Molecular detection of *Neospora caninum* abortion in dairy cattle from different historical regions of Romania

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**Abstract.** Neosporosis is a major cause of abortion in many countries, transplacental transmission being considered the major route of transmission of *N. caninum* in cattle. Little information are available about the prevalence of *N. caninum* abortion in dairy cattle in Romania, the only existing data are coming from Transylvania region. In the present study, we analyzed by molecular technique (PCR) the presence of *N. caninum* DNA in fetal tissues of spontaneous dairy cattle abortion from different regions of Romania. Twenty-one abortions collected from four historical regions from Romania were tested. The Nc-5 gene of *N. caninum* was amplified from samples of eight aborted fetuses.

**Keywords:** *Neospora caninum,* Neosporosis; Abortion; Dairy cattle; Romania.

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**Introduction**

Bovine neosporosis, caused by the apicomplexan protozoan parasite *Neospora caninum*, was initially recognized in 1989 (Thilsted and Dubey, 1989) and is reported as a leading infectious cause of reproductive failure in dairy cattle in countries worldwide (Dubey at al., 2007).

*Neospora caninum* is a protozoan parasite whose lifecycle includes both the dog (definitive host) and the cow (intermediate host). *N. caninum* can be transmitted postnatally (horizontally) by ingestion of tissues infected with tachyzoites or tissue cysts or by ingestion of food or drinking water contaminated sporulated oocysts (Dubey et al., 2007).

The PCR plays an important role in the diagnosis of *N. caninum*-infection. Most PCR protocols are used to detect *N. caninum* DNA in the tissues of aborted fetuses (Dubey and Schares, 2006). The first investigation in Romania, for detecting *N. caninum* DNA by PCR, was done in only one dairy farm from of the country and the test revealed Nc-5 fragments of the expected size (about 327 bp) from the brain tissue samples of three from nine aborted fetuses (Şuteu et al., 2010).

Serological investigation in dairy cattle from Romania, for detecting antibodies against *N. caninum* showed a prevalence ranging between
27.7% and 41.7% (Gavrea et al., 2011; Mitrea et al., 2012; Imre et al., 2011).

The aim of the study was to bring more information on *N. caninum* DNA presence in abortions from farms located in different regions of Romania.

**Materials and methods**

**Study area**

Romania is a country located at the crossroads of Central and South-eastern Europe, north of the Balkan Peninsula, on the Lower Danube, within and outside the Carpathian arch, bordering on the Black Sea. Romania is divided into 9 historical regions with 41 counties. The dairy cattle abortion analyzed in this study are originated from four historical regions (Bucovina, Moldova, Muntenia and Transylvania) (figure 1).

![Figure 1. Historical regions of Romania – sampling plan](image)

**Biological samples**

In 2011 and 2012, twenty-one dairy cattle abortions (between 3 and 7 months of age) were obtained from six dairy farms located in four historical region of Romania (table 1). The abortions were necropsied and samples of brain and heart were collected. Brain and heart samples were stored at -80°C for PCR analysis.

**Polymerase chain reaction (PCR)**

In abortions, genomic DNA extraction was performed on brain and heart and samples.

DNA was extracted from 40 mg tissue using a commercial kit (Isolate Genomic DNA Kit, Bioline, UK), according to the manufacturer’s protocol. PCR was performed on all abortions samples to detect *N. caninum* DNA.

<table>
<thead>
<tr>
<th>Historical area</th>
<th>No. of abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucovina</td>
<td>6</td>
</tr>
<tr>
<td>Moldova</td>
<td>2</td>
</tr>
<tr>
<td>Muntenia</td>
<td>4</td>
</tr>
<tr>
<td>Transylvania</td>
<td>9</td>
</tr>
</tbody>
</table>

**Table 1.** The number and origin of tested abortuses

PCR protocol for detecting *N. caninum* was conducted using primers from the Nc-5 region of the genomic DNA. Pair of Np6/Np21 primers (5’ GGGTGTGCGTCCAATCCTGTAAC 3’ - 5’ CTGCCAGTGACCTAGGTTCCT 3’) (Generi-Biotech, Czech Republic) was used to amplify the 327 bp DNA fragment (Yamage et al., 1996). PCR was carried out in a 25 μl reaction mixture consisting of 12.5 μl of MyTaq Red HS Mix (Bioline, UK) and 25 pM of each primer. The volumes of DNA template were 4 μl. The amplification was performed in BIO RAD C1000™ Thermal Cycler. Cycling conditions were: 1 min at 95°C; 15 s at 95°C, 1 min at 63°C, and 10 s at 72°C (40 cycles); and 2 min at 72°C.

