



Seroprevalence of *Neospora caninum* Infection in Dairy Cattle in West of Iran

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Abstract

The *Neospora caninum* parasite causes abortion in cattle in virtually all parts of the world with enormous economic consequences. The purpose of this study was to determine the seroprevalence of antibodies of *Neospora caninum* in dairy cattle in Lorestan Province, west of Iran. A total of 347 dairy cows were randomly selected. The serum of each case was analyzed for the possibility of the presence of antibody against *N. caninum* antigen, using the commercial kit: ELISA. The results of the ELISA test indicated that from 347 dairy cattle examined, the antibodies to *N. caninum* were found in 34 (9.8%). The percentage of seropositive aborted cattle was 13.33%. This study also indicated that there was no significant relationship between seropositivity and such factors as the age, breed, and abortion history of the cattle. Moreover, no significant relationship between seroprevalence of infection among rural and industrial cows was found. The neosporosis could be one of the possible causes of abortion in cattle. Further studies are recommended to determine the relationship between this parasite and the occurrence of abortion in cattle in the province of Lorestan.

Özet

İran'ın Batısındaki Süt Sığırlarında *Neospora caninum* Enfeksiyonunun Seroprevalansı

Neospora caninum parazitleri sığırlarda dünyanın hemen hemen bütün bölgelerinde çok büyük ekonomik sonuçlara yol açan düşüklere neden olur. Bu çalışmanın amacı İran'ın batısında, Lorestan bölgesindeki süt sığırlarında *Neospora caninum* antikorlarının seroprevalansını belirlemektir. Toplamda 347 süt ineği rastgele seçildi. Her olgunun serumu ticari kit: ELISA kullanılarak *N.caninum* antijenine karşılık antikor mevcudiyeti olasılığı yönünden incelendi. ELISA testinin sonuçları incelenen 347 süt sığırının 34'ünde (%9,8) *N.caninum*'a karşı antikorların bulunduğunu gösterdi. Seropozitif abort yapan sığırların oranı %13,33 bulunmuştur. Bu çalışma aynı zamanda seropozitivite ile büyükbaşların yaşı, cinsiyeti ve düşük geçmişleri gibi faktörler arasında anlamlı bir ilişki olmadığını gösterdi. Ayrıca, kırsal ve entansif sistemde yetiştirilen inekler arasında enfeksiyon seroprevalansı yönünden de anlamlı bir ilişki bulunmadı. Sığırlardaki abortların olası nedenlerinden birisi neosporosis olabilir. Lorestan bölgesindeki görülen abort vakaları ile bu parazit arasındaki bağlantıyı belirleyebilmek için daha kapsamlı çalışmalar önerilmektedir.

Introduction

Relatively speaking, *Neospora caninum* is a newly identified parasite; for the first time in 1984, it was detected in dogs in Norway as an apicomplexan protozoan (Bjerkas et al., 1984). The neosporosis is a major cause of reproductive failure in cattle in the world, and has caused considerable economic losses (Dubey et al., 1996). The bovine neosporosis is seen in clinical observations in the form of abortion in cattle (Sanderson et al., 2000). Serological tests have the

benefit of being applied ante mortem and may offer data during the period of infection (Dubey and Schares, 2006). Indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assays (ELISA) are the main tests used for serologic survey for *N. caninum* infection (Dubey et al., 1996).

Many aspects of the life cycle of *Neospora caninum* are unknown. It has been established that the dog and the coyote are the only hosts known that can excrete the environmentally resistant oocysts (Gondim et al.,

2004). The bovine are infected as intermediate hosts by oocysts shedding of the infected dogs (Jensen et al., 1999). It has also been observed that *N. caninum* is transmittable vertically from an infected cow to her fetus during pregnancy (Anderson et al., 1997). Waldner (2005), observed that the likelihood of abortion among seronegative cows is less than that in dairy and beef cattle with antibodies to *Neospora caninum* (seropositive).

The presence of neosporosis has frequently been reported from many regions of the world. In contrast, the number of published reports about neosporosis and its impact on the cattle in Iran, is limited to the following: Hajikolaie et al. (2008), Nematollahi et al. (2011); Nourollahi Fard et al. (2008), Razmi et al. (2006), Sadrebazzaz et al. (2004) and Youssefi et al. (2009).

The reason for conducting the present study is twofold: first, there is no information about the prevalence of antibodies to *Neospora caninum* in dairy cattle in the Lorestan Province, and second, husbandry is one of the most important industries in the west of Iran including Lorestan Province.

