

Serological study of *Neospora caninum* in pregnant dairy cattle in Tehran, Iran

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Abstract

Seven hundred and sixty-eight blood samples of pregnant cattle from four Holstein dairy herds that are farmed in the vicinity of Tehran were used to evaluate the seroprevalence of *Neospora caninum* infection by enzyme-linked immunosorbent assay (ELISA). Two hundred and ninety-eight of the 768 blood samples (38.8%) were positive for this infection. The prevalence of infection in the herds varied from 18.7% to 65.1%. The abortion rate in seropositive and seronegative animals was 20.67% and 10.11%, respectively. Thus, the risk of abortion was approximately double the rate in seropositive cows ($p < 0.0005$). There was a high correlation between the infection rate and the age in one herd. In other three herds, no significant correlation was found between infection rate and age. This is the first extended study with regards to the rate of *Neospora caninum* infection in pregnant cattle in the vicinity of Tehran.

Introduction

Neospora caninum is one of the most important protozoan parasites of cattle in terms of pathology, geographical distribution and the economic losses incurred. Neosporosis of cattle has been associated with abortion, neonatal mortality and between a 3% and 4% decrease in the volume of milk production (Hernández *et al.*, 2001; Dubey and Schares, 2006). The diagnosis of this infection in live animals can be achieved by detection of anti-*N. caninum* antibodies using different serological tests, such as the indirect fluorescence antibody test (IFAT), the *Neospora* agglutination test (NAT), enzyme-linked immunosorbent assays (ELISA), and Western blotting. ELISA is an approved serological test (von Blumroder *et al.*, 2004) that has been used in epidemiological studies to estimate the prevalence of *N. caninum* infections and to examine the relationship between exposure to *N. caninum* and abortion, milk yields and culling in cattle (Pare *et al.*, 1997, Thurmond and Hietala, 1997, Hernandez *et al.*, 2001 and Hernandez *et al.*, 2002).

The seroprevalence of neosporosis in cattle varies depending on the country and region under study. There are some serological studies in dairy herds in some parts of Iran (Sadrebazzaz *et al.*, 2004, 2006; Razmi *et al.*, 2006; Nouroollahi Fard *et al.*, 2008). However, despite the fact that Tehran is the largest dairy-producing region in Iran, there is no published information on the epidemiology of *N. caninum* in the cattle of this province. There are only two reports of *N. caninum* infection in dogs in Tehran

(Malmasi *et al.*, 2007; Haddadzadeh *et al.*, 2007). The aim of this study was to investigate the seroepidemiology of *N. caninum* infection in dairy herds in the vicinity of Tehran.

Materials and Methods

Field study area

In the spring and summer of 2007, blood samples were taken from cattle that were between three and five months of pregnancy from four different dairy herds, termed A, B, C, and D. These herds were located in an area of 80 km² within the southeastern, western and southern localities surrounding Tehran (Varamin, Eshtahard, Nazarabad and Eslamshahr, respectively). In each farm, the selected animal were categorized into four age groups (<3 yr, 3-4 yr, 4-5 yr and >5yr). All the sampled animals were followed to record the occurrence of spontaneous abortion until the end of gestation.

Serum samples

Blood samples were taken from the caudal vein of the animals and immediately transported to the laboratory. Serum was removed after centrifugation at 1200×g for 10 min. Each serum sample was kept in microtubes and stored at -20°C until they were tested for an antibody specific to *N. caninum*.

Serology

All sera were tested for IgG antibodies to *Neospora caninum* in the Parasitology Department Laboratory, Faculty of Veterinary Medicine, The University of Tehran

using the Herdcheck-ELISA commercial kit (IDEXX Lab., Germany) according to the instructions of the manufacturer. Briefly, 100 µl of undiluted negative control, undiluted positive control and diluted serum sample were added to the well and the plate was incubated at room temperature for 30 min. The wells were washed four times with PBS Tween Buffer and 100 µl of HRP conjugate was added to each well and incubated at room temperature for 30 min. The plate was washed again and 100 µl of substrate solution was added and incubated at room temperature for 15 min. Then, 100 µl of stop solution were added to stop the reaction and the plate was analyzed in an ELISA microplate reader at a wavelength of 650 nm. The presence or absence of antibody against *Neospora* was determined by the sample to positive (S/P) ratio for each sample. The sample to positive (S/P) ratio was calculated with the use of the following formula:

$$S/P = \frac{\text{Sample A (650)} - \text{negative control mean}}{\text{positive control mean} - \text{negative control mean}}$$

Sera that had a corrected optical density (OD) >0.5 were considered to be positive for *N. caninum* infection.

