Seroprevalence of *Neospora caninum* in cows with reproductive failure in Center and Northwest of Romania

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Abstract. The purposes of this study were to demonstrate the presence of IgG type antibodies against *Neospora caninum* and to establish the prevalence of the infection in reproductively challenged bovines from five counties in Central and Northwestern Romania. One hundred and ninety three blood samples have been collected in the direction of detecting anti-*N. caninum* antibodies using indirect ELISA testing. The cattle were raised in two different breeding systems and their reproductive problems varied from multiple inseminations to abortions in different stages of gestation. The cattle originated from different raising systems and varied in age and breed. The overall seroprevalence that we obtained reached the value of 55.95% (95% CI 48.7%-63.1%) and was different from county to county.

Keywords: Neosporosis; Cattle; Reproduction; ELISA.

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Introduction

Neosporosis is a parasitic disease that is caused by *Neospora caninum* an apicomplexan closely related to *Toxoplasma gondii* with which it has been confused until 1988. This infection has been frequently associated in cattle with abortion, reproductive disorders, culling and losses in milk production (Garcia-Vasquez et al., 2008).

Abortions can take place from the third month of pregnancy to just before term. However, abortion mostly occurs at 5–6 months of gestation (Moore, 2005). Cows infected with *N. caninum* have up to 5.7-18.9% higher risk of abortion than non-infected cows (Duong et al., 2008). This parasite can be transmitted transplacentary in several hosts, with the vertical transmission being the major way of spreading this disease in cattle. Bovines can also acquire this infection by the ingestion of food contaminated with oocysts from dog feces. Similarly, canines are infected by eating fetuses or tissues containing bradyzoites (Dubey, 1999).

Materials and methods

Serum samples

The survey was performed in five counties from Center and Northwest of Romania. The cattle
were raised in two different breeding systems: intensive and backyard, and their age varied between 3 and 12 years old. They were also divided into other two groups: pure breed animals and mixed breed animals.

The animals originated from five counties: Cluj, Satu-Mare, Mureş, Sibiu and Alba. From Cluj and Satu-Mare a number of 62 samples were collected, from Mureş 41 samples, from Sibiu 21 and from Alba a number of 7 samples.

Most of the animals were vaccinated against anthrax and infectious bovine rhinotracheitis. A blood sample was collected without additives, from each cow. After collection, the samples were left in an incubator at 37°C for the serum to express. Subsequently, the serum was collected and frozen at -20°C in Eppendorf tubes for until the analysis.

Serological testing

The serum samples were tested for antibodies against *N. caninum* by ELISA using a commercial kit (HerdCheck Anti-Neospora, IDEXX Laboratories Inc. 09566-FC826) following the manufacturer's instructions. The serum samples were diluted 1:100 and 100 µl from each diluted sample were added to the *Neospora* antigen-coated plates and then incubated for 30 minutes at room temperature. The plates were washed four times with washing buffer (300 µl each well) and then dried. After that, the 100 µl of the anti-bovine HRPO conjugate was added to each well and incubated for 30 minutes at room temperature. The adding of 100 µl TMB followed and plates were incubated again for 15 minutes at room temperature. At the end, the color reaction that might appear was stopped with 100 µl stop solution. Plates were read at 450 nm and the test results were expressed as S/P ratio obtained by an equation provided by the manufacturer. Samples with an S/P ratio lower than 0.5 were considered negative and ones equal or higher than 0.5 were considered positive.

Statistical analysis

The data were statistically analyzed using Epi Info 3.5.1 software. We calculated the statistical significance of the prevalence of *N. caninum* infection in cattle between the raising systems and breed categories considered for this study. Values of p≤0.05 were considered statistically significant.

Results

The overall *N. caninum* seroprevalence was 55.95% (108/193). The values of the seroprevalence in different counties are mentioned in table 1.

