

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

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 3.5×10^7 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 H₃BO₃, 24 mL of 1 N 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; 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; Sadrebazaz *et al.*, 2004; Sadrebazaz *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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REFERENCES

- Barr, B.C., M.L. Anderson, J.P. Dubey and P.A. Conrad, 1991. *Neospora* like protozoan infection associated with bovine abortions. *Vet. Pathol.*, 28: 110-116.
- Barr, B.C., M.L. Anderson, L.W. Woods, J.P. Dubey and P.A. Conrad, 1992. *Neospora*-like protozoal infections associated with abortion in goats. *J. Vet. Diagn. Invest.* 4: 365-367.
- Bjerkas, I., S.F. Mohn and J. Presthus, 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Z. Parasitenkd.*, 70: 271-274.
- Cheadle, M.A., D.S. Lindsay, S. Rowe, C.C. Dykstar and M.A. Williams *et al.*, 1999. Prevalence of antibodies to *Neospora* sp. In horses from Alabama and characterization of an isolate recovered from a naturally infected horse. *Int. J. Parasitol.*, 29: 1537-1544.
- Ciaramella, P., M. Corona, L. Cortese, D. Piantedosi, D. Santoro, A. DiLoria and R. Rigato, 2004. Seroprevalence of *Neospora* spp. in asymptomatic horses in Italy. *Vet. Parasitol.*, 123: 11-15.
- Cole, R.A., D.S. Lindsay, B.L. Blagburn, D.C. Sorjonen and J.P. Dubey, 1995. Vertical transmission of *Neospora caninum* in dogs. *J. Parasitol.*, 81: 208-211.
- Corbellini, L.G., D.R. Smith, C.A. Pescador, M. Schmitz, A. Correa, D.J. Steffen and D. Driemeier, 2006. Herd-level risk factors for *Neospora caninum* seroprevalence in dairy farms in Southern Brazil. *Prev. Vet. Med.*, 74: 130-141.
- Dubey, J.P. and D.S. Lindsay, 1989. *Neospora caninum* induced abortion in sheep. *J. Vet. Diagn. Invest.*, 2: 230-233.
- Dubey, J.P. and M.L. Porterfield, 1990. *Neospora caninum* (Apicomplexa) in an aborted equine fetus. *J. Parasitol.* 76: 732-734.
- Dubey, J.P., D.S. Lindsay and T.P. Lipscomb, 1990. Neosporosis in cats. *Vet. Pathol.*, 27: 335-339.
- Dubey, J.P., S. Mitchell, J.K. Morrow, J.C. Rhyhan and L.M. Stewart *et al.*, 2003. Prevalance of antibody to *Neospora caninum*, *Sarcocystis neurona* and *Toxoplasma gondii* in wild horses from central wyoming. *J. Parasitol.*, 89: 716-720.

- Dubey, J.P., S. Romand, P. Thuuiez, O.C. Kowk, S.K. Shen and H.R. Gamble, 1999. Prevalence of antibodies to *Neospora caninum* in horses in North America. *J. Parasitol.*, 85: 968-969.
- Fard, N.S.R., M. Khalili and A. Aminzadeh, 2008. Prevalence of antibodies to *Neospora caninum* in cattle in Kerman province, South East Iran. *Vet. Arhiv.*, 78: 253-259.
- Gupta, G.D., J. Lakritz, J.H. Kim, D.Y. Kim, J.K. Kim and A.E. Marsh, 2002. Seroprevalence of *Neospora Toxoplasma gondii* and *Sarcocystis neurona* antibodies in horses from Jeju Island, South Korea. *Vet. Parasitol.*, 106: 193-201.
- Haddadzadeh, H.R., A. Sadrebazzaz, A. Malmasi, H.T. Ardakani, P.K. Nia and N. Sadreshirazi, 2007. Seroprevalence of *Neospora caninum* infection in dogs from rural and urban environments in Tehran, Iran. *Parasitol. Res.*, 101: 1563-1565.
- Hoane, J.S., M.R. Yeargan, S. Stamper, W.J. Saville, J.K. Morrow, D.S. Lindsay and D.K. Howe, 2001. Recombinant NhSAG1 ELISA: A sensitive and specific assay for detecting antibodies against *Neospora hughesi* in equine serum. *J. Parasitol.*, 91: 446-452.
- Hosseini, M.H., M. Moraveje, Y. Tahamtan, A. Rahimian, G.H. Mohammadi and M.M. Namanari, 2011. Seroprevalence of *Neospora* spp. in horses in North East of Iran. *Parasitol.*, 6: 64-68.
- Lindsay, D.S., 2001. Neosporosis: An emerging protozoal disease of horses. *Equine Vet. J.*, 33: 116-118.
- Malmasi, A., M. Hosseinejad, H. Haddadzadeh, A. Badii and A. Bahonar, 2007. Serological study of anti *Neospora caninum* antibodies in household dogs and living in dairy and beef cattle farms in Tehran, Iran. *Parasitol. Res.*, 100: 1143-1145.
- Marsh, A.E., D.K. Howe, G. Wang, B.C. Barr, N. Cannon and P.A. Conrad, 1999. Differentiation of *Neospora hughesi* from *Neospora caninum* based on their immunodominant surface antigens, SAG1 and SRS2. *Int. J. Parasitol.*, 29: 1575-1582.
- Packham, A.E., K.W. Sverlow, P.A. Conrad, E.F. Loomis and J.D. Rowe et al., 1998. A modified agglutination test for *Neospora caninum*: Development, optimization, and comparison to the indirect fluorescent-antibody test and enzyme-linked immunosorbent assay. *Clin. Diagn. Lab. Immunol.*, 5: 467-473.
- Pitel, P.H., S. Pronost, S. Romand, P. Thulliez, G. Fortier and J.J. Ballet, 2001. Prevalence of antibodies to *Neospora caninum* in horses in France. *Equine Vet. J.*, 33: 205-207.
- Pitel, P.H., S. Romand, S. Pronost, N. Foucher and G. Gargala, 2003. Investigation of *Neospora* sp. antibodies in aborted mares from Normandy, France. *Vet. Parasitol.*, 118: 1-6.
- Razmi, G.R., G.R. Mohammadi, T. Garrosi, N. Farzaneh, A.H. Fallah and M. Maleki, 2006. Seroepidemiology of *Neospora caninum* infection in dairy cattle herds in Mashhad area, Iran. *Vet. Parasitol.*, 135: 187-189.
- Sadrebazzaz, A., H. Haddadzadeh, K. Esmailnia, G. Habibi, M. Vojgani and R. Hashemifesharaki, 2004. Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. *Vet. Parasitol.*, 124: 201-204.
- Sadrebazzaz, A., H. Haddadzadeh and P. Shayan, 2006. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in camels (*Camelus dromedarius*) in Mashhad, Iran. *Parasitol. Res.*, 98: 600-601.
- Vardeleon, D., A.E. Marsh, J.G. Thorne, W. Loch, R. Young and P.J. Johnson, 2001. Prevalence of *Neospora hughesi* and *Sarcocystis neurona* antibodies in horses from various geographical locations. *Vet. Parasitol.*, 95: 273-282.