SEROPREVALENCE OF Neospora caninum AND Toxoplasma gondii INFECTIONS IN A DAIRY CATTLE FARM FROM SOUTH-EAST OF ROMANIA

Vasilica GOTU1, Daniela POREA2, Mădălina SITEAVU1, Mariana IONIȚĂ1, Ioan Liviu MITREA1

1University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine of Bucharest, 105 Spl. Independentei, District 5, 050097, Bucharest, Romania
2Danube Delta National Institute for Research and Development, 165 Babadag Street, 820112, Tulcea, Romania

Corresponding author email: gotuvasilica@yahoo.com

Abstract

Toxoplasma gondii and Neospora caninum are two closely related protozoan parasites that can cause reproductive disorders in ruminants. The aim of this study was to determine the seroprevalence of N. caninum and T. gondii infections in a dairy cattle farm in South-Eastern Romania. For this, a total of 157 blood samples from 84 dairy cows and 73 heifers with reproductive disorders were analyzed for N. caninum specific antibodies. In addition, of these, 127 samples (54 dairy cows and 73 heifers), were also analyzed for T. gondii specific antibodies by using commercial ELISA kits. Overall, 5.1% (95% CI: 1.66-8.54) and 11.8% (95% CI: 6.20-17.42) of tested animals were seropositive for N. caninum and T. gondii, respectively, and 1.6% (95% CI: 0.59-3.74) were positive for both parasites. The apparent seroprevalence of N. caninum and T. gondii, respectively, according to the analyzed category, varied between 7.1% and 9.3% (dairy cows) and from 2.7% and 13.7% (heifers). These results emphasize the need of periodical screenings for N. caninum and T. gondii infections, associated with applying appropriate control measures, in the frame of good management practices.

Key words: Toxoplasma gondii, Neospora caninum, dairy cattle farm, reproductive disorders, seroprevalence.

INTRODUCTION

Reproductive disorders in ruminants are a major cause of economic losses and also a major limitation for the achievement of optimum efficiency in the livestock production system largely. Amongst the causes of reproductive disorders, protozoan parasites are a significant cause of abortion and infertility in domestic ruminants. Toxoplasma gondii and Neospora caninum are closely related obligate intracellular protozoan (Apicomplexa: Sarcocystidae) that can cause reproductive disorders in ruminants (Kaltungo and Musa, 2013; Sánchez-Sánchez et al., 2018). Although these parasites show morphological similarities, they are antigenically different in the tissue cyst phase N. caninum having a thicker cyst-wall (Temesgen and Tariku Geinoro, 2019).

T. gondii is a zoonotic parasitic protozoan with worldwide distribution. T. gondii is capable of infecting all warm-blooded animals. In cattle, the majority of T. gondii infections are subclinical (Olsen et al., 2019; Lindsay and Dubey, 2020). Neosporosis is recognized as one of the most important disease of cattle worldwide (Dubey and Scharas, 2011). Cows with N. caninum antibodies (seropositive) are more likely to abort than seronegative cows (Lindsay and Dubey, 2020). Epidemiological studies conducted on toxoplasmosis and neosporosis in cattle have been published in many parts of the world. However, only a few studies targeting the detection of both pathogens, T. gondii and N. caninum were conducted, in countries such as China (Su et al., 2012; Ma et al., 2020), Mongolia (Pagmadulam et al., 2018), Turkey (Zhou et al., 2017), Switzerland (Gottstein et al., 1998) and Czech Republic (Bártová et al., 2015). Commonly, the seroprevalence varies among countries, among regions and between dairy and beef cattle, depending on the testing method and its cut-off, but also on the number
of the examined cattle, their age, gender, breed, and health status (Bártová et al., 2015). Epidemiological data about the seroprevalence of *N. caninum* and *T. gondii* in dairy cattle is limited in Romania and no studies have been performed for simultaneously testing. *N. caninum* infection in dairy cattle has been serologically diagnosed in cattle from different areas of the country, with mean prevalences up to 56% (Gavrea et al., 2011; Imre K. et al., 2011; Mitrea et al., 2012; Enachescu et al., 2014). However, a coproscopical and serological study in dogs (Southern Romania) reported on *N. caninum*-like oocysts in 4.9% of the dogs, with higher prevalence in rural guard dogs (9.8%) and cattle farm dogs (5.1%); also, the study reported a seroprevalence of 20.2% of *N. caninum* infection (38.1% in dogs from cattle farms) (Mitrea et al., 2013). These data suggest a potential high infection pressure for dairy cattle in the investigated area. There are little available data regarding *T. gondii* infection in cattle in Romania. Some old studies report about finding dye test antibodies in 18.9% (31/164) of cattle (Elias (1966) and a seropositivity of 12.6% (84/667) of cattle, by the complement fixation test (Medrea and Constantinescu, 1991; Dubey et al., 2014).

