Seroprevalence of neosporosis in cattle from a dairy farm in center of Romania

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ABSTRACT

Bovine neosporosis is a parasitic disease produced by *Neospora caninum*, that induces abortion in cows, and consequently has a negative impact on the herd’s reproductive efficiency. The main objective of this research was to determine the serological evidence of *N. caninum* in a cattle herd from a dairy farm in Mureș county using an ELISA test. This herd has a numerous history of abortions. During February 2008 one hundred and eighty six (186) serum samples from adult cows were collected to be tested for anti-Neospora antibodies. The testing was made using a commercial ELISA kit (HerdCheck Anti-Neospora) by IDEXX Laboratories Inc. 09566-FC826, following the manufacturer’s instructions. From the 186 samples tested, 103 (56.2%) came out positive to anti-Neospora antibodies.

Keywords: *Neospora caninum*, seroprevalence, cattle, neosporosis, abortion.

Introduction

*Neospora caninum* is an apicomplexan parasite closely related to *Toxoplasma gondii*. It was first described in dogs in 1984 by Bjerkas et al. and later in calves with myeloencephalitis. But it was first isolated and named in 1988 by Dubey et al. as *Neospora caninum* and is a serious cause of illness in dogs and it can cause abortion in cattle and occasionally in goats, sheep, deer and llamas. The parasite was isolated from cattle (Conrad et al., 1993; Barr et al., 1993), dogs (Dubey et al., 1988; Hay et al., 1990), sheep, water buffaloes and white-tailed deer. Antibodies to *N. caninum* were reported in raccoons, camels, pigs, camels, horses, cats, foxes and coyotes. The parasite isolated from horses was named *Neospora hughesi* (Marsh et al., 1995).

Abortion is the unique clinical sign observed in adult cows infected with *N. caninum* and it can take place from the third month of pregnancy to just before the term. However, abortion mostly occurs at 5-6 months of pregnancy (Moore et al., 1995). Bovines can get infected transplacentary (which is the major way of contamination) and by ingestion of food and water contaminated with oocysts from dog feces. The economical impact of neosporosis infection in dairy herds is mainly caused by the abortions that may occur in infected cattle and will also depend on the direct costs including labor, technical advice, diagnosis, rebreeding, possible loss of milk yield and replacement costs, if the abortive cow is culled (Thurmond and Hietala, 1997).
In Europe, bovine neosporosis has been detected in Belgium (De Meerschman et al., 2000), Czech Republic (Vlaclavek et al., 2003), Denmark (Jensen et al., 1999), France (Pitel et al., 2000 and 2001), Germany (Conraths et al., 1996), Hungary (Hornok et al., 1999 and 2006), Italy (Magnino et al., 1999 and 2000; Fioretti et al., 2000), Poland (Wierzchon et al., 2006), Portugal (Canada et al., 2002), Spain (Canada et al., 2004), Sweden (Stenlund et al., 1997) and United Kingdom (Davison et al., 1999; Trees et al., 2000). In Romania the first studies regarding bovine neosporosis were made by Şuteu O et al. in 2004 and 2005.

In this study we performed a serological survey to detect specific antibodies against *N. caninum* in cattle serum from a herd from Mureş county.

**Materials and method**

In February 2008 blood samples were collected from 183 cows from a herd in Mureş county. The cows were all pure breed, red Holstein. All the animals were vaccinated against anthrax and infectious bovine rhinotracheitis. From each cow a blood sample was collected on no additives. After collecting, the samples were left in incubator at 37°C for the serum to express. After expressing we collected the serum and freeze it at -20°C in Eppendorf tubes for it can be used later. The serum samples were tested for antibodies against *N. caninum* by using a commercial ELISA kit (HerdCheck Anti-Neospora) by IDEXX Laboratories Inc. 09566-FC826, following the manufacturer’s instructions. Briefly, 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. Then the plate was washed 4 times with diluted washing buffer (300 µl each well) and dried. After that, the conjugate provided in the kit was added (100 µl) to each well and incubated for 30 minutes at room temperature and then 100 µl TMB were added and incubated again 15 minutes at room temperature. At the end, the reaction was stopped with 100 µl Stop solution. Plates were read at 620 nm and the test results were expressed in optical density values which were analyzed using the equation provided by the manufacturer to determine which sample was negative and which one was positive to *N. caninum*-specific antibodies.