Materials and Methods

Sampling

This cross-sectional study was conducted on 347 dairy cows, chosen at random in Lorestan Province, west of Iran, between May, 2012 and April, 2013. Using

$$PP = \text{Mean OD value (sample or Negative Control)} \times 100 / \text{Mean OD value Positive Control}$$

The findings were demonstrated as the percent positivity (PP) of the high positive control sera. For the analysis of the test, the manufacturer's current suggestion is that a test result of below 20 PP demonstrates negative result, and a test result of equal or above to 20 PP points to a positive result.

The data about such factors as abortion history, breed, age, and the way in which the animals are cared for, were obtained from the owners, and recorded.

Statistical analysis

A chi-square test of independence was used to analyze associations between infection by *N. caninum* and other factors studied in the present study. For statistical analysis, the SPSS 12 computer program was used and $P < 0.05$ was considered to be significant.

Results

Antibodies to *N. caninum* were detected in 34 of the 347 (9.8%) sera based on ELISA method. In the cattle

throw-away needles, blood samples were collected from the cattle. All samples were taken to the diagnostic laboratory at once. Serum was taken out after centrifugation at $1000 \times g$ for 10 min. All sera were equally allocated into two micro tubes and kept at -70°C until laboratory testing.

Serological screening

The sera were analyzed for detecting antibodies to *N. caninum* making use of ELISA. Anti-*Neospora* antibodies were identified using the commercial *N. caninum* ELISA kit (IDEXX, Switzerland). The kit was used as directed by the manufacturer. In brief, 100 microliters of pre-diluted serum specimen were added as first antibody and the plate incubated at 37°C on shaker for 1 hour. The wells were washed 3 times with PBS Tween Buffer and 100 microliters of HRP conjugate were included to each well and incubated for 1 hour at 37°C . The plate was washed once more and 100 microliters of substrate solution included and incubated at room temperature for 10 minutes. Later 50 microliters of stop solution were included to halt the reaction and the plates were registered in an ELISA Microplate Reader (Anthos 2020, Austria) at a wavelength of 450 nm. The optical density (OD) of the ELISA was registered on an automated plate reader and the Percent Positivity values (PP) of the test specimens were estimated by the following formula:

group with <18 months age group, 3 out of 26 (11.54%) were seropositive, whereas in the cattle group with >18 months age, 31 out of 321 (9.66%) were seropositive. Seroprevalence of *Neospora caninum* in the indigenous cattle was 8.91% and in Holstein cattle was 4.54%. It was observed that 13.33% of the cattle with an abortion history and 9.64% of the cattle with no abortion history were seropositive. Seroprevalence in rural and industrial dairy cows was 12.34% and 7.57% respectively. Results gathered from the sera using ELISA are presented in Table 1.

The results were articulated as the percent positivity (PP) of the high positive control sera. No statistically significant relationship was found between seropositivity and age, breed, and abortion history. It was also noted that there was no significant difference regarding the seroprevalence of infection between rural and industrial dairy cows ($P > 0.05$).

Table 1. Seroprevalence of *Neospora caninum* in relationship to age, breed, and abortion history in rural and industrial cattle in Lorestan Province.

Tablo 1. Lorestan Bölgesinde kırsal ve entansif sistemde yetiştirilen sığırlarda yaş, cinsiyet ve abort geçmişi ile bağlantılı olarak *Neospora caninum* seroprevalansı.

Group	The number of animal tested	No. of positives	Seroprevalence (%)
<18 month	26	3	11.54
>18 month	321	31	9.66
Aborted cattle	15	2	13.33
Non-aborted cattle	332	32	9.64
Rural cattle	162	20	12.34
Industrial cattle	185	14	7.57

Discussion

The *Neospora caninum* is considered as one of the major causes of abortion in cattle worldwide which results in significant economic loss. The annual economic loss due to the adverse financial effect of neosporosis on dairy cattle in Australia and New Zealand was \$ 25 million and \$17.8 million respectively (Dubey et al., 2007).

The magnitude of economic impact which is related to the degree of prevalence of neosporosis varies. In a research conducted in South Vietnam on 215 cows with ELISA, 88 specimens (41%) were declared positive (Dung et al., 2008). The prevalence of *N. caninum* infection among the cattle by ELISA from a region in south-eastern Iran (an arid desert area with violent sandstorms in the springs, and hot summers; otherwise a moderate climate), was 12.6% (Nourollahi Fard et al., 2008). In another research conducted on 237 cattle with ELISA in northern Iran (with mild and humid climate), 40 industrial cattle (25.8%) and 36 rural cattle (43.9%) were declared positive (Youssefi et al., 2009). Similarly, other findings were: using ELISA in Ahvaz (known for its long, very hot summers, humid climate and mild short winters with no snow) 21% (Hajikolaie et al., 2008) and using IFA test in Mashhad (with a steppe climate, hot summers and cool winters) 15.8% (Sadrebazzaz et al., 2004).