Therefore, our goal was to investigate the seroprevalence of both, *N. caninum* and *T. gondii* infection in a dairy cattle farm with reproductive disorders.

**MATERIALS AND METHODS**

The study was conducted in a dairy cattle farm with reproductive disorders, from South-East of Romania. Reproductive disorders have been defined by infertility and in the majority of cases were associated with retroperitoneal fat necrosis (nutritional fat necrosis, also called steatitis or yellow fat disease).

Between 2017-2020, 157 cattle blood samples were collected and subjected for serological testing. Animals were divided into two age category: heifers (aging between 1 and 2 years) (n = 73) and dairy cows (older than 2 years) (n = 84).

All 157 animals were tested for *N. caninum* infection by using an indirect ELISA immunoenzymatic assay (Ingezim, 12.NC.K., Ingenasa S.A, Spain) for the detection of antibodies against *N. caninum*, following the manufacturer's instruction. The test results were expressed as RIPC (Relative Index Per Cent) based on positive-control and negative-control sera. The samples with RIPC ≥ 20 were considered positive, those with RIPC between 17 and 20 - were categorized as doubtful, and samples with RIPC ≤ 17 were considered negative.

Additional, a subset of the samples (n = 127; 54 from dairy cows and 73 from heifers), were also tested for *T. gondii* specific antibodies by using a commercial test (Toxoplasmosis Indirect, IDVET, kit multi-species ID Screen® Montpellier), following the manufacturer's instruction. Test results were expressed as sample/positive control (S/P) ratios. Samples with the S/P (%) ≥ 50% were rated positive; samples with S/P% between 40 and 50 were considered doubtful; samples with S/P ≤ 40 were considered negative.

**RESULTS AND DISCUSSIONS**

Neosporosis and toxoplasmosis are two major causes of reproductive disorders in ruminants and serologic surveys reveal that they are worldwide distributed, including Romania. In this study, a serological testing was performed to determine the prevalence of *N. caninum* and *T. gondii* infection in a dairy cattle farm in South-East of Romania, with history of reproductive disorders, by using two commercially available ELISA-based tests. Overall, 5.1% (8/157; 95% CI: 1.66%-8.54%) and 11.8% (15/127; 95% CI: 6.20%-17.42%) of tested animals were seropositive for *N. caninum* and *T. gondii*, respectively (Table 1). Of the 127 tested animals for the both pathogens, two cattle (1.6%; 95% CI: -0.59% -3.74%) were seropositive for both parasites. The apparent seroprevalence, according to the analyzed category, heifers and dairy cows, ranged between 2.7% and 7.1%, respectively, for *N. caninum*, and from 13.7% to 9.3% for *T. gondii* (Table 1).
Table 1. Determined seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infection in tested cattle, South-East of Romania