### Table 1. Seroprevalence of *N. caninum* in cattle with reproductive failure from Center and Northwest of Romania

<table>
<thead>
<tr>
<th>County</th>
<th>Cluj</th>
<th>Satu-Mare</th>
<th>Mureş</th>
<th>Sibiu</th>
<th>Alba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>33</td>
<td>29</td>
<td>32</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>(%)</td>
<td>(53.2%)</td>
<td>(46.8%)</td>
<td>(78%)</td>
<td>(61.9%)</td>
<td>(14.3%)</td>
</tr>
<tr>
<td>Negative</td>
<td>29</td>
<td>33</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>(%)</td>
<td>(46.8%)</td>
<td>(53.2%)</td>
<td>(22%)</td>
<td>(38.1%)</td>
<td>(85.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>62</td>
<td>41</td>
<td>21</td>
<td>7</td>
</tr>
</tbody>
</table>

$p < 0.004$

Depending on the breed, the seroprevalence was higher in pure breed cattle (57.9%; 95% CI 49.8-65.6%) than in mixed breed cattle (47.1%; 95% CI 29.8-64.9%), but these values have no statistical significance, *p*>0.22.

Out of the 193 bovines from which blood was collected, 109 had at least one abortion in their history, 73 needed more than 3 (up to 11) artificial inseminations to get pregnant, 4 had vaginal inversion, 3 had retention of the placenta and the rest of them had some other minor reproductive problems.

Out of the 109 cows that had aborted in their history, 61.5% (95% CI 51.7-70.6%) were positive to the infection with *N. caninum*, 37 bovines (from the total of 73) presented anti-*N. caninum* specific antibodies, also two of the cows that presented vaginal inversion, 2 of the three that had retention of the placenta (66.7%) and none of the cows that presented afterbirth conditions, ovarian hypotrophy and mastitis. The differences between these seroprevalence data have no statistical significance (*p*>0.2).

From 109 cows that aborted, most of the abortions took place in the second trimester of gestation, 51.4% (56/109), followed by third
trimester abortions 27.5% (30/109). Also, most of the abortions were spontaneous 66/109 (60.6%) and most the fetuses were mummified (37.6%); 9.17% (10/109) of the abortive cows had at least two abortions in their reproductive history.

Most of the cattle that were artificially inseminated needed between 3 and 11 inseminations to get pregnant and their seropositivity to N. caninum infection was 49.3%.

Discussions

Bovine neosporosis is a common problem in many countries that have intensive dairy production (Wouda, 2000). Recent serological surveys indicate that N. caninum infection occurs in dairy cattle herds throughout Europe (Jensen et al., 1999; Magnino et al., 1999; Dijkstra et al., 2001; Pitel et al., 2001; De Meerschman et al., 2002; Milne et al., 2006; Panadero et al., 2010).

Even if N. caninum was reported as an abortion agent in many parts of the world (Dubey, 2003) little is known about the role of this parasite on abortions outbreaks in Romania.

The overall seroprevalence that we obtained in this study was 55.95%. A similar prevalence (56.2%) was obtained in by Gavrea et al. (2008) after testing 186 cows from a dairy farm in Mureş county.

Other surveillance tests were made by Ionescu et al. (2002) on goats and bovines from Harghita, Bihor, Arad and Brașov counties. The tested goats were shown as being negative, but the prevalence in cattle population was 20% (30 of the 150 bovines were diagnosed as positive to N. caninum).

In another study, the same authors (Ionescu et al., 2003) have performed an indirect ELISA test on sera samples from cattle from five counties of Romania. They obtained a prevalence of 33.71%.

Şuteu et al. (2005) tested bovines raised in backyard system from Cluj county. From these 877 animals, a number of 47 reacted as positive in IFAT tests, with a prevalence of 5.4%. The bovines originated from different raising systems: 187 were from intensive raising systems and only six were from backyard raising systems. All of them manifested reproductive problems from which the most important were abortion and the need of multiple inseminations until reaching pregnancy. Most of the animals were vaccinated against anthrax and infectious bovine rhinotracheitis.

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References


