Study on outbreak of Neospora caninum-associated abortion in dairy cows in Calabria (Southern Italy)

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SUMMARY
Introduction - Neospora caninum is an apicomplexan protozoon causing abortion in cattle worldwide. Infected cows represent economic losses due to fetal abortion, stillbirth, clinical and subclinical disease, impaired milk production, neonatal deaths, reduced fertility and reduced value of infected cows. The abortion caused by neosporosis occurs at 5-6 months gestation. Serological techniques are primarily employed to detect specific antibodies against N. caninum to differentiate infected from non-infected animals and to assess the situation into a herd with regard to abortions due to infection with N. caninum. The herd prevalence rates of N. caninum infection reported in several European countries range from 16% in Sweden to 80% in Spain.

Aim - The present study was designed to assess the role of N. caninum for causing abortion in dairy farms in the central area of Calabria region (Southern Italy), by applying the ELISA serologic test.

Materials and methods - For the study of outbreak of neosporosis, blood samples were collected from 163 aborted dairy cows. Antibodies to N. caninum were assayed by using a home-made ELISA kit (Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy). Prevalence of N. caninum-positive herds were determined using WinEpiscope software version 2.0.

Results and discussion - IgG against N. caninum were found in nearly 34% (55/163) cases. The dams of the three calves with poor vital signs and neurological disorders were serologically seropositive in ELISA test. The frequency of infection by N. caninum recorded in the sample demonstrates a discrete circulation of the parasite in the territory concerned. Although the presence of antibodies to N. caninum in cattle only indicate expose to the parasite, probability of abortion in seropositive cattle is twice higher than in seronegative cattle. So, is plausible to correlate the abortion with the frequency of infection recorded. In all dairy herds examined, dogs can be found roaming around farms and have the close contact with cattle. These dogs might be the source of infection, contributing to the high prevalence of N. caninum antibodies in cattle by contaminating the farm with oocysts.

Conclusion - Results of the present survey agree with that reported from similar studies about the serological prevalence of neosporosis and it seems to support the outbreak of N. caninum-associated abortion in dairy cows in Calabria region (Southern Italy).

KEY WORDS
Abortion; Cattle; Neospora caninum; Serology.

INTRODUCTION
Parasitic infections are widespread in domestic animals. A variety of endo- or ecto-parasites can affect the animals and their adverse effects on health, production and welfare have been repeatedly documented¹-³. Furthermore, many parasitic diseases are zoonosis and they represent a severe public health concern⁴.

In this study we looked at a protozoan infection, as neosporosis, that represent a disease of considerable worldwide concern. Neospora caninum, an apicomplexan parasite, is the leading cause of epidemic abortion in cattle, with a very high annual economic impact. N. caninum related diseases have also been reported in other livestock species, including sheep, goats, horses and deer with canids such as dogs, wolves and coyotes being the definitive hosts⁵-⁶.

Infected cows represent economic losses due to fetal abortion, stillbirth, clinical and subclinical disease, impaired milk production, neonatal deaths, reduced fertility and reduced value of infected cows⁷.

In cattle the major natural route of infection seem to be the trans-placental transmissions from infected dams to their offspring. Neospora caninum can be transmitted postnatally (horizontally and laterally) by ingestion of tissues infected with tachyzoites or tissue cysts or by ingestion of food or drinking water contaminated by sporulated oocysts⁸. The trans-placental infection of the foetus can be endogenous or exogenous. The first kind of transmission occurs in a persistently infected dam. Neospora caninum infection in several European countries reporting
herd prevalence rates from 16% in Sweden to 80% in Spain. At present, there is no effective treatment or vaccine for N. caninum infection, and control measures are based on herd management and diagnosis. Since the presence of antibody activity specific to N. caninum is indicative of infection, detection of antibody of N. caninum in sera samples is the major methods for study of epidemiology of neosporosis. So, the serological diagnosis of neosporosis in adult cattle and precolostral calves is an integral part of control programs of this disease into a herd.

Serological techniques are primarily employed to detect specific antibodies against N. caninum to differentiate infected from non-infected animals and to assess the situation into a herd with regard to abortions due to infection with N. caninum. These techniques include a wide variety of enzyme-linked immunosorbent assays (ELISAs) (home-made and commercial tests), indirect fluorescent antibody tests (IFATs) and a N. caninum-agglutination test (NAT). In particular, the enzyme-linked immunosorbent assay (ELISA) has proven to be a useful technique for antibody detection against a variety of antigens, since it enables rapid determination and antibody titration, making it very suitable for serological surveys.

