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 Immunobots (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 (Sadreuzzaz *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 \( \chi^2 \)-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>
<thead>
<tr>
<th>Age</th>
<th>The number of animals tested</th>
<th>Number of positives</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18 months</td>
<td>80</td>
<td>9</td>
<td>11.2</td>
</tr>
<tr>
<td>≥18 months</td>
<td>156</td>
<td>33</td>
<td>21.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>The number of animals tested</th>
<th>Number of positives</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bull</td>
<td>74</td>
<td>8</td>
<td>10.8</td>
</tr>
<tr>
<td>Cow</td>
<td>162</td>
<td>34</td>
<td>20.9</td>
</tr>
</tbody>
</table>

There was no statistically significant relationship between seroprevalence of sex and age groups (\( p<0.05 \)).

*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 feces after ingesting infected meat

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; Flossing *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.
Akoa *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.

ACKNOWLEDGEMENTS

The researchers wish to thanks the Islamic Azad University, Tabriz Branch, Tabriz, Iran for the financial supports and all laboratory technicians for technical aids in this project.

REFERENCES