The present study is the first of its kind about the seroprevalence of *N. caninum* in dairy cattle in Lorestan Province. This study showed that the seroprevalence of antibody to *N. caninum* is 9.8% in dairy cattle in this province. The discrepancies between the rates of infection in the present study and those of others could be attributed to two factors: first, the climatological differences of the province of Lorestan and other areas in Iran, and second, the differences in the detection methods.

Lorestan, a mountainous province, has a sub-humid climate with moderately cold winters, which is remarkably different than the climates of the regions

about which the other studies (cited above) were conducted.

The results of the present study are similar to the results of study by Nematollahi et al. (2011), using ELISA on dairy cattle in Tabriz, the center of Azerbaijan Province that reported 10.5% seropositivity among dairy cattle. The results of the current study are also comparable with the study conducted by Youssefi et al. (2009), in which the percentage of seropositive rural cattle (43.9%) was significantly higher than the percentage of seropositive industrial cattle (25.8%), although the statistical difference was not observed.

In the Province of Lorestan, dairy cattle consist of the traditional rural cows and the industrial cows. The latter usually consists of 20-100 kept and cared for according to the appropriate standards. The milking is automatic; however, the feeding is intensive. The entrance of dogs into the facilities is limited. In comparison, rural cow herders, usually own two or three cows, housed in the owner's place of residence, which usually includes a small grazing area adjacent to it that provides additional source of feeding. Otherwise, feeding and milking of the cows are done manually. Occasionally, dogs can be seen in the same place with the cows. The degree of humidity and the possibility of intermingling of cows and dogs could enhance the seroprevalence rate of *N. caninum* (Youssefi et al., 2009).

It is noted that the presence of antibodies to *N. caninum* in cattle only indicates exposure to the parasite; however, the chances of abortion in cattle diagnosed with seropositive is two times higher than in seronegative cattle (Thurmond et al., 1997). Only a few cows with a history of abortion are diagnosed as seropositive (Paré et al., 1996). There are different reasons for abortion in cattle, of which *Neospora* is one. This necessitates more consideration on all factors contributing to abortion in cattle. For instance, a more reliable picture could have emerged, if serological tests

of bovine viral diarrhoea virus had been added (Paré et al., 1996).

In this study, IDEXX ELISA kit was employed. In a research carried out by Wu et al. (2002), sensitivity for the IDEXX ELISA on the field samples was recorded 97.6% while specificity was 98.5%. IFAT compared to ELISA is time-consuming and more costly, thus used infrequently for checking cattle populations for *N. caninum* infection (Haddad et al., 2005). The ELISA shows sensitivity $\geq 95\%$ and specificity $\geq 97\%$, and therefore making it an authentic test for detecting the infected animals and herds (Haddad et al., 2005). Jensen et al. (1999), reported that seroprevalence rises with age. On the contrary, the present research manifested no significant relationship between age and seropositivity to neosporosis. This finding is in line with those of the studies conducted by Hajikolaie et al. (2008), and Sadrebazzaz et al. (2004). In a study conducted by Osawa et al. (2002), from a herd of 879 beef and dairy cattle tested by ELISA, "262 (29.8%) cattle were positive to *N. caninum*." In the same study, examining the serum samples taken from a herd that had the highest experience of abortion, showed the highest likelihood to be positive to *N. caninum* (up to 56.7%). In the present study seropositivity among aborting cows was higher than non-aborting cows, although not statistically significant.

The Neosporosis is one of the possible causes of abortion in dairy cattle with severe economic consequences. The economic impact of *Neospora caninum* on dairy production through the adverse financial hardship stemming from cattle abortion clearly points to the necessity of controlling this disease. The present study is a step in the right direction, in the province of Lorestan, where there is a serious need for preventive, control and monitoring measures. Further comprehensive studies needed to investigate the extent and the severity of abortions caused by *Neospora caninum*, and determine the relationship between this parasite and abortion in cattle in various regions of the Lorestan.

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REFERENCES

Anderson, M.L., Peynolds, J.P., Rowe, J.D., Sverlow, K.W., Packham, A.E., Barr, B.C., Conrad, P.C., 1997. Evidence of vertical transmission of *Neospora* sp. Infection in dairy cattle. Journal of the American Veterinary Medical Association 210, 1169-1172.

