

SEROLOGICAL SCREENING FOR *Neospora caninum* INFECTION IN A DAIRY CATTLE FARM WITH REPRODUCTIVE DISORDERS, IN SOUTH-EASTERN ROMANIA

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Abstract

Neospora caninum is an apicomplexan protozoa parasite that causes reproductive disorders in cattle worldwide. Despite the high seroprevalence reported in Romania, there are limited data on *N. caninum* infection in dairy cattle, and no studies have evaluated the presence of infection over time in a limited area. Therefore, this study aimed to assess the seroprevalence of *N. caninum* in a dairy cattle farm in Southeastern Romania where a previously study reported about the cattle exposure to *N. caninum* infection. A total of 85 cattle, assigned in four groups, according to the reproductive status (repeated breeding syndrome, history of abortion, pregnant cows, and fresh cows) were tested for the presence of *N. caninum* antibodies, using an indirect ELISA test. The overall seroprevalence was 28.23% (95% CI: 18.67–37.80). According to the reproductive groups, the seroprevalence varied between 19.04% (95% CI: 2.25–35.84) in fresh cows to 38.09% (95% CI: 17.32 - 58.87) in cows with repeated breeding syndrome. By these findings new information is provided about the disease's presence in the studied farm, implying that infection with *N. caninum* may be the primary cause of reported reproductive disorders, as well as about the infection's persistent nature.

Key words: *Neospora caninum*; seroprevalence; dairy cattle farm; reproductive disorders, Romania.

INTRODUCTION

Neosporosis is one of the most important causes of reproductive disorders, including abortion and neonatal mortality, in cattle worldwide, which can cause financial losses, especially in farms when the disease is endemic or epidemic (Dubey & Schares, 2011). The disease is caused by *Neospora caninum*, an apicomplexan protozoa parasite that has a heteroxenous life cycle involving canids as definitive hosts and a variety of warm-blooded animals as intermediate hosts, especially bovids (Dubey, 2003; Dubey & Schares, 2011; Goodswen et al., 2013).

Worldwide, *N. caninum* is responsible for 15-21% of abortions in cattle (Dubey et al., 2017; Reichel et al., 2013; Liu et al., 2020).

Infection in cattle occurs horizontally by ingestion of oocysts shed by definitive hosts (canids) or vertically from the infected dam to the fetus, which is generally considered to be the most important transmission route (Dubey,

2003; Djijkstra et al., 2001). About 95% of calves born from infected dams are infected for life and become parasite carriers.

N. caninum can be maintained in cattle herds through generations (Almeria et al., 2017; Ikonnikova et al., 2023).

Carrier cattle can suffer reproductive disorders at any time due to the reactivation of the infection; therefore, the identification of positive dams is necessary to prevent the spread of disease (Gliga et al., 2022).

Serological studies conducted on neosporosis in cattle have been published in many parts of the world and reported differences in prevalence between countries and within countries, between regions, and between dairy and beef cattle (Dubey & Schares, 2011; Kowalczyk et al., 2016). In European countries, *N. caninum* seroprevalence rates in dairy cattle varied between 16% and 76% (Gliga et al., 2022), and the association between reproductive disorders and neosporosis has already been reported.

In Romania, the prevalence of infection with *N. caninum* reported in several serological studies in cattle, carried out in different areas of the country, varies from 5.1% to 60.3% (Gavrea & Cozma, 2010; Gavrea et al., 2011; Imre et al., 2011; Mitrea et al., 2012; Enachescu et al., 2012a; 2014; Gotu et al., 2021) and dogs (Enachescu et al., 2012b; Mitrea et al., 2013). Despite the high seroprevalence reported, there are limited data on *N. caninum* infection in dairy cattle, and no studies have evaluated the presence of infection over time in a limited area. Therefore, this study aimed to assess the seroprevalence of *N. caninum* on a dairy cattle farm in Southeastern Romania, where cattle exposure to *N. caninum* infection was reported, in order to evaluate the dynamics of the disease.

