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, Iran

INTRODUCTION

Neospora caninum an apicomplexan protozoan parasite was first detected in 1984 in dogs (Bjorkas 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 (Nourollahi et al., 2008; Razmi et al., 2006; Sadrebazaz et al., 2004) dogs (Malmasi et al., 2007; Haddadzadeh et al., 2007) camels (Sadrebazaz 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 species, the Neospora Modified Agglutination Test (N-MAT) described by Packham et al. (1998) was performed. In brief, sera were doubling diluted from 1:10-1:80 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 3.5×107 mL⁻¹ suspension of tachyzoites of the NC-1 strain of N. caninum resuspended in alkaline buffer (7.02 g of NaCl, 3.09 g of H₂BO₃, 24 mL of 1N NaOH, 4 g of Horse Serum (HS) albumin (fraction V), 50 mg of eosin Y, dH₂O 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% CO₂. 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; Villabos et al., 2006; 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 (Hoyne 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 report also supports other reports related to the prevalence of *Neospora* infection in cattle and camels in Iran (Razmi et al., 2004; 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.

**ACKNOWLEDGEMENTS**

The researchers wish to thanks the Islamic Azad university, Tabriz Branch, Tabriz, Iran for the financial supports and all laboratory technicians for technical aids in this project.

**REFERENCES**