Statistical analysis

Analysis of the data was performed using the Chi-squared analysis on contingency tables and linear regression (SPSS 11.5, Standard Version, Copyright SPSS Inc., 1982-2002). Statistical significance was reached at $p \leq 0.05$.

Results

IgG antibodies against *N. caninum* were detected in 298 of 768 blood samples (38.8%). In total, 40.7% of the cattle in farm A, 18.7% of farm B, 34.2% of farm C and 65.1% of farm D were seropositive (Table 1). The infection rates in different age groups of all farms are shown in Table 2. A significant correlation was demonstrated between infection rates in different age groups in herd A and when the entire population was considered ($r^2=0.97$, $p=0.03$). No significant correlation was found within the other three herds. The abortion rate in seropositive animals was 20.67%, and 10.11% in seronegative cattle. Therefore, the risk of abortion was twice as high in the seropositive cows ($p<0.0005$). When the data of all four herds were collated, there was a high positive correlation between the infection rate and the occurrence of abortion (overall $X^2=30.06$, $p=0.0005$, $df=1$).

Discussion

This is the first extensive study on the seroepidemiology of *N. caninum* in dairy farms in the surroundings of Tehran. In this study, an IgG antibody against *N. caninum* was detected in 298 of 768 pregnant cattle (38.8%). The seroprevalence of *N. caninum* infection in cattle varies largely, depending on the country and region under study. In a comparative study that was carried out by Bartels et al. (2006), the rate of seroprevalence in European dairy farms ranged from 0.5% in Sweden to 16.2% in Spain. The prevalence of *Neospora* infection were estimated to range between 14.1% and 40.4% in the USA (Paré et al., 1996; Chi et al., 2002; Moore, 2005), between 5.7% and 35.6% in Asia (Hur et al. 1998; Koiwai et al., 2005), and between 6.0% and 21.1% in Oceania (Reichel 1998; Hall et al., 2006). Akca et al. (2005) reported that 8.2% of tested Simmental cows were positive in the Kars province of Turkey. Sevgili et al. (2005) found antibodies to *N. caninum* in 23 of the 305 (7.5%) cow serum samples, based on the results of ELISA tests in the province of Sanliurfa, Turkey. There are only few reports on the rates of seroprevalence of *N. caninum* infection in dairy herds in Iran. These studies were mainly carried out in Mashhad, in the northeastern region of Iran

Table 1: Prevalence of seropositive pregnant cattle in four different herds.

Herd	Pregnant animals		
	Total No.	Seropositive No.	Seropositive (%)
A	199	81	40.7
B	198	37	18.7
C	199	68	34.2
D	172	112	65.1
Total	768	298	38.8

Table 2: Seroprevalence of pregnant cattle in different age groups of four different herds.

Herds	Age											
	<3years			3 – 4 years			4-5 years			>5 years		
	Total No.	Pos. No.	Pos. %	Total No.	Pos. No.	Pos. %	Total No.	Pos. No.	Pos. %	Total No.	Pos. No.	Pos. %
A	82	27	32.9	31	11	35.5	32	15	46.9	54	28	51.9
B	77	5	6.5	52	17	32.7	31	7	22.6	38	8	21.1
C	67	16	23.9	49	19	38.8	49	22	44.9	34	11	32.4
D	28	18	64.3	46	23	50.0	16	13	81.3	82	58	70.7
Total	254	66	26.0	178	70	39.3	128	57	44.5	208	105	50.5

Table3: Abortion rate in pregnant cattle in four different herds.

Herd	Seropositive animals (No.)	Seropositive aborted animals (No.)	Abortion in seropositive animals (%)	Seronegative animals (No.)	Seronegative aborted animals (No.)	Abortion in seronegative animals (%)
A	81	24	29.62	118	19	16.1
B	37	9	24.32	161	16	9.93
C	68	5	7.35	131	8	6.1
D	112	24	21.42	60	5	8.33
Total	298	62	20.67	470	48	10.11

(Sadrebazzaz *et al.*, 2004; Razmi *et al.*, 2006), and in Kerman, in the southeastern region of Iran (Nourollahi Fard *et al.*, 2008). In the 3 mentioned studies, using IFAT and ELISA, the rate of *N. caninum* infection were 15.7% to 19.6% (Sadrebazzaz *et al.*, 2004), 46% (Razmi *et al.*, 2006) and 12.6% (Nourollahi Fard *et al.*, 2008), these result are not similar to ours.