MATERIALS AND METHODS

The study was conducted in a dairy cattle farm with reproductive disorders, where the cattle exposure to *N. caninum* infection was previously reported (Gotu et al., 2021). Reproductive disorders have been defined by infertility, and at the farm level it is considered that this symptom is mainly due to retroperitoneal fat necrosis. In order to assess the dynamics of *N. caninum* infection in the investigated farm, a monitoring serological study was conducted one year after the previous one, and other categories of animals were tested. Therefore, a number of 85 cattle were assigned into four groups, according to their reproductive status, as following: (i) 21 cows with repeated breeding syndrome; (ii) 21 cows with history of abortion; (iii) 22 pregnant cows, and (iv) 21 fresh cows. From them, blood samples were collected centrifuged and the sera was stored at -20° until use.

Cattle serum samples were tested for *N. caninum* antibodies using an ELISA

commercial kit (*Neospora caninum* Indirect Multi-Species, ID-VETLab., Montpellier, France), following the instructions of the manufacturer.

The kit is based on purified extract of *N. caninum* and is validated for the detection of antibodies against *N. caninum* in bovine, ovine or caprine sera, plasma or milk by indirect ELISA. The optical density (OD) values of indirect ELISA were read at 450 nm, using EPOCH spectrophotometer.

The samples' S/P% was calculated (using a formula according to the kit manual).

The cut-off of the kit was S/P>50%. Subsequently, the interpretation of results were as following: the samples with S/P% value ≤40% were considered negative; those with S/P% ranging from 40% to 50% were considered doubtful, and those with S/P%≥50% were considered positive.

RESULTS AND DISCUSSIONS

In this study, in order to evaluate the *N. caninum* infection in dairy cattle located in the South-east of Romania a total of 89 cattle serum samples were analyzed for the detection of specific antibodies. Of them, twenty-four samples were tested positive, with an overall seroprevalence of 28.23% (95% CI: 18.67-37.80).

Seropositive animals were found in all the four groups tested, with prevalence ranging between 19.04% (95% CI, 2.25-35.84) in fresh cows and 38.09% (95% CI, 17.32-58.87) in the cows with repeated breeding syndrome.

The seroprevalence in cows with a history of abortion was 33.33% (95% CI, 13.17-53.50), while in pregnant cows the seroprevalence was 22.73% (95% CI, 5.22-40.24) (Table 1).

Table 1. Determined seroprevalence of *Neospora caninum* infections in tested cattle, by an indirect ELISA test

Cattle category	No. of tested samples	No. of positive samples	Seroprevalence %, [95% CI]
Cows with repeated breeding syndrome	21	8	38.09 [17.32 - 58.87]
Cows with a history of abortion	21	7	33.33 [13.17 - 53.50]
Pregnant cows	22	5	22.73 [5.22 - 40.24]
Fresh cows	21	4	19.04 [2.25 - 35.84]
Total	85	24	28.23 [18.67 - 37.80]

The overall seroprevalence obtained in this study is significantly higher than the previously recorded one (5.1%) in the analyzed farm and can be most likely associated with the risk categories presently analyzed. Furthermore, these results are suggesting as infection with *N. caninum* may be a primary cause of reproductive disorders in the tested animals from the analyzed farm. Also, increased external exposure to oocysts may be a contributing factor, as reported by other studies (Mitrea et al., 2012).

In addition, the screening performed on samples collected over several years in the previous study (2017-2020) and in this study (2021) indicates that the disease persists on the analyzed farm for at least 5 years. Other studies indicate that seropositive animals can remain seropositive throughout their lives and that elevated antibody titres can persist for years (Stenlund et al., 2003; Pabón et al., 2007).

Considering these factors, additional epidemiological studies should be conducted on this farm by re-testing the animals to determine if the disease has a chronic evolution or not. This may be a solution to improve the mitigation measures for this disease on the investigated farm.

Our results are in accordance with those previously reported at both national and European levels, with the seroprevalence obtