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Short Communication

Seroprevalence of *Neospora* spp. in Horses in North East of Iran

MH Hosseini¹, M Moraveji², Y Tahamtan¹, A Rahimian¹, Gh Mohammadi³, *MM Namavari¹

¹Razi Vaccine and Serum Research Institute, Shiraz, Iran

²Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

³Department of Clinical study, School of Veterinary Medicine, Ferdowsi University, Mashhad, Iran

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ABSTRACT

Background: *Neospora caninum*, an obligate intracellular protozoan parasite, is recognized as a major cause of abortion in cattle, while limited information is presently available on the seroprevalence of *Neospora* antibodies in horses' worldwide. The aim of the present study was to determine serologic prevalence of *Neospora* infection in horses in Iran.

Methods: Sera from 150 horses from Mashhad suburb in Razavi Khorasan Province, northeast Iran were examined for antibodies to *Neospora* spp. using *Neospora* modified direct agglutination test (N-MAT).

Results: Antibodies to this parasite were detected in 45 (30%) of the examined serum samples. Thirty four percent of the samples had titer of 1:40 while then reduced to 30% when 1:80 serum dilution was applied as significant cut off titer.

Conclusion: This study is the first investigation carried out on the *Neospora* in horses in Iran and indicates that horses in Iran are exposed to this parasite.

Keywords: *Neospora* spp., Horse, Seroprevalence, N-MAT, Iran

Corresponding author: E-mail: namavari@yahoo.com

Introduction

Neospora caninum, an apicomplexan protozoan parasite was first detected in 1984 in dogs (1). *N. caninum* has worldwide distribution and has been identified in a wide range of animal species, being associated with neonatal mortality and abortion in cattle, sheep, goats, horses, dogs and cats (2-7). Antibody to *Neospora* species in equine populations were reported in many parts of the world such as USA and New Zealand (8-10), South Korea (11), France (12,13), and Italy (14).

Although there are some reports related to survey of *Neospora* infection in cattle (15-17), dogs (18, 19) and camels (20) in Iran, but there is no information about the prevalence of this infection in horses. Therefore, this study was carried out to determine the seroprevalence of such infection in horses in Iran.

Materials and Methods

From October 2008 to September 2009, blood samples were obtained from 150 adult horses. The samples were from Mashhad suburb in Razavi Khorasan Province, north-east of Iran. Blood samples were centrifuged 15 min at $1000 \times g$ and the sera obtained was stored at -20°C and subsequently thawed at 37°C immediately before testing.

For detection of antibodies to *Neospora* species, the *Neospora* modified agglutination test (N- MAT) described by Packham et al., 1998 was performed (21). In brief, sera were doubling diluted from 1:10 to 1:80 with phosphate-buffered saline containing 0.2 M 2-mercaptoethanol, and 50 μl of each dilution was put in a well of 96 U-bottom micro-titer plate. Then 50 μl of 3.5×10^7 /ml suspension of tachyzoites of the NC-1 strain of *N. caninum* resuspended in alkaline buffer (7.02 g of NaCl, 3.09 g of H_3BO_3 , 24 ml of 1N NaOH, 4 g of horse serum [HS] albumin

[fraction V], 50 mg of eosin Y, dH₂O to 1 liter, 0.1% sodium azide as a preservative; pH 8.7) were added to each serum dilution of samples as well as positive and negative controls. The wells were then mixed thoroughly by pipetting them up and down several times, covered, and incubated overnight at 37°C with 5% CO_2 . A cut-off titer of 1:80 was considered as significant for the presence of antibodies according (12, 21). Reactions were considered positive when the tachyzoites were spread on entire bottom of well of the micro titer plate and those showing button formation were considered negative.

Results

Antibodies to *Neospora* spp. were found in 51 (34%) of the 150 horses with 1:40 serum dilution, where 1:80 serum dilution was applied as significant cut off dilution the serum positivity was reduced to 45(30%) of the 150 horses. The mares showed a seropositivity of 45% while the seropositivity for males was 55 %. These values indicated that there is not any association between the presence of antibodies to *Neospora* spp. and the sex of the animals. Clinical examination of all the seropositive horses did not exhibit any neurological signs associated with infection by the parasite and reproductive disorders and abortion have not been reported in the seropositive mares.

Discussion

Equine neosporosis in horses caused by *N. caninum* and *N. hughesi*, clinically is characterized by abortion, neonatal diseases, and neurological findings of severe encephalomyelitis (7, 13, 22, 23). As *N. caninum* and *N. hughesi* share surface tachyzoites epitopes (24), and the only method

being able to distinguish between the serum reactivity against these two species of *Neospora* is the Western-Blot (25); therefore it is impossible to discern which species infected these animals.

This study is the first to describe the presence of antibodies to *Neospora* spp. in horses in Iran. In this survey, prevalence of serum antibodies against *Neospora* spp. was 34% with 1:40 serum dilution while 30% of them showed titers of 1:80, which is considered significant by Packham et al. (21). This prevalence rate is comparable to those have been reported from USA, France and Italy, where 23–29% of the horses were seropositive (12, 14, 26). This report also supports other reports related to the prevalence of *Neospora* infection in cattle and camels in Mashhad (16, 17, 20). The results indicate that exposure to this parasite is common in this region however; the absence of neurological signs and laboratory findings show that a significant degree of *Neospora* spp. infection occurs sub-clinically (14). Horizontal transmission of *Neospora* spp. appears to be a major mode of transmission in horses; therefore, it is important to determine which factors increase the probability of infection (27). As 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 investigation indicates that serum antibodies against *Neospora* spp. are present in horses in Iran. The seropositive animals should be kept under control for any eventual clinical signs of neosporosis.

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The authors declare that there is no conflict of interests.

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