The within-herd prevalence of *N. caninum* in the present study was between 18.7% and 65.1%. This range is more scattered than the range that was reported in studies of Australian and New Zealand dairy herds of 31-35% (Atkinson *et al.*, 2000 and Reichel, 2000), 16-35% in Vietnam (Doung, 2008) and 15.7-19.6% in the northeastern region of Iran (Sadrebazzaz *et al.*, 2004). It is conceivable that the wide range of infection rates in the four studied herds in Tehran was mainly due to differences in management conditions between herds. In farm D, with the highest prevalence of infection (65.1%), the management conditions were not well-controlled, and stray dogs could easily enter the herd due to lack of fencing.

Several studies have been performed on the seroepidemiology of anti-*N. caninum* antibodies in dogs in the vicinity of Tehran, which suggested the presence of *N. caninum* infection in these areas (Malmasi *et al.*, 2006; Haddadzadeh *et al.*, 2007). Other authors have shown that the presence of farm dogs is a risk factor for *N. caninum*-associated abortion in cattle (Pare *et al.*, 1998; Lindsay *et al.*, 1999).

In our study, a significant correlation was demonstrated between infection rates in different age groups in herd A, but no significant correlation was found within the other three herds. The relationship between age and seroprevalence in bovine neosporosis is speculative. Wouda *et al.* (1998) and Sadrebazzaz *et al.* (2004) reported no significant difference in seropositivity for different age groups of cattle. Jensen *et al.* (1999) suggested that seroprevalence increases with age. In contrast, Sanderson *et al.* (2000) reported that cows below 3 yr of age had higher CI-ELISA inhibition percentage values than cows above 6 yr of age. The association between seropositivity to *N. caninum* and abortion in dairy cattle that was found in this study correlated with findings in previous reports (Dubey *et al.*, 1997; Paré *et al.*, 1997; Mainar-Jaime *et al.*, 1999; Anderson *et al.*, 2000; López-Gatius *et al.*, 2004). However, a significant correlation between infection and fertility disorders was not found. This finding is compatible with the results from a seroepidemiological study carried out in Spain (López-Gatius *et al.*, 2005).

In this study, the abortion rate in total seropositive cattle was 20.67% and in seronegative cattle was 10.11%; the comparison between pregnant cows that were seropositive versus those that were seronegative for *N. caninum* demonstrated that the risk of abortion was double the normal risk in seropositive cattle that could be considered as part of the endemic pattern of abortion due

to *N. caninum* (Dubey *et al.*, 2007). In previous studies, the risk of abortion in seropositive cattle in comparison with the seronegative cattle was reported to be greater by 5.3-fold (Lopez *et al.*, 2005), eight-fold (Vaclavek *et al.*, 2003) and four-fold (Davison, 1999; Sager, 2001 and Haessig and Gottstein, 2002).

In conclusion, the results of the present study suggest that *N. caninum* is an important factor in the economic losses of the dairy industry in the region of Tehran, and appropriate management and control strategies need to be practiced by dairy farmers in this area.

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References

1. Akca, A.; Gokce, H.I.; Guy, C.S.; McGarry, J.W. and Williams, D.J.L. (2005) Prevalence of antibodies to *Neospora caninum* in local and imported cattle breeds in the Kars province of Turkey. *Res. Vet. Sci.* 78: 123-126.
2. Anderson, M.L.; Andrianarivo, A.G. and Conrad, P.A. (2000) Neosporosis in cattle. *Anim. Reprod. Sci.* 60-61: 417-431.
3. Atkinson, R.A.; Atkinson, R.W.; Cook, L.A.; Reddacliff, J.; Rothwell, K.W.; Broady, P.A.W. and Ellis, J.T. (2000) Seroprevalence of *Neospora caninum* infection following an abortion outbreak in a dairy cattle herd. *Australian Vet. J.* 78: 262-266.
4. Bartels, C.J.M.; Arnaiz-Seco, J.I.; Ruiz-Santa-Quitera, A.; Björkman, C.; Frössling, J.; von Blumröder, D.; Conraths, F.J.; Schares, G.; van Maanen, C.; Wouda, W. and Ortega-Mora, L.M. (2006) Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. *Vet. Parasitol.* 137: 17-27.
5. Chi, J.; Van Leeuwen, J.A.; Weersink, A. and Keefe, G.P. (2002) Management factors related to seroprevalences to bovine viral diarrhoea virus, bovine-leukosis virus, *Mycobacterium avium* subspecies paratuberculosis, and *Neospora caninum* in dairy herds in the Canadian Maritimes. *Prev. Vet. Med.* 55: 57-68.
6. Davison, H.C.; Otter, A. and Trees, A.J. (1999) Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. *Int. J. Parasitol.* 29: 1683-1689.
7. Dubey, J.P.; Jenkins, M.C.; Adams, D.S.; McAllister, M.M.; Anderson-Sprecher, R.; Baszler, T.V.; Kwok, O.C.; Lally, N.C.; Björkman, C. and Uggla, A. (1997) Antibody responses of cows during an outbreak of neosporosis evaluated by indirect fluorescent antibody test and different enzyme-linked immunosorbent assays. *J. Parasitol.* 83: 1063-1069.
8. Dubey, J.P.; Schares, G. (2006) Diagnosis of bovine