The aim of the present study was to determine the role of N. caninum infection plays in abortion or premature birth in dairy farms in the central area of Calabria region (Southern Italy), by applying the ELISA serologic test.

MATERIALS AND METHODS

The study was carried out in the area included between the Reggio Calabria (latitude: 38°6′41″40 N and longitude: 15°39′43″56 E) and Cosenza (latitude: 39°18′39″60 N and longitude: 16°15′3″60 E) provinces, located in the central area of Calabria region, Southern Italy. In this region there is essentially two types of climate: the continental climate in the mountains and the Mediterranean climate of the coast. Winter is cold in the mountains, with occasional snow; the summer is hot and muggy. In coastal and hilly areas the winter is never very cold and summer is very hot, sometimes torrid.

The area has an estimated almost 6700 dairy farms with a total of 100,000 cattle bred for milk production, according to the data of the National livestock register established by Italian Ministry of Health at Istituto Zooprofilattico “G. Caporale” - Teramo. The cattle breedings may have a very small size and are generally family-run business. In this study the examined farms had a variable consistency of lactating cows, belonging to the following breeds: Italian Friesian, Italian Brown, Red Pied Fleckvieh and crossbreds. In all dairy farms selected, all animals were bred by artificial insemination; all animals were free from tuberculosis ad brucellosis, as shown by yearly tests. All farms had dogs with unrestricted access to areas belonging to the cows.

This study was performed over a 2 years period in 10 dairy herds (identified with letter from A to L). The average age of animals bred was 4.8 years ± 2 (SD). The cows were selected on the basis of a history of abortion between the second and the seventh month of pregnancy or premature birth, with a total of pathological events greater than 18% into a herd. Three cows gave birth to alive but weak calves and with neurological signs, although on these subjects was not able to run any diagnostic test, because of their missing. No investigation or prevention measures related to N. caninum had been carried out on the herds.

Blood samples were collected from a total of 163 cows, using a system Venoject® Multi-Sample Needles 20G and a vacuum blood collection tubes. The samples were transported to the laboratory of the “Istituto Zooprofilattico Sperimentale del Mezzogiorno”.

Serum obtained from each sample were analyzed for antibody activity to Neospora caninum using an home-made ELISA test (NEOEL), developed by the IZS del Mezzogiorno. The test was performed according to manufacturer’s instruction and using his optimal cutoff values.

Prevalence of N. caninum-positive herds were determined using WinEpiscope software version 2.0. The upper and lower confidence limits (95%) for prevalence data were calculated assuming a binomial distribution. The maximum number of positive and the maximum possible prevalence that can be exist in a population where all collected samples are negative were determined using WinEpiscope 2.0.

RESULTS

Table 1 shows seroprevalence values for N. caninum-positive and -negative samples. Nearly 34% (55/163) were positive by ELISA test. The positivity found on the total number of animals into every herds are displayed in Figure 1. The dams of the three calves with poor vital signs and neurological disorders were serologically seropositive in ELISA test.

Table 2 shows the percentage disease measures with maximum number possible of expected positive obtained on all negative samples with WinEpiscope 2.0 in examined dairy herds.

DISCUSSION

The seroprevalence of N. caninum in cattle or dairy cows had been studied in many countries. Prevalences were varied among countries, regions, herds and even at different times of the same herds, but there were a few reports on bovine neosporosis in Southern Italy. Serodiagnosis may be useful not only for diagnosing infection, but also for providing further information on the life cycle or epidemiology of neosporosis.

The results of this study indicated that the used ELISA test was sensitive for detection of N. caninum in cattle and can be a useful diagnostic method for the serodiagnosis of this infection. This is in accordance with the literature.

The present study revealed that the seroprevalence of N. caninum infection in dairy cows in an area of southern Italian territory was nearly 34% by ELISA.

The frequency of infection by N. caninum recorded in the sample demonstrates a discrete circulation of the parasite in the territory concerned. Although the presence of antibodies to N. caninum in cattle only indicate expose to the parasite, probability of abortion in seropositive cattle is twice higher than in seronegative cattle. So, is plausible to correlate the abortion with the frequency of infection recorded.

