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Seroepidemiology of *Neospora caninum* in Cattle in East-Azerbaijan Province, North West Iran

Garedaghi Yagoob
Department of Parasitology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Abstract: *Neospora caninum* is an intracellular parasite which causes abortion in cattle worldwide. The aim of this study was to determine the seroprevalence of *Neospora caninum* in cattle in the province of East-Azerbaijan in North-West Iran. Blood samples were collected from 236 cattle in the province of East-Azerbaijan for determining the seroprevalence of *Neospora caninum*. A total of 236 serum samples were tested for anti-neospora antibodies. Serum samples were analyzed for antibodies against *N. caninum* antigen using a commercial *N. caninum* ISCOM ELISA kit. Antibodies to *N. caninum* were found in 42 of the 236 (17.7%) sera based on ELISA test results. This study is the first report of Neospora infection in this area. With regard to seropositivity, no significant difference was observed regarding origin, sex and age ($p>0.05$).

Key words: *Neospora caninum*, cattle, ELISA, East-Azerbaijan province, sex, Iran

INTRODUCTION

Neospora caninum (Apicomplexa) is a worldwide-distributed pathogen which causes abortions in cows leading to economic losses in the cattle industry (Dubey, 1999a). The parasite was first detected in 1984 in dogs with myositis, lameness and encephalitis and named as *N. caninum* (Bjerkas *et al*., 1984; Dubey, 1999b). *Neospora caninum* has worldwide distribution and has been known as one of the most commonly diagnosed causes of bovine abortion. The parasite was subsequently identified in aborted bovine foetuses (Barr *et al*., 1990; Thilsted, 1989) and is now recognized as a significant cause of economic loss in dairy and beef cattle herds worldwide due primarily to abortion and reduced reproductive efficiency (Barling *et al*., 2000; Dubey, 1999b; Waldner *et al*., 1998). The economic impact of Neospora-induced abortions depends on direct costs and the value of fetuses lost. Indirect costs include those associated with establishing the diagnosis, rebreeding cows that aborted and possible loss of milk yield. As clinical diagnosis is difficult, Serological tests are necessary for an exact diagnosis. Several serological tests including the Enzyme-Linked Immunosorbent Assay (ELISA), the Indirect Fluorescent Antibody Technique (IFAT), the Direct Agglutination Test (DAT) and Immunoblots (IB) can be used to detect anti *Neospora caninum* antibodies (Bjorkman and Uggla, 1999).

N. caninum is transmitted vertically from an infected cow to her foetus during pregnancy (Anderson *et al*., 1997). Dogs have been shown to excrete *N. caninum* oocysts (De Marez *et al*., 1999; Lindsay *et al*., 1999; McAllister *et al*., 1998).

Although, neosporosis has been reported from many parts of the world (Dubey and Lindsay, 1996; Dubey *et al*., 2005), there is only one published report available on its occurrence in Iran, Mashhad that indicated that 123 (15.18%) of 810 cattle were seropositive by Indirect Fluorescent Antibody test in 4 herds (Sadrebazzaz *et al*., 2004). So, this study was performed to determine the prevalence of antibodies to *N. caninum* in cattle in the province of East-Azerbaijan in North-West Iran.

MATERIALS AND METHODS

Serum samples: Serum samples were collected from a total of 236 cattle, the animals being randomly selected. Blood samples were taken using disposable needles. The owners were questioned about animal management and age and the information obtained was recorded. This study was performed between September, 2009 and October, 2010. All samples were immediately transported to the diagnostic laboratory. Serum was removed after centrifugation at 1000×g for 10 min. All sera were divided equally into two microtubes and stored at -20°C until laboratory testing.

ELISA: Serum samples were stored at -20°C until tested. They were analyzed for antibodies to *N. caninum* using ELISA. Anti-Neospora antibodies were detected using a commercially available *N. caninum* iscom ELISA kit (Svanova Biotech AB, Sweden).

The kit was used according to the manufacturer's instructions. Briefly, 100 μL of pre-diluted serum sample

added as first antibody and the plate incubated at 37°C on shaker for 1 h. The wells were washed 3 times with PBS Tween Buffer and 100 μL of HRP conjugate added to each well and incubated for 1 h at 37°C. The plate was washed again and 100 μL of substrate solution added and incubated at room temperature for 10 min. Then, 50 μL of stop solution were added to stop the reaction and the plates were read in an ELISA microplate reader (Anthos 2020, Austria) at a wavelength of 450 nm. The Optical Density (OD) of the ELISA was read on an automatic plate reader and the Percent Positivity values (PP) of the test samples were calculated by the following equation:

$$PP = \frac{\text{Mean OD value (sample or negative control)}}{\text{Mean OD value positive}} \times 100$$

Control: The results were expressed as the Percent Positivity (PP) of the high positive control sera. The manufacturer's current recommendations for the interpretation of the test are that a test result of below 20 PP indicates a negative result and a test result of above or equal to 20 PP indicates a positive result.

