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Short Communication

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

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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, northeast 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 (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 microtiter 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 H3BO3, 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% CO2. 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% with1:40 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.

References

1. Bjerkas I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z Parasitenk.1984; 70: 271-274.
2. Barr BC, Anderson ML, Dubey JP, Conrad PA. *Neospora* –like protozoan Infection associated with bovine abortions. Vet Pathol. 1991; 28: 110-116.
3. Barr BC, Anderson ML, Woods LW, Dubey JP, Conrad PA. *Neospora*-like protozoal infections associated with abortion in goats. J Vet Diagn Invest. 1992; 4: 365-367.
4. Cole RA, Lindsay DS, Blagburn BL, Sorjonen DC, Dubey JP. Vertical transmission of *Neospora caninum* in dogs. J Parasitol. 1995; 81: 208-211.
5. Dubey JP, Lindsay DS. *Neospora caninum* induced abortion in sheep. J Vet Diagn Invest.1990; 2: 230-233.
6. Dubey JP, Lindsay DS, Lipscomb TP. Neosporosis in cats.Vet Pathol. 1990; 27: 335-339.
7. Dubey JP, Porterfield ML. *Neospora caninum* (Apicomplexa) in an aborted equine fetus. J Parasitol. 1990; 76: 732-734.
8. Dubey JP, Mitchell S, Morrow J, Rhyan J, Stewart L, Granstrom D, Romand S, Thulliez P,Lindsay D. Prevalance of antibody to *Neospora caninum*, *Sarcocystis neurona*, and *Toxoplasma gondii* in wild horses from central Wyoming. J Parasitol. 2003; 89; 716-720.
9. Vardeleon D, Marsh AE, Thome JG, Loch W, Young R, Johnson PJ. Prevalence of *Neospora hughesi* and *Sarcocystis neurona* antibodies in horses from various geographical Locations. Vet Parasitol. 2001; 95: 273-282.
10. Cheadle MA, Lindsay DS, Rowe S, Dykstar CC, Williams MA, Spencer MD,

Blagburn BL. Prevalence of antibodies to *Neospora* sp. In horses from Alabama and characterization of an isolate recovered from a naturally infected horse. Int J Parasitol. 1999; 29: 1537-1544.

11. Gupta GD, Lakritz J, Kim JH, Kim DY, Kim JK, Marsh AE. Seroprevalence of *Neospora*, *Toxoplasma gondii* and *Sarcocystis neurona* antibodies in horses from Jeju Island, South Korea. Vet Parasitol. 2002; 106: 193-201.
12. Pitel PH, Pronost S, Romand S, Thulliez P, Fortier G, Ballet JJ. Prevalence of antibodies to *Neospora caninum* in horses in France. Equine Vet J. 2001; 33: 205-207.
13. Pitel PH, Romand S, Pronost S, Foucher N, Gargala G, Maillard K, Thulliez P, Collobert-Laugier C, Taintuier D, Fortier G, Ballet JJ. Investigation of *Neospora* sp. Antibodies in aborted mares from Normandy, France. Vet Parasitol. 2003; 118: 1-6.
14. Ciaramella P, Corona M, Cortese L, Piantedosi D, Santoro D, DiLoria A, Rigato R. Seroprevalence of *Neospora* spp. in asymptomatic horses in Italy. Vet Parasitol. 2004; 123: 11-15.
15. Nourollahi Fard SR, Khalili M, Aminzadeh A. Prevalence of antibodies to *Neospora Caninum* in cattle in Kerman province, South East Iran. Veterinarski Arhiv. 2008; 78(3): 253-259.
16. Razmi GR, Mohammadi GR, Garrosi T, Farzaneh N, Fallah AH, Maleki M. Seroepidemiology of *Neospora caninum* infection in dairy cattle herds in Mashhad area, Iran. Vet Parasitol. 2006; 135: 187-189.
17. Sadrebazaz A, Haddadzadeh H, Esmailnia K, Habibi G, Vojgani M, Hashemifesharaki R. Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. Vet Parasitol. 2004; 124: 201-204.
18. Malmasi A, Hosseininejad M, Haddadzadeh H, Badii A, Bahonar A. Serological Study of anti-*Neospora caninum* antibodies in household dogs and living in dairy, and beef cattle farms in Tehran, Iran. Parasitol Res. 2007; 100: 1143-1145.
19. Haddadzadeh HR, Sadrebazaz A, Malmasi A, Taleiardakani H, Khazraiinia P, Sadreshirazi N. Seroprevalence of *Neospora caninum* infection in dogs from rural and urban environments in Tehran, Iran. Parasitol Res. 2007; 101: 1563-1565.
20. Sadrebazaz A, Haddadzadeh H, Shayan P. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in camels (*Camelus dromedarieus*) in Mashhad, Iran. Parasitol Res. 2006; 98: 600-601.
21. Packham AE, Sverlow KW, Conrad PA, Loomis EF, Rowe JD, Anderson ML, Marsh AE, Cray C, Barr BC. 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. 1998; 5: 467–473.
22. Villabolos EMC, Ueno TEH, Sauza SLP, Cunha EMS, Sauza Hunold Lora MCC, Gennari SM, Soares RM. Association between the presence of serum antibodies against *Neospora* spp. and fetal loss in equines. Vet Parasitol. 2006; 142: 372-375.
23. Lindsay DS. Neosporosis: an emerging protozoal disease of horses. Equine Vet J. 2001; 33 : 116–118.
24. Marsh AE, Howe DK, Wang G, Barr BC, Cannon N, Conrad PA. Differentiation of *Neospora hughesi* from *Neospora caninum* based on their immunodominant surface antigens, SAG1 and SRS2. Int J Parasitol. 1999; 29: 1575-1582.
25. Hoane JS, Yeargan MR, Stamper S, Saville WJ, Morrow JK, Lindsay DS,

Howe DK. Recombinant NhSAG1 ELISA: a sensitive and specific assay for detecting antibodies against *Neospora hughesi* in equine serum. J Parasitol. 2005; 91: 446-452.

26. Dubey JP, Romand S, Thuuiez P, Kowk OC, Shen SK, Gamble HR. Prevalence of antibodies to *Neospora caninum* in horses in North America. J Parasitol. 1999; 85: 968-969.
27. Corbellini LG, Smith DR, Pescador CA, Schmitz M, Correa A, Steffen DJ, Driemeier D. Herd-level risk factors for *Neospora caninum* seroprevalence in dairy farms in southern Brazil. Prev Vet Med. 2006;74:130-141.