Molecular detection of *Neospora caninum* in slaughtered goat kids from Romania

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**Abstract.** Although studies have been reported on *Neospora caninum* from goats, no study regarding *N. caninum* infection in goat kids was performed in Romania. The objective of this study was to determine the prevalence of *N. caninum* in goat kid tissues (diaphragm) by PCR. Diaphragm tissues were collected from 181 slaughtered goat kids, from 7 counties from center and north-west of Romania. All tissues were tested by PCR in order to identify *N. caninum* DNA. In two (1.1%) of the samples, collected from Alba and Hunedoara county, we found *N. caninum* DNA. The results obtained in this study have shown the presence of *N. caninum* in small ruminants. To our knowledge this is the first report about molecular prevalence of *N. caninum* in goat kids in Romania.

**Keywords:** *N. caninum*; PCR; Goat-kids; Romania.

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**Introduction**

*Neospora caninum* is a protozoan parasite of a wide range of mammals (Dubey et al., 2007). The parasite was first discovered in Norway in dogs but was not isolated and named until 1988 (Bjerkas et al., 1984; Dubey et al., 1988). Neosporosis is a major cause of reproductive failure in dairy and beef cattle in many countries, causing considerable economic losses (Trees et al., 1999), and neurological alterations/disorders in dogs (Barber and Trees, 1996). Goats are intermediate hosts for *N. caninum*. This parasite can cause abortion, stillbirth and low yielding in goat herds (Barr et al., 1992; Dubey et al., 1992; Dubey et al., 1996).

The first investigation for detecting *N. caninum* DNA by PCR in cattle from Romania, 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 studies in goats from Romania revealed *N. caninum* prevalence in 2.3% (Iovu et al., 2012). However there is no information on *N. caninum* DNA prevalence in goats from Romania.
Materials and methods

Animals and study area

In Romania goat herds are grazed during spring to early summer and fed on supplements with barley and corn for the rest of the year. The main source of drinking water for goats was spring water. Goat kids were traditionally slaughtered, in Romania, in the spring time. In the spring of 2012, one-hundred-eighty-one goat-kids (between two and three months of age) from thirteen flocks originated from seven counties from center, north-west and west of Romania (Alba, Brasov, Cluj, Hunedoara, Mures, Satu-Mare, Valcea) were tested (table 1, figure 1). Diaphragm tissues were collected from all 181 slaughtered goat kids. All diaphragm tissues were stored at -20°C and tested by PCR in order to identify *N. caninum* DNA.

DNA isolation and amplification

Genomic DNA extraction was performed on all diaphragm 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 diaphragm samples to detect *N. caninum* DNA.

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’ GGGTGTTGCGTCCAATCCTGTAAC 3’ - 5’ CTCGCCAGTCAACCTACGTCTTCT 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 C1000™ Thermal Cycler (Bio-Rad, USA). 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 (BioDoc-ITM Imagine System, Bio-Rad, USA). 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 diaphragm tissue in two from 181 goat-kids tested (1.1%).

*Neospora caninum* DNA was detected by PCR in goat-kids provided from Băiţa (Hunedoara county) and Ciugudu de jos (Alba county). None of the goat kids tested from Brasov, Cluj, Mures, Satu Mare and Valcea counties were positive (table 1).

<table>
<thead>
<tr>
<th>County</th>
<th>Herds</th>
<th>Number of samples</th>
<th>Frequency of <em>N. caninum</em> DNA</th>
<th>Prevalence of <em>N. caninum</em> DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alba</td>
<td>Poiana Aiudului</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ciugudu de Jos</td>
<td>14</td>
<td>1</td>
<td>7.14 %</td>
</tr>
<tr>
<td>Braşov</td>
<td>Făgăraş</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mureş</td>
<td>Sighișoara</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Râşnov</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Târgu-Mureş</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hunedoara</td>
<td>Băiţa</td>
<td>22</td>
<td>1</td>
<td>4.55%</td>
</tr>
<tr>
<td></td>
<td>Spini</td>
<td>18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hunedoara</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Satu Mare</td>
<td>Satu Mare</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vâlcea</td>
<td>Vâlcea</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cluj</td>
<td>Someşul Rece</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sâlciu</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>181</td>
<td>2</td>
<td>1.1%</td>
</tr>
</tbody>
</table>
Discussion

Neosporosis is a relatively new disease and little information is available about the infection with *N. caninum* in goats. Dogs, coyotes, dingoes and wolves are the only known definitive hosts for *N. caninum* (Dubey, 2003; Gondim et al., 2004; King et al., 2010; Dubey et al., 2011). Goats are one of the intermediate hosts for *N. caninum*. To our knowledge this is the first report about molecular prevalence of *N. caninum* in goat kids in Romania.

In our study we miss clear data about reproductive pathology in the evaluated herds, only pour information about abortions in the past, but no clear diagnosis was done. Considering the results of serological studies (2.3%) obtained in goats from Romania by Iovu et al., (2012), the percentage (1.1%) of *N. caninum* DNA in goat-kids obtained in our study is expected. In two (1.1%) of the samples, collected from Alba and Hunedoara county, we found *N. caninum* DNA. Also, Iovu et al. (2012) detected seropositive samples only in adult goats (2.6%; 12/469), all the goats being raised in herds and originated from three counties, including Hunedoara county. However, experimental horizontal and vertical transmissions of *N. caninum* are reported in goats (Dubey, 2003). The presence of *N. caninum* DNA detected by molecular techniques was reported in aborted goat fetus from Italy (Eleni et al., 2004). In Spain three from twenty-six (11.5%) aborted goat fetuses, tested by PCR, were positive for *N. caninum* DNA (Moreno et al., 2012).

Taking into account the age of goat kids from our study, both transplacental and horizontal transmission can be possible.

In conclusion, our study demonstrated molecular presence of *N. caninum* in goats and indicates that *N. caninum* should be a possible causative agent of abortion in domestic goats from Romania. Consequently, neosporosis should be included in a differential diagnosis of causes of abortion in this species. Veterinary supervision, dog control and attention goats health, are expected to reduce neosporosis prevalence.
Acknowledgments

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References