Statistical analysis: A χ^2-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 AND DISCUSSION

Results obtained from the sera using ELISA are shown in Table 1 and 2. The results were expressed as the Percent Positivity (PP) of the high positive control sera. Antibodies to *N. caninum* were found in 42 of the 236 (17.7%) sera based on ELISA results. Among the 80 sera in the cattle <18 months age group, 9 (11.2%) were seropositive whereas among the 156 sera >18 months old, 33 (21.1%) were seropositive (Table 1).

Among the 74 bulls, 8 (10.8%) were seropositive whereas of the 162 cows, 34 (20.9%) were seropositive (Table 2). There was no statistically significant relationship between seroprevalence of sex and age groups ($p>0.05$)

Table 1: Seroprevalence of *Neospora caninum* in relation to age

Age	The number of animals tested	No. of positives	Seroprevalence (%)
<18 months	80	9	11.2
≥18 months	156	33	21.1

Table 2: Comparison of *Neospora caninum* antibodies in relation to sex

Sex	The number of animals tested	No. of positives	Seroprevalence (%)
Bull	74	8	10.8
Cow	162	34	20.9

N. caninum is considered to be one of the major causes of abortion in cattle worldwide (Barling *et al*., 2000; Dubey, 1999a). In contrast to vertical transmission, horizontal transmission involves a two-host life cycle whereby the cow is infected from the ingestion of coccidial oocyst stages shed by the definitive host. Dogs are known to be a definitive host and produce oocysts in their faeces after ingesting infected meat (McAllister *et al*., 1998; Gondim *et al*., 2004).

In this study, researchers decided to obtain information on seroprevalence of *N. caninum* antibodies in cattle in North-West Iran (East-Azerbaijan). Several serologic tests including ELISA, IFAT and DAT can be used to detect *N. caninum*. The capability of a test to distinguish infected from non infected individuals is often described by its diagnostic sensitivity and specificity. All the serological tests mentioned above are valuable for identifying sera with moderate to high levels of anti-neospora antibodies. At present, the 2 main types of serological tests most commonly used for the diagnosis of Neospora infection are IFAT and ELISA.

Characterization studies have shown that *N. caninum* NC-1 iscoms contain membrane antigens from both the cell surface and from intracellular compartments. Iscom ELISA for the detection of *Neospora caninum* antibodies in blood serum and milk was developed to decrease cross-reactivity (Bjorkman *et al*., 1997; Bjorkman and Lunden, 1998; Frossling *et al*., 2003) therefore, researchers used a Commercial Iscom ELISA kit (Svanova, Sweden) for diagnostics of bovine neospora-species antibodies in blood serum.

The sensitivity and specificity of this technique were high (Bjorkman and Uggla, 1999). This study showed that the seroprevalence of *N. caninum* infection is 17.7% in East-Azerbaijan's cattle was >15.18% which has been reported by Sadrebazzaz *et al*. (2004) in Mashhad, Iran. Akca *et al*. (2005) reported that 8.2% of Simmental cows tested were positive in Kars province, Turkey. Sevgili and Altas (2005) found antibodies to *N. caninum* in 23 of the 305 (7.5%) cow sera based on ELISA test results in the province of Sanliurfa, Turkey. With regard to seropositivity, no significant difference was observed in origin, animal breed and age ($p>0.05$). The presence of antibodies against *N. caninum* in cows only indicate exposure to the parasite. In this study there was no significant difference in seroprevalence between the different age groups. Wouda *et al*. (1998) and Sadrebazzaz *et al*. (2004) reported for most herds that the seroprevalence levels were equal across all age groups. The relationship between age and seroprevalence in

bovine neosporosis is speculative. Jensen *et al.* (1999) suggested that seroprevalence increases with age. In contrast, Sanderson *et al.* (2000) reported that cows <3 years of age had higher CI-ELISA inhibition percentage values than cows >6 years of age.

They also suggested that infected cows can infect fetuses and if these calves have not been reinfected, antibody titers decline over time resulting in an apparent decrease in seroprevalence with cow age.

CONCLUSION

This study shows that due to the lack of information about the prevalence of infection in the definitive host, the dog in Iran, it is not possible to know which method of transmission (horizontal or vertical) is the main route of infection. However, further studies on the epidemiological evidence for a relationship between *N. caninum* infection in dogs and cattle and the relationship between abortion in cows and infection with *N. caninum* in Iran are required.

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REFERENCES

Akca, A., H.I. Gokce, C.S. Guy, J.W. McGarry and D.J. Williams, 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.