<table>
<thead>
<tr>
<th>Cattle category</th>
<th><em>Neospora caninum</em></th>
<th></th>
<th><em>Toxoplasma gondii</em></th>
<th></th>
<th><em>N. caninum and T. gondii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seroprevalence</td>
<td>95% CI</td>
<td>Seroprevalence</td>
<td>95% CI</td>
<td>Seroprevalence</td>
</tr>
<tr>
<td></td>
<td>(no. positive/no. tested)</td>
<td></td>
<td>(no. positive/no. tested)</td>
<td></td>
<td>(no. positive/no. tested)</td>
</tr>
<tr>
<td>Dairy cows</td>
<td>7.1% (6/84)</td>
<td>1.64 - 12.65</td>
<td>9.3% (5/54)</td>
<td>1.53 - 16.99</td>
<td>1.9% (1/54)</td>
</tr>
<tr>
<td>Heifers</td>
<td>2.7% (2/73)</td>
<td>-1.00 - 6.48</td>
<td>13.7% (10/73)</td>
<td>5.81 - 21.59</td>
<td>1.4% (1/73)</td>
</tr>
<tr>
<td>Total</td>
<td>5.1% (8/157)</td>
<td>1.66 - 8.54</td>
<td>11.8% (15/127)</td>
<td>6.20 - 17.42</td>
<td>1.6% (2/127)</td>
</tr>
</tbody>
</table>

* 95% CI: 95% Confidence Interval; no: number

Our results regarding *N. caninum* indicated an overall seroprevalence of 5.1%, lower compared with those previously reported in some studies in the country. Therefore, in Romania, reported seroprevalence rates appear to be higher in healthy animals than in those with reproductive disorders, suggesting that reproductive disorders may be also caused by other factors, in the field of management issues (Mitrea et al., 2012). This assumption seems to be valid for our study as well, considering that the main cause of reproductive disorders was retroperitoneal fat necrosis.

In other studies, antibodies against *N. caninum* have been reported in cattle from different European countries such as Czech Republic (0.5%), Sweden (0.5%), Belgium (12.2%), Germany (1.6%), Hungary (15.1%), Italy (34.0%), Spain (16.2%), Poland (9.23%), and Turkey (60.0%), with prevalence rates ranging from 0.5% to 60% (Czech Republic vs. Turkey) Bartels et al., 2006; Kul et al., 2009, Bártová et al., 2015.

The antibodies against *N. caninum* have been detected in both healthy animals and those with reproductive disorders, with a higher level for the latter (2.3% vs. 20.1%) (Reiterova et al., 2009; Vaclavek et al., 2003; Bártová et al., 2015).

Regarding *T. gondii*, the detected seroprevalence of 11.8% in this study is close to the previous ones detected in our country, using different detection method (Dubey et al., 2014). The detected seroprevalence is also similar to those recently reported (8.1%) in water buffaloes (Bărburaş et al., 2019).

Comparing with other studies in European countries, the *T. gondii* seroprevalence detected in our study is lower than the average level, in a detection scale of 2-76%, being similar to those reported in Switzerland (10.7%) and Czech Republic (9.7%) (Gottstein et al., 1998; Bártová et al., 2015).

Generally, the reported seroprevalence rates of *N. caninum* were found to be higher than those of *T. gondii*. However, the results obtained in this study showed a higher prevalence for *T. gondii* infection than for *N. caninum* infection, which is similar to those previously described in Turkey and Czech Republic (Kul et al., 2009; Bártová et al., 2015). Similar aspects have been previously found in dairy goats from Romania (Iovu et al., 2012).

Regarding the correlation between infections and the age category, different opinions were issued, and, generally, in respect to neosporosis the seropositivity is higher in cows over 2 years of age and horizontal transmission was suggested (Mitrea et al., 2012).

In our study, the seroprevalence of *N. caninum* was higher in dairy cows and the one for *T. gondii* in heifers; however, the difference is not statistically significant.

The co-existence of *N. caninum* seropositivity was frequently observed with specific antibodies against other pathogens, including *T. gondii* (Yildiz et al., 2009). In our study, 1.6% of animals were positive for both parasites. Similar results were previously described in Switzerland, with 1.1% of the tested cows positive for both infections (Gottstein et al., 1998).

However, further studies are necessary in order to investigate in more details, at farm level, the eco-epidemiology of neosporosis and toxoplasmosis, to fundament a suitable control program for the both abortion causative agents, in Romanian farms.

**CONCLUSIONS**

Our study showed that *N. caninum* and *T. gondii* infections were present in the analyzed dairy cattle farm and can overlap with other reproductive disorders. These results emphasize the need in the frame of good management practices, of screenings for
N. caninum and T. gondii in cattle with reproductive diseases.

REFERENCES