With regard to the three cases of calves born from positive mothers and with poor vital signs, this finding reinforces the thesis of a trans-placental infection of fetuses as cause of the
In all dairy herds examined, dogs can be found roaming around farms and have the close contact with cattle. These dogs might be the source of infection, contributing to the high prevalence of *N. caninum* antibodies in cattle by contaminating the farm with oocysts. In this present study, each farms had at least one dog in nearby environment. Hence, the presence of dogs on a farm has been a potential risk to provide the increasing chance of horizontal transmission. Furthermore, the animal handling among the farms after the sale of adult or young subjects was frequently performed with a walking transport, allowing the parasite diffusion.

In conclusion, this study demonstrated a seroprevalence for *N. caninum* infection of about 34% in naturally infected dairy cows in southern Italy. Our results agree with that reported from similar studies about the serological prevalence of neosporosis and it seems to support the outbreak of *N. caninum*-associated abortion in dairy cows in Calabria region (Southern Italy). Further serological surveys in dogs, lived in the surrounding area of infected farm need to be accomplished and also seroprevalence study in other Italian regions, as well as, the association between seropositive status and bovine abortions in this country.

**References**


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**Table 1** - Number of cows with abortion and prevalence (Frequency %) with confidence intervals (CIA95.00%) around the percent of serological findings in different dairy herds (A-L).

<table>
<thead>
<tr>
<th>Herds</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nr. of Cows with abortion</td>
<td>19</td>
<td>4</td>
<td>52</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>32</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Nr. of cows positives Frequency (%)</td>
<td>10</td>
<td>0</td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>C.J. +95.00%</td>
<td>52.63</td>
<td>0.00</td>
<td>28.85</td>
<td>20.00</td>
<td>33.33</td>
<td>70.00</td>
<td>83.33</td>
<td>21.88</td>
<td>38.89</td>
<td>10.00</td>
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<tr>
<td>C.J. -95.00%</td>
<td>71.14</td>
<td>60.24</td>
<td>43.08</td>
<td>55.61</td>
<td>77.72</td>
<td>93.33</td>
<td>99.58</td>
<td>39.97</td>
<td>64.25</td>
<td>44.50</td>
</tr>
<tr>
<td>Nr. of cows negatives Frequency (%)</td>
<td>24.45</td>
<td>0.00</td>
<td>17.13</td>
<td>2.52</td>
<td>4.33</td>
<td>34.75</td>
<td>35.88</td>
<td>9.28</td>
<td>17.30</td>
<td>0.25</td>
</tr>
<tr>
<td>C.J. +95.00%</td>
<td>47.37</td>
<td>100.00</td>
<td>71.15</td>
<td>80.00</td>
<td>66.67</td>
<td>30.00</td>
<td>16.67</td>
<td>78.13</td>
<td>61.11</td>
<td>90.00</td>
</tr>
<tr>
<td>C.J. -95.00%</td>
<td>75.55</td>
<td>100.00</td>
<td>82.87</td>
<td>97.48</td>
<td>95.67</td>
<td>65.25</td>
<td>64.12</td>
<td>90.72</td>
<td>82.70</td>
<td>99.75</td>
</tr>
<tr>
<td>Nr. of cows negatives Frequency (%)</td>
<td>28.86</td>
<td>39.76</td>
<td>56.92</td>
<td>44.39</td>
<td>22.28</td>
<td>6.67</td>
<td>0.42</td>
<td>60.03</td>
<td>35.75</td>
<td>55.50</td>
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</tbody>
</table>

**Table 2** - Percentage disease measures with maximum number possible of expected positive obtained on all negative samples with WinEpiscope 2.0 in different dairy herds (A-L).

<table>
<thead>
<tr>
<th>Disease Measures</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling fraction (%)</td>
<td>47.37</td>
<td>100.00</td>
<td>71.13</td>
<td>80.00</td>
<td>66.67</td>
<td>30.00</td>
<td>16.67</td>
<td>78.13</td>
<td>61.11</td>
<td>90.00</td>
</tr>
<tr>
<td>Max possible prevalence (%)</td>
<td>26.30</td>
<td>0</td>
<td>5.80</td>
<td>20.00</td>
<td>33.33</td>
<td>60.00</td>
<td>83.33</td>
<td>94.00</td>
<td>22.20</td>
<td>10.00</td>
</tr>
<tr>
<td>Max nr. possible of positive</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 1** - Serological positivity at Neospora caninum on the total number of animals examined into every herds.