Anderson, M.I., J.P. Reynolds, J.D. Rowe, K.W. Sverlow, A.E. Packham, B.C. Barr and P.A. Conrad, 1997. Evidence of vertical transmission of Neospora sp. infection in diary cattle. J. Am. Vet. Med. Assoc., 210: 1169-1172.

Barling, K.S., J.W. McNeill, J.A. Thompson, J.C. Paschal, F.T. McCollum, T.M. Craig and L.G. Adams, 2000. Association of serologic status for *Neospora caninum* with postweaning weight gain and carcass measurements in beef calves. J. Am. Vet. Med. Assoc., 217: 1356-1360.

Barr, B.C., M.L. Anderson, P.C. Blanchard, B.M. Daft, H. Kinde and P.A. Conrad, 1990. Bovine fetal encephalitis and myocarditis associated with protozoal infections. Vet. Pathol., 27: 354-361.

Bjerkas, I., S.F. Mohn and J. Presthus, 1984. Unidenti?ed cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z. Parasitenkd., 70: 271-274.

Bjorkman, C. and A. Lunden, 1998. Application of iscom antigen preparations in ELISAs for diagnosis of *Neospora* and *Toxoplasma* infections. Int. J. Parasitol., 28: 187-193.

Bjorkman, C. and A. Uggla, 1999. Serological diagnosis of *Neospora caninum* infection. Int. J. Parasitol., 29: 1497-1507.

Bjorkman, C., O.J.M. Holmdahl and A. Uggla, 1997. An indirect enzyme-linked immunoassay (ELISA) for demonstration of antibodies to *Neospora caninum* in serum and milk of cattle. Vet. Parasitol., 68: 251-260.

De Marez, T., S. Liddell, J.P. Dubey, M.C. Jenkinsgas and L. Barre, 1999. Oral infection of calves with *Neospora caninum* oocysts from dogs: Humoral and cellular immune responses. Int. J. Parasitol., 29: 1647-1657.

Dubey, J.P. and D.S. Lindsay, 1996. A review of Neospora caninum and neosporosis. Vet. Parasitol., 67: 1-59.

Dubey, J.P., 1999b. Neosporosis in cattle: Biology and economic impact. J. Am. Vet. Med. Assoc., 214: 1160-1163.

Dubey, J.P., 1999a. Recent advances in *Neospora* and neosporosis. Vet. Parasitol., 84: 349-367.

Dubey, J.P., E. Knickman and C.E. Greene, 2005. Neonatal *Neospora caninum* infections in dogs. Acta Parasitol., 50: 176-179.

Dubey, J.P., J.L. Carpenter, C.A. Speer, M.L. Topper and A. Uggla, 1988. N ewly recognised fatal protozoan disease of dogs. J. Am. Vet. Med. Assoc., 192: 1269-1285.

Frossling, J., B. Bonnett, A. Lindberg and C. Bjorkman, 2003. Validation of a *Neospora caninum* iscom ELISA without a gold standard. Prev. Vet. Med., 57: 141-153.

Gondim, L.F., M.M. McAllister, N.E. Mateus-Pinilla, W.C. Pitt, L.D. Mech and M.E. Nelson, 2004. Transmission of *Neospora caninum* between wild and domestic animals. J. Parasitol., 90: 1361-1365.

Jensen, A.M., C. Bjorkman, A.M. Kjeldsen, A. Wedderkopp, C. Willadsen, A. Uggla and P. Lind, 1999. Associations of *Neospora caninum* seropositivity with gestation number and pregnancy outcome in Danish dairy herds. Prev. Vet. Med., 40: 151-163.

Lindsay, D.S., J.P. Dubey and R.B. Duncan, 1999. Confirmation that the dog is a definitive host for *Neospora caninum*. Vet. Parasitol., 82: 327-333.

McAllister, M., J.P. Dubey, D. Lindsay, W. Jolley, R. Wills and A. McGuire, 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol., 28: 1473-1479.

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.

Sanderson, M.W., J.M. Gay and T.V. Baszler, 2000. *Neospora caninum* seroprevalence and associated risk factors in beef cattle in the Northwestern United States. Vet. Parasitol., 90: 15-24.

Sevgili, M. and M.G. Altas, 2005. Seroprevalence of *Neospora caninum* in cattle in the province of Sanliurfa. Turk. J. Vet. Anim. Sci., 29: 127-130.

Thilsted, J.P., 1989. Neosporosis-like abortions in a herd of dairy cattle. J. Vet. Diagn. Invest., 1: 205-209.

Waldner, C.L., E.D. Janzen and C.S. Ribble, 1998. Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. J. Am. Vet. Med. Assoc., 213: 685-690.

Wouda, W., A.R. Moen and Y.H. Schukken, 1998. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. Theriogenology, 49: 1311-1316.