

## Seroepidemiology of *Neospora* sp., in Horses in East Azerbaijan Province of Iran

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**Abstract:** *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 East Azerbaijan province of Iran. Sera from 100 horses from Tabriz city in East Azerbaijan province Northwest Iran were examined for antibodies to *Neospora* sp. using *Neospora* Modified direct Agglutination Test (N-MAT). Antibodies to this parasite were detected in 28 (28%) of the examined serum samples. About 32% of the samples had titer of 1:40 while then reduced to 28% when 1:80 serum dilution was applied as significant cut off titer. This study is the first investigation carried out on the *Neospora* in horses in East Azerbaijan province of Iran and indicates that horses in Iran are exposed to this parasite.

**Key words:** *Neospora* sp., horse, seroepidemiology, East Azerbaijan province, N-MAT, Iran

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### INTRODUCTION

*Neospora caninum*, an apicomplexan protozoan parasite was first detected in 1984 in dogs (Bjerkas *et al.*, 1984). *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 (Barr *et al.*, 1991, 1992; Cole *et al.*, 1995; Dubey and Lindsay, 1989; Dubey *et al.*, 1990; Dubey and Porterfield, 1990). Antibody to *Neospora* species in equine populations were reported in many parts of the world such as USA and New Zealand (Dubey *et al.*, 2003; Vardeleon *et al.*, 2001; Cheadle *et al.*, 1999), South Korea (Gupta *et al.*, 2002), France (Pitel *et al.*, 2001, 2003) and Italy (Ciaramella *et al.*, 2004). Although, there are some reports related to survey of *Neospora* infection in cattle (Fard *et al.*, 2008; Razmi *et al.*, 2006; Sadrebazaz *et al.*, 2004), dogs (Malmasi *et al.*, 2007; Haddadzadeh *et al.*, 2007) camels (Sadrebazzaz *et al.*, 2006) and horses (Hosseini *et al.*, 2011) in Iran but there is no information about the prevalence of this infection in horses in East Azerbaijan province. Therefore, this study was carried out to determine the seroprevalence of such infection in horses in Northwest of Iran.

### MATERIALS AND METHODS

From October, 2009 to September, 2010, blood samples were obtained from 100 adult horses. The samples were from Tabriz city in East Azerbaijan province, Northwest of Iran. Blood samples were centrifuged 15 min at 1000×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* sp., the *Neospora* Modified Agglutination Test (N-MAT) described by Packham *et al.* (1998) was performed. 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 microtiter plate. Then 50 µL of  $3.5 \times 10^7$  mL<sup>-1</sup> 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<sub>3</sub>BO<sub>3</sub>, 24 mL of 1 N NaOH, 4 g of Horse Serum (HS) albumin (fraction V), 50 mg of eosin Y, dH<sub>2</sub>O to 1 L, 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<sub>2</sub>. A cut-off titer of 1:80 was considered as significant for the presence of antibodies according (Pitel *et al.*, 2001; Packham *et al.*, 1998). 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 AND DISCUSSION

Antibodies to *Neospora* sp., were found in 32 (32%) of the 100 horses with 1:40 serum dilution where 1:80 serum dilution was applied as significant cut off dilution the serum positivity was reduced to 28 (28%) of the 100 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* sp. 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. Equine neosporosis in horses caused by *N. caninum* and *N. hughesi*, clinically is characterized by abortion, neonatal diseases and neurological findings of severe encephalomyelitis (Dubey and Porterfield, 1990; Pitel *et al.*, 2003; Lindsay, 2001). As *N. caninum* and *N. hughesi* share surface tachyzoites epitopes (Marsh *et al.*, 1999) and the only method being able to distinguish between the serum reactivity against these two species of *Neospora* is the Western-blot (Hoane *et al.*, 2001), therefore it is impossible to discern which species infected these animals. This study is the first to describe the presence of antibodies to *Neospora* sp., in horses in East Azerbaijan province of Iran.

In this survey, prevalence of serum antibodies against *Neospora* sp., was 32% with 1:40 serum dilution while 28% of them showed titers of 1:80 which is considered significant by Packham *et al.* (1998). This prevalence rate is comparable to those have been reported from USA, France and Italy where 23-29% of the horses were seropositive (Pitel *et al.*, 2001; Ciaramella *et al.*, 2004; Dubey *et al.*, 1999). This study also supports other researches related to the prevalence of *Neospora* infection in cattle and camels in Iran (Razmi *et al.*, 2006; Sadrebazzaz *et al.*, 2004; Sadrebazzaz *et al.*, 2006).

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* sp., infection occurs sub-clinically (Ciaramella *et al.*, 2004). Horizontal transmission of *Neospora* sp., appears to be a major mode of transmission in horses therefore, it is important to determine which factors increase the probability of infection (Corbellini *et al.*, 2006). 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.

## CONCLUSION

This investigation indicates that serum antibodies against *Neospora* sp. are present in horses in East Azerbaijan province of Iran. The seropositive animals should be kept under control for any eventual clinical signs of neosporosis.

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