Also a smaller number of the ELISA tested cows (31) were vaccinated using Bovilis NeoGuard vaccine produced by Intervet. This vaccine is obtained from killed *N. caninum* tachyzoites and has gentamicin and thimerosal as preservatives. We have administrated 5 ml subcutaneously during the first trimester, followed by a second 5 ml dose, 3-4 weeks later. It is recommended to revaccinate with two doses for subsequent pregnancies.

**Results**

The blood samples were collected from a dairy farm which has in history a large number of abortive cows. Only in 2007 there were 36 abortions in 27 cows and 9 heifers. The abortions were easy, with no complications, the fetuses were eliminated with their placentas and were autolised. The spreading of the abortions during the year was very random (Table 1). During January 2008 there were 6 abortions.

![Fig.1. Bovine fetus with possible neosporian etiology](image_url)
Table 1. Incidence of bovine abortions during 2007

<table>
<thead>
<tr>
<th></th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>38</td>
<td>-</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>-</td>
<td>10,5</td>
<td>13,15</td>
<td>18,43</td>
<td>10,53</td>
<td>2,63</td>
<td>5,26</td>
<td>15,78</td>
<td>-</td>
<td>2,63</td>
<td>2,63</td>
<td>18,43</td>
</tr>
</tbody>
</table>

After abortion or birth, for a successful pregnancy a cow had to be rebred for 4 or 5 times.

The incidence of neosporosis was 56.2%, 103 samples out of 183 being positive.

Table 2. Seroprevalence of *N. caninum* according to the age of the cattle

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Seronegative</th>
<th>Seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>7,8</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>10,5</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>3,4</td>
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<tr>
<td>6</td>
<td>12</td>
<td>6,5</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>5,6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>1,6</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>1,6</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>0,5</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>0,5</td>
</tr>
<tr>
<td>No data</td>
<td>11</td>
<td>5,8</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>43,8</td>
</tr>
</tbody>
</table>

Out of these seropositive cows that have aborted 18 were serologically tested, 17 were positive at the ELISA test (94.4%) and only one (5.6%) was negative.

From 183 cows tested with ELISA test, 31 (16.94%) were vaccinated with Bovilis NeoGuard. 21 of these vaccinated cows (67.7%) were seropositive and later 4 (19.04%) of them aborted.

Conclusions

Neosporosis is a very important disease in cattle and dogs. In cattle it can cause abortion or transplacental infection of the new calves and in dogs it can cause neurological symptoms.

The history and tests made on 183 serum samples gathered during February 2008 led us to the next results: during 2007 a number of 36 cows aborted. From these 36 females, 9 were heifers and 27 were cows. In January 2008 a number of 8 abortions took place.

103 cows came out positive after ELISA testing indicating a prevalence of 56.2%. This percentage is very high fact that underlines the importance of this parasitic disease in Romania and all over the world.

Rezumat

Neosporoza bovină este o parazitoză produsă de protozoarul *Neospora caninum* care induce apariţia avorturilor la bovine, având un impact negativ asupra eficacităţii reproductive a efectivului. Obiectivul acestei lucrări a fost identificarea serologică a prezenţei *N. caninum* într-un efectiv de vaci de lapte din judeţul Mureş la care s-a raportat de-a lungul anilor un număr mare de avorturi. În luna februarie 2008 au fost prelevate, de la vacile adulte, 186 de probe de sânge. Aceste seruri au fost testate pentru identificarea anticorpilor produs şi distribuit de IDEXX Laboratories Inc.
Etapele prin care au fost procesate probele au fost cele indicate de producător. Dintre cele 186 de probe de ser testate, un număr de 103 (56,2%) au fost pozitive la identificarea anticorpilor anti-Neospora.

Cuvinte cheie: Neospora caninum, seroprevalența, bovine, neosporoză, avorturi.

References