Aliquots of each PCR product were electrophoresed on 1.5% agarose gel stained with SYBR® Safe DNA gel stain (Invitrogen) and observed for the presence of the specific fragment under UV light (BIO DOC-It™ Imagine System). DNA fragment size was compared with a standard molecular weight (100 bp DNA ladder – Fermentas). Two controls were performed: a positive control of *N. caninum* – Nc-1 strain (Şuteu et al., 2004) and negative control – distilled water.

**Results**

Nc-5 fragments of the expected size (about 327 bp) were amplified from the brain or heart tissues of eight fetuses (38.09%).

*Neospora caninum* DNA was detected by PCR in abortuses provided from Moldova, Muntenia and Transylvania (figure 2). None from the six abortuses tested from Bucovina were positive (table 2).
Figure 2. PCR products amplified with primer pair Np6/Np21, migration into agarose gel 1.5%, from bovine fetuses. Lane 1 – 1kb DNA ladder; lane 2 – positive control; lanes 3, 5 (samples from bovine fetuses – Moldova region) – PCR positive; lanes 4, 6 – PCR negative (samples from bovine fetuses – Moldova region)

Table 2. PCR results in tested abortuses

<table>
<thead>
<tr>
<th>Historical area</th>
<th>No. of abortions</th>
<th>Frequency</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucovina</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moldova</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Muntenia</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Transylvania</td>
<td>9</td>
<td>4</td>
<td>44.4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>21</td>
<td>8</td>
<td>38.1</td>
</tr>
</tbody>
</table>

**Discussion**

*Neospora caninum* is considered a major cause of bovine abortion throughout the world. The first report of *N. caninum* identification by PCR in brain tissues of aborted bovine fetuses in Romania was made by Şuteu et al. (2010) and *N. caninum* DNA was found in one dairy farm from central part of Romania, in three from the nine bovine abortions tested, with prevalence about 33%. In the present study *N. caninum* DNA was found in eight abortuses from 21 tested (38.09%). Results showed the presence of *Neospora caninum* in three different historical regions of Romania, proving that the parasite might be involved in the reproductive failure in farms from a large part of the country. PCR was confirmed to be a valuable diagnostic tool for the primary diagnosis of *N. caninum* in aborted fetuses.

Abortion due to *N. caninum* can occur at any time of gestation from 3 months to term, but the majority of abortions occur at 4–6 months of gestation (Anderson et al., 1991; 1995; Wouda et al., 1999). Abortion before 3 months of gestation has not been observed, so the role of *N. caninum* at this age of pregnancy is unknown. About 15% of *N. caninum* positive cattle can abort (Buxton et al., 1997) and it is thought that cows in their first and second lactation are more likely to abort. However, few cattle (less than 5%) can abort for a second time (Anderson et al., 1995).

In Europe, *N. caninum* was detected by PCR in the brains of 21% of 242 aborted fetuses in Switzerland (Sager et al., 2001), while in Spain, the prevalence of *N. caninum* DNA was 15.3% (Pereira-Bueno et al., 2003). The highest prevalence of *N. caninum* in bovine fetuses, by PCR, was obtained in Mexico (80%; 35/44) (Medina et al., 2006). A similar prevalence with our study was obtained in China, where Yang et al. (2012) obtained *N. caninum* DNA in 31.3% of the bovine fetuses.

*N. caninum* is a cause of bovine abortion in Romania and underlines the need to use molecular biology techniques to increase the chance to detect the infection in aborted fetuses.

**Acknowledgments**

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The authors declare that they have no competing interests.

**References**