- neosporosis. *Vet. Parasitol.* 140: 1–34.
9. Duong, M.C.; Alenius, S.; Thu Huong, L.T. and Björkman, C. (2008) Prevalence of *Neospora caninum* and bovine viral diarrhoea virus in dairy cows in Southern Vietnam. *Vet. J.* 175: 390–394.
 10. Haddadzadeh, H.R.; Sadrebazzaz, A.; Malmasi, A.; Talei Ardakani, A.; Khazraii Nia, P. and Sadreshirazi, N. (2007) Seroprevalence of *Neospora caninum* infection in dogs from rural and urban environments in Tehran, Iran. *Parasitol. Res.* 101: 1563–5.
 11. Hall, C.A.; Reichel, M.P. and Ellis, J.T. (2006) Prevalence of *Neospora caninum* infection in Australian (NSW) dairy cattle estimated by a newly validated ELISA for milk. *Vet. Parasitol.* 142: 173–178.
 12. Hässig, M. and Gottstein, B. (2002) Epidemiological investigations of abortions due to *Neospora caninum* on Swiss dairy farms. *Vet. Rec.* 150: 538–542.
 13. Hernández, J.; Risco, C. and Donovan, A. (2001) Association between exposure to *Neospora caninum* and milk production in dairy cows. *J. Am. Vet. Med. Ass.* 219: 632–635.
 14. Hernandez, J.; Risco, C. and Donovan, A. (2002) Risk of abortion associated with *Neospora caninum* during different lactations and evidence of congenital transmission in dairy cows. *J. Am. Vet. Med. Ass.* 221: 1742–1746.
 15. Hur, K.; Kim, J.H.; Hwang, W.S.; Hwang, E.K.; Jean, Y.H.; Lee, B.C.; Bae, J.S.; Kang, Y.B.; Yamane, I. and Kim, D.J. (1998) Seroepidemiological study of *Neospora caninum* in Korean dairy cattle by indirect immunofluorescent antibody assay. *Korean J. Vet. Res.* 38: 859–866.
 16. Koiwai, M.; Hamaoka, T.; Haritani, M.; Shimizu, S.; Kimura, K. and Yamane, I. (2005) Proportion of abortions due to neosporosis among dairy cattle in Japan. *J. Vet. Med. Sci.* 67: 1173–1175.
 17. López-Gatius, F.; Pabon, M. and Almeria, S. (2004) *Neospora caninum* infection does not affect early pregnancy in dairy cattle. *Theriogenology.* 62: 606–613.
 18. López-Gatius, F.; Santolaria, P. and Almeria, S. (2005) *Neospora caninum* infection does not affect the fertility of dairy cows in herds with high incidence of Neospora-associated abortions. *J. Vet. Med. B. Infect. Dis. Vet. Public Health.* 52: 51–53.
 19. Malmasi, A.; Hosseinijad, M.; Haddadzadeh, H.; Badii, A. and Bahonar, A. (2007) Serologic study of anti-*Neospora caninum* antibodies in household dogs and dogs living in dairy and beef cattle farms in Tehran, Iran. *Parasitol. Res.* 100: 1143–1145.
 20. Moore, D.P. (2005) Neosporosis in South America. *Vet. Parasitol.* 127: 87–97.
 21. Nourollahi Fard, S.R.; Khalili, M. and Aminzadeh, A. (2008) Prevalence of antibodies to *Neospora caninum* in cattle in Kerman province, South East Iran. *Vet. Arhiv.* 78: 253–259.
 22. Ould-Amrouche, A.; Klein, F.; Osdoit, C.; Mohammed, H.O.; Touratier, A.; Sanaa, M. and Mialot, J.P. (1999) Estimation of *Neospora caninum* seroprevalence in dairy cattle from Normandy, France. *Vet. Res.* 30: 531–538.
 23. Paré, J.; Thurmond, M.C. and Hia, S.K. (1996) Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. *Can. J. Vet. Res.* 60: 133–139.
 24. Paré, J.; Thurmond, M.C. and Hia, S.K. (1997) *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. *J. Parasitol.* 83: 82–87.
 25. Razmi, G.R.; Mohammadi, G.R.; Garrosi, T.; Farzaneh, N.; Fallah, A.H. and Maleki, M. (2006) Seroepidemiology of *Neospora caninum* infection in dairy cattle herds in Mashhad area, Iran. *Vet. Parasitol.* 135: 187–189.
 26. Razmi, G.R.; Maleki, M.; Farzaneh, N.; Talebkhan Garoussi, M. and Fallah, A.H. (2007) First report of *Neospora caninum*-associated bovine abortion in Mashhad area, Iran. *Parasitol. Res.* 100: 755–7.
 27. Reichel, M.P. (1998) Prevalence of *Neospora* antibodies in New Zealand dairy cattle and dogs. *N. Z. Vet. J.* 46: 38.
 28. Reichel, M.P. (2000) *Neospora caninum* infections in Australia and New Zealand. *Australian Vet. J.* 78: 258–261.
 29. Sadrebazzaz, A.; Haddadzadeh, H.; Esmailnia, K.; Habibi, G.; Vojgani, M. and Hashemifesharaki, R. (2004) Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. *Vet. Parasitol.* 124: 201–204.
 30. Sager, H.; Fischer, I.; Furrer, K.; Strasser, M.; Waldvogel, A.; Boerlin, P.; Audigé, L. and Gottstein, B. (2001) A Swiss case-control study to assess *Neospora caninum*-associated bovine abortions by PCR, histopathology and serology. *Vet. Parasitol.* 102: 1–15.
 31. Sanderson, M.W.; Gay, J.M. and Baszler, T.V. (2000): *Neospora caninum* seroprevalence and associated risk factors in beef cattle in the northwestern United States. *Vet. Parasitol.* 90: 15–24.
 32. Sevigili, M.; Gul Altas, M. (2005) Seroprevalence of *Neospora caninum* in cattle in the province of Sanliurfa. Turkey. *J. Vet. Anim. Sci.* 29: 127–130.
 33. Thurmond, M.C.; Hietala, S.K. (1997) Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *Am. J. Vet. Res.* 58: 1381–1385.
 34. Vaclavek, P.; Koudela, B.; Modry, D. and Sedlak, K. (2003) Seroprevalence of *Neospora caninum* in aborting dairy cattle in the Czech Republic. *Vet. Parasitol.* 115: 239–245.
 35. Von Blumroder, D.; Schares, G.; Norton, R.; Williams, D.J.; Esteban-Redondo, I.; Wright, S.; Bjorkman, C.; Frossling, J.; Risco-Castillo, V.; Fernandez-Garcia, A.; Ortega-Mora, L.M.; Sager, H.; Hemphill, A.; van Maanen, C.; Wouda, W. and Conraths, F.J. (2004) Comparison and standardisation of serological methods for the diagnosis of *Neospora caninum* infection in bovines. *Vet. Parasitol.* 120: 11–22.
 36. Wouda, W.; Moen, A.R. and Schukken, Y.H. (1998) Abortion risk in progeny of cows after a *Neospora caninum* epidemic. *Theriogenol.* 49: 1311–1316.