- Bjerkas, I., Mohn, S.F., Presthus, J., 1984. Unidentified cyst-forming sporozoan causing encephalomyelitis and myositis in dogs. Zeitschrift für Parasitenkunde 70, 271-274.
- Doung, M.C., Aleninus, S., Huong, L.T., Björkman, M.C., 2008. Prevalence of *Neospora caninum* and bovine viral diarrhoea virus in dairy cows in southern Vietnam. The Veterinary Journal 175, 390-394.
- Dubey, J.P., Lindsay, D.S., Adams, D.S., Gay, J.M., Baszler, T.V., Thulliez, P., 1996. Serologic responses of cattle and other animals infected with *Neospora caninum*. American Journal of Veterinary Research 57, 329-336.
- Dubey, J.P., Schares, G., 2006. Diagnosis of bovine neosporosis. Veterinary Parasitology 140, 1-34.
- Dubey, J.P., Schares, G., Ortega Mora, L.M., 2007. Epidemiology and control of neosporosis and *Neospora caninum*. Clinical Microbiology Reviews 20, 323-367.
- Gondim, L.F.P., McAllister, M.M., Pitt, W.C., Zemlicka, D.E., 2004. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. International Journal for Parasitology 34, 159-161.
- Haddad, J.P.A., Dohoo, I.R., Vanleewen, J.A., 2005. A review of *Neospora caninum* in dairy and beef cattle - a Canadian perspective. The Canadian Veterinary Journal 46, 230-243.
- Hajikolaie, M.R., Hamidinejat, H., Goraninejad, S., 2008. Serological study of *Neospora caninum* infection in cattle from Ahwaz, Iran. International Journal of Veterinary Research 2, 63-66.
- Jensen, A.M., Björkman, C., Kjeldsen, A.M., Wedderkopp, A., Willadsen, C., Ugglå, A., Lind, P., 1999. Association of *Neospora caninum* seropositivity with gestation number and pregnancy outcome in Danish dairy herds. Prevention Veterinary Medicine 40, 151-163.
- Nematollahi, A., Jaafari, R., Moghaddam, G.H., 2011. Seroprevalence of *Neospora caninum* infection in dairy cattle in Tabriz, northwest Iran. Iranian Journal of Parasitology 6, 95-98.
- Nourollahi Fard, S.R., Khalili, M., Aminzadeh, A., 2008. Prevalence of antibodies to *Neospora caninum* in cattle in Kerman province, South East Iran. Veterinary Archives 78, 253-259.
- Osawa, T., Wastling, J., Acosta, L., Ortellado, C., Ibarra, J., Innes, E.A., 2002. Seroprevalence of *Neospora caninum* infection in dairy and beef cattle in Paraguay. Veterinary Parasitology 110, 17-23.
- Paré, J., Thurmond, M.C., Hietala, S.K., 1996. Congenital *Neospora caninum* infection in dairy cattle and associated calf hood mortality. Canadian Journal of Veterinary Research 60, 133-139.
- Razmi, G.R., Mohammadi, G.R., Garrosi, T., Farzaneh, N., Fallah, A.H., Maleki, M., 2006. Seroprevalence of *Neospora caninum* infection in dairy cattle herds in Mashhad area, Iran. Veterinary Parasitology 135, 187-189.

- Sadrebazzaz, A., Haddadzadeh, H., Esmailnia, K., Habibi, G., Vojgani, M., Hashemifesharaki, R., 2004.** Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. *Veterinary Parasitology* 124, 201-204.
- Sanderson, M.W., Gay, J.M., Baszler, T.V., 2000.** *Neospora caninum* seroprevalence and associated risk factors in beef cattle in north western United States. *Veterinary Parasitology* 90, 15-24.
- Thurmond, M.C., Hietala, S.K., Blanchard, P.C., 1997.** Herd-based diagnosis of *Neospora caninum* induced endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. *Journal of Veterinary Diagnostic Investigation* 9, 44-49.
- Waldner, C.L., 2005.** Serological status for *Neospora caninum*, bovine viral diarrhoea virus and infectious bovine rhinotracheitis virus at pregnancy testing and reproductive performance in beef herds. *Animal Reproduction Science* 90, 219-242.
- Wu, J.T., Dreger, S., Chow, E.Y., Bowlby, E.E., 2002.** Validation of 2 commercial *Neospora caninum* antibody enzyme linked immunosorbent assays. *Canadian Journal of Veterinary Research* 66, 264-271.
- Youssefi, M.R., Arabkhazaeli, F., Tabar Molla Hassan, A., 2009.** Seroprevalence of *Neospora caninum* infection in rural and industrial cattle in northern Iran. *Iranian Journal of Parasitology* 4, 15-18.