Seroprevalence of *Neospora* spp. in horses in South of Iran

Moraveji, M.1, Hosseini, M.H.2, Amrabadi, O.3, Rahimian, A.2, Namazi, F.1, Namavari, M.2*
1Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
2Razi Vaccine and Serum Research Institute, Shiraz, Iran
3Iranian Veterinary Organization, Shiraz, Iran
*Corresponding author email: namavari@yahoo.com
Received 27 November 2010; received in revised form 25 April 2011; accepted 29 April 2011

Abstract. *Neospora caninum*, an apicomplexan protozoan parasite, is recognized as a major cause of abortion in cattle. However, limited information is presently available on the seroprevalence of *Neospora* antibodies in horses worldwide. The aim of the present study is to determine serological prevalence of *Neospora* infection in horses in Iran. Blood samples were obtained from 200 horses and tested for serum antibodies against *Neospora* spp. by the *Neospora* modified direct agglutination test (N-MAT). Antibodies were found in 64 (32%) horses being tested with titers of 1:80. This is the first serological survey for *Neospora* antibodies performed on horses in Iran.

INTRODUCTION

*Neospora* spp., an apicomplexan protozoan parasite that has been associated with neurological disease in dogs and horses as well as with abortion in cattle, sheep, goats, deer and horse (Dubey & Lindsay, 1996). Two species, *Neospora caninum* and *Neospora hughesi*, have been identified as infecting the horse (Marsh *et al.*, 1998). Although neosporosis caused by *N. caninum* is considered an important disease of cattle and dogs worldwide (Dubey *et al.*, 1999a; Pitel *et al.*, 2003) limited information is currently available on the seroprevalence of *Neospora* antibodies in horses (Cheadle *et al.*, 1999; Dubey *et al.*, 1999b, 2003; Pitel *et al.*, 2001; Vardeleon *et al.*, 2001; Mc Dole & Gay, 2002; Ciaramella *et al.*, 2004; Jakubek *et al.*, 2006; Kliger *et al.*, 2007). Although clinical equine neosporosis is rare and has been only reported in USA, exposure to *Neospora* spp. in horses seems to be common (Lindsay, 2001).

*Neospora* infection in cattle (Sadrebazzaz *et al.*, 2004; Razmi *et al.*, 2006; Nourollahi Fard *et al.*, 2008), dogs (Haddadzadeh *et al.*, 2007; Malmasi *et al.*, 2007) and camels (Sadrebazzaz *et al.*, 2006) has been reported in Iran but no data is available on the prevalence of *Neospora* antibodies in horses. Therefore, this study was performed to determine the seroprevalence of antibody titres to *Neospora* spp. in horses in the south of Iran.

MATERIALS AND METHODS

Between April and September 2009, blood samples were collected from the jugular vein of 200 adult horses in Shiraz suburb in Fars Province, (Fars is located in the southern Iran and covering an area of 133 000 km²). A complete history was recorded and clinical status was estimated on each animal and all the seropositive animals were further subjected to neurological examination. Blood samples were centrifuged 15 min at 1000x g and the sera obtained were stored at -20°C and subsequently thawed at 37°C immediately before testing.

Tachyzoites of the NC-1 strain of *N. caninum* (Dubey *et al.*, 1988) were used as
Antigen for agglutination test. Tachyzoites were cultured in vitro in Vero cell monolayers, using RPMI medium (Sigma Co., USA) and 2% fetal calf serum. RPMI medium was supplemented with penicillin (10 000 U), streptomycin (100 µg) and amphotericin B (25 µg) (Invitrogen, USA). The tachyzoites were harvested when about 80% of the Vero cells were infected.

For detection of antibodies to Neospora species, the Neospora modified direct agglutination test (N-MAT) described by Packham et al. (1998) was performed. Sera were screened at 1:40 and positive sera were tested at 1:80 dilution and a cut off titer of 80 was considered as significant for the presence of antibodies (Pitel et al., 2001). Sera from an experimentally infected and a seronegative horse were used as controls.

The Chi-square test was applied to analyze the correlation between Neospora seroprevalence and sex.

RESULTS

Of the 200 sera tested, 80 (40%) had titres of 1:40; when these samples were tested at 1:80 dilution, 64 (32%) were positive. The mares exhibited a seropositivity of 43.7% (28/64) while the seropositivity for males was 56.3% (36/64) (Table 1). These values showed no association between the presence of antibodies to Neospora spp. and the sex of the animals. The clinical examination of all the seropositive horses showed no neurological signs associated with infection of the parasite and also reproductive disorders and abortion have not been reported in the seropositive mares.

Table 1. Seroprevalence of Neospora spp. in 200 horses from two sexes by the Neospora modified direct agglutination test (N-MAT) (at 1:40 and 1:80 serum dilution)

<table>
<thead>
<tr>
<th>Sex of horses</th>
<th>Serum dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:40</td>
</tr>
<tr>
<td>Mare</td>
<td>45.0 (36/80)</td>
</tr>
<tr>
<td>Male</td>
<td>55.0 (44/80)</td>
</tr>
<tr>
<td>Total</td>
<td>40.0 (80/200)</td>
</tr>
</tbody>
</table>

Neospora infection in horses caused by two species, N. caninum and N. hughesi, has been identified as infecting the horse and was associated with neurological disorders and fetal loss (Dubey & Porterfield, 1990; Pitel et al., 2003; Villalobos et al., 2006). Since most Neospora isolates from horses have been identified as N. hughesi, it has been suggested that neosporosis of horses may predominantly be caused by this species (Lindsay, 2001). Also N. caninum and N. hughesi cross-react serologically (Walsh et al., 2000), therefore it is not possible to determine these infections are due to which species.

There is limited information about the seroprevalence of Neospora antibodies in horses and just some serological surveys have been reported from the United States (USA) (Cheadle et al., 1999; Dubey et al., 1999b, 2003; Mc Dole & Gay, 2002), Europe (Pronost et al., 1999; Pitel et al., 2001, Ciaramella et al., 2004, Jakubek et al., 2006), New Zealand (Vardeleon et al., 2001) and Israel (Kligler et al., 2007). Only one report was published in the Middle East of Neospora infection in horses, but no report is yet available in Iran.

The serological results of this study indicate that 64 of 200 (32%) horses were exposed to Neospora spp. which is comparable to those have been reported from USA, France and Italy, where 23–29% of the horses were seropositive (Dubey et al., 1999b; Pitel et al., 2001; Ciaramella et al., 2004). In the Middle East, the prevalence was reported from Israel as 11.9%, which is lower than the prevalence in the present study (Kligler et al., 2007).

The prevalence of antibodies against N. caninum in cattle in the southeast of Iran is 12.6% (Nourollahi Fard et al., 2008) and in water buffaloes in the southwest of Iran is 37% (Haji Hajikolaei et al., 2007). The results indicate that exposure to this parasite is common in the south of Iran. Dogs could be definitive host in this region but no information is available on the prevalence of Neospora infection in dog. Further studies on the epidemiological evidence for
investigation of relationship between Neospora infection in dogs and horses in Iran are required.

In conclusion, this study is the first investigation on this species in horses in Iran and indicates that exposure to the parasite is also present in this country.

Acknowledgments. The authors would like to thank the Razi Vaccine and Serum Research Institute, Shiraz for providing financial support.

REFERENCES


