001). Although neosporosis caused by *N. caninum* is considered an important disease of cattle and dogs worldwide, little is known regarding pathogenicity and transplacental infection by this parasite in horses (Dubey et al., 1999a; Pitel et al., 2003). In fact, the role of *Neospora* organisms as an abortifacient agent for pregnant mares remains to be elucidated (Dubey and Porterfield, 1990; Pronost et al., 1999; Duarte et al., 2004).

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Antibodies to *Neospora* spp. in equine populations were reported in many parts of the world: USA and New Zealand (Dubey et al., 1999b, 2003; Cheadle et al., 1999; Vardeleon et al., 2001), South Korea (Gupta et al., 2002), France (Pitel et al., 2001, 2003) and Italy (Ciaramella et al., 2004). Although worldwide spread, sero-surveys recently indicated that *Neospora* spp. infection might be uncommon in horses from North America (Hoane et al., 2005) and Brazil (Hoane et al., 2006).

Differences between frequency of occurrence of serum antibodies against *Neospora* spp. in groups of aborting and healthy mares have suggested a role for *Neospora* spp. in equine abortion (McDole and Gay, 2002; Pitel et al., 2003; Duarte et al., 2004). However, those differences fell short of statistical significance, probably because of the small sample size surveyed. The purpose of the present study was similar to that published elsewhere (McDole and Gay, 2002): to determine whether seropositivity to *Neospora* spp. was associated with sample submission for diagnostic workup from mares experiencing fetal loss, but in this case we have tested a larger sample size.

2. Material and methods

Blood serum was obtained from 1106 adult horses. The samples were from different municipalities from the state of São Paulo, Brazil. The samples were received from August 2000 to August 2005 by the Rabies and Viral Encephalitis Laboratory from the Instituto Biológico, in São Paulo, SP, Brazil. Five hundred samples were from animals submitted for a diagnostic workup for the detection of anti-Equine Herpesvirus-1 (EHV-1) antibodies (this group was named case group). The remaining 606 samples were from animals submitted to immunologic diagnosis of Equine Infectious Anemia (EIA), caused by Equine Infectious Anemia Virus (EIAV) (named control group).

All the samples from the case group were tested for the detection of EHV-1 infection by serum antibody neutralization assays. Serum samples from the control group were tested by the agar gel precipitation test (Coggins test). Both tests were performed as described elsewhere (Coggins et al., 1972; Thomson et al., 1976)

The horses of the control group (325 females and 281 males) were declared healthy by the veterinary assistance which submitted the samples, while the 483 females of the case group were known to have recent problems of either abortion on the last third of gestation or neonatal mortality. The 17 males of the case group were animals living in close contact with females that had shown signs of reproductive disease.

For the detection of antibodies to *Neospora* spp., the indirect fluorescent antibody test (IFAT) against *N. caninum* tachyzoites strain NC-1 was used according to the method described by Dubey et al. (1988), except for using goat anti-Horse IgG-FITC conjugated as secondary antibody. All samples were screened at 1:50 h then tested at two-fold dilutions to determine the maximal titer for the reaction. Reactions were considered positive when the tachyzoites showed total peripheral fluorescence (distinct from apical). Positive and negative controls were included on all runs.

Chi-square test and one-sided Fischer's exact test with statistical significance when $p < 0.05$ were used for investigating relationships between two qualitative variables. The statistical analyses were done by using SPSS Version 9.0. The odds ratio of the association between qualitative variables was calculated by using the software Win Episcope 2.0.

3. Results

The overall frequency of occurrence of anti-*Neospora* spp. antibodies was 10.3% (114/1106). Among the males, 7.4% (22/298) was positive (serum titer $\geq$50), while 11.4% (92/808) of the females had titers against *Neospora* spp. The results showed no association between the presence of antibodies to *Neospora* spp. and the sex of the animals ($\chi^2 = 3.775$; $p = 0.052$).

Among the animals belonging to the case group, 15.4% (77/500) were positive (serum titer $\geq$50). Of the control group, only 6.1% (37/606) had titers against *Neospora* spp. (Table 1). Considering only the results of the females, 15.1% (73/483) of the mares in the diseased group was reactant while 5.8% (19/325) was seropositive in the control group (Table 1). None of the animals had IFAT titer greater than 400.

Considering positive animals with titer $\geq$50, the titer prevalence in the case group was higher than the control group and the difference was statistically significant ($\chi^2 = 25.599$; $p = 0.000$). Considering only the results of the females, the seropositivity in the EHV-1 group was also statistically greater than the control group ($\chi^2 = 25.599$; $p = 0.000$). The odds ratio of an association between the presence of a positive titer and submission for diagnostic workup for the detection of antibodies anti-EHV-1 was 2.80 (95% CI of 1.88–4.17) if all animals was included. Considering only the mares, the odds ratio was 2.87 (95% CI of 1.73–4.77).