RESEARCH NOTE

Seroprevalence of *Neospora caninum* in cats from the Czech Republic

Kamil Sedlák¹, Eva Bartova²* and Tereza Machacova²

¹State Veterinary Institute Prague, Department of Virology and Serology, Sídlistní 136/24, 165 03 Prague 6, Czech Republic
²University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary, Department of Biology and Wildlife Diseases, Hygiene and Ecology, Palackého tř. 1/3, 612 42 Brno, Czech Republic

**Abstract**

Sera of 414 cats coming from different parts of the Czech Republic were tested for *N. caninum* antibodies. Sera samples were collected during years 2002–2011. *N. caninum* antibodies were detected by a commercial competitive-inhibition enzyme-linked immunosorbent assay (cELISA) with cut off ≥30% inhibition. Samples positive in cELISA were confirmed by an indirect fluorescence antibody test (IFAT); titre ≥50 was considered positive. In total, 137 (33%) cats reacted positively in cELISA; *N. caninum* antibodies in IFAT were detected in 16 (3.86%) cats with titres 50 and 100. In 6 cats, positive for *N. caninum* antibodies, *T. gondii* antibodies were also detected by IFAT. It is the first report of *N. caninum* antibodies in domestic cats from the Czech Republic and third report in Europe.

**Keywords**

Neosporosis, antibodies, ELISA, IFAT, feline

*Neospora caninum* have been confused with *Toxoplasma gondii* for long time. Both parasites are very similar cyst-forming coccidian of the phylum Apicomplexa with indirect life cycle, with carnivores as definitive hosts (Dubey et al. 2007). A wide range of domestic and wild animals have been experimentally exposed to *N. caninum* infection. However, viable *Neospora* sp. has been isolated only from a few hosts as summarized by Dubey and Schar (2011). The cats could be sensitive to neospora infection, but clinical symptoms such as myositis and encephalitis are not so specific. According to the experimental studies, the infection with *N. caninum* is more serious in case of cats infected in neonatal and prenatal period or in case of nursing cats (Dubey 1992). Only some studies focused on detection of *N. caninum* antibodies in cats. The aim of this study was to test sera of cats from the Czech Republic for *N. caninum* antibodies by two different serological tests.

In this study, domestic cats without evident clinical symptoms of any diseases coming from different parts of the Czech Republic (especially from Prague, Central Bohemia and North Moravia) were tested for *N. caninum* antibodies. Blood was taken by puncture of the saphenous vein from 414 cats during years 2002–2011. Serum obtained by centrifugation was stored at −20°C until examination at State Veterinary Institute in Prague.

Sera were tested for the presence and level of IgG antibodies against *N. caninum* by a commercial competitive-inhibition enzyme-linked immunosorbent assay (cELISA, VMRD, Pullman, USA) with cut off ≥30% inhibition. Serum samples were also tested by indirect fluorescent antibody test (IFAT) that is used as a reference method. Commercially available antigen (VMRD, Pullman, USA) and species-specific conjugates anti-cat IgG immunoglobulin (Sigma Aldrich, Czech Republic) were used. The sera were diluted in a two-fold series starting at 1:50 as a basic dilution; the titre ≥50 was considered positive. All serum samples were also examined for *T. gondii* antibodies by IFAT using a commercially available antigen (VMRD, Pullman, USA) and species-specific conjugates anti-cat IgG immunoglobulin (Sigma Aldrich). The sera were diluted in a two-fold series starting at 1:50 as a basic dilution; the titre ≥50 was considered positive.

*Neospora caninum* antibodies were detected in 137 (33%) and 16 (3.86%) of 414 cats by cELISA and IFAT, respectively. In IFAT, we found 11 and 5 positive cats with titre 50 and 100,

*Corresponding author: bartovae@vfu.cz*
Table I. *Neospora caninum* antibodies in domestic cats in cELISA and IFAT

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive/n (%)</th>
<th>Inhibition (%)</th>
<th>30–40</th>
<th>40.1–50</th>
<th>50.1–60</th>
<th>60.1–70</th>
<th>70.1–80</th>
<th>80.1–90</th>
</tr>
</thead>
<tbody>
<tr>
<td>cELISA</td>
<td>137/414 (33%)</td>
<td>Positive</td>
<td>81</td>
<td>44</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>IFAT</td>
<td>16/414 (3.86%)</td>
<td>Titre 50*</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

cELISA – competitive-inhibition enzyme-linked immunosorbent assay (cut-off 30% inhibition), IFAT – indirect fluorescent antibody test (cut-off titre 50). *Samples positive in IFAT (titre 50 or 100) and simultaneously positive in cELISA (% inhibition 30–80)

respectively and with 30–90% inhibition in cELISA. The results are summarized in Table I. This is the first serologic survey for *N. caninum* antibodies performed in cats in the Czech Republic and third report in Europe. *N. caninum* antibodies were found by IFAT with cut-off titre 40 in 0.6% of 330 cats from Hungary (Hornok et al. 2008) and in 24.8% of 282 stray cats from Italy by latex-agglutination test (Ferroglio et al. 2005). The same methods cELISA and/or IFAT were used also in Brazil; *N. caninum* antibodies were found in 25% of 400 (Brescia et al. 2007) and in none of 70 (Coelho et al. 2011) cats, respectively. In Thailand, 36 tested cats were negative for *N. caninum* antibodies in both IFAT and cELISA (Arunvitas et al. 2011).

The discrepancy in *N. caninum* prevalence found in our study in cELISA and IFAT could be explained by different sensitivity and specificity of these tests. The method cELISA was primarily developed and validated for cattle sera (Baszler et al. 2001). Till yet, it has not been validated for testing of cat sera that it is why this method could be used as a screening method since it has high sensitivity. However it is recommended to confirm the results with reference method IFAT that is more specific.

There are also some studies focusing on prevalence of *N. caninum* antibodies in feral and zoo felids. Antibodies to *N. caninum* were found in 19% of 100 feral cats (*Felis catus*) by cELISA in Iran (Hamidine et al. 2011) and in 19% of 26 Eurasian lynx (*Lynx lynx*), 6.8%–16.7% European wild cat (*Felis silvestris*) and 12% of 25 Iberian lynx (*Lynx pardinus*) from Spain by cELISA and confirmed by IFAT (Sobrino et al. 2008; Millan et al. 2009a, b). Andre et al. (2010) used IFAT to test captive wild felids in zoos in Brazil and found 11%–71% prevalence in different felids (lion, jaguarondi, puma, jaguar, tiger and ocelot).

Domestic cat and other felids are definitive hosts of *T. gondii* that is why there is usually higher prevalence of *T. gondii* compared to *N. caninum* in cats. *T. gondii* and *N. caninum* antibodies were detected in 43.6% and 6.9% zoo felids from U.S.A (Spencer et al. 2003) and in 92% and 12% zoo felids from the Czech Republic (Sedlak and Bartova 2006), respectively. In both studies, the same method IFAT was used but with different cut-off titre (50 and 40, respectively). In our study, we found only 6 cats (1.4%) positive for *T. gondii* antibodies with titres 400, 1600 and 3200 in 3, 2 and 1 cats, respectively. These cats were simultaneously infected with *N. caninum* with titre 50.

Acknowledgements. This study was funded by the grant no. MSM6215712402 from the Ministry of Education, Youth and Sports of the Czech Republic. We would like to thank Barbara Zahradnikova for help with sample examinations.

References


Neospora caninum in cats


Received: December 12, 2013
Revised: March 5, 2014
Accepted for publication: March 17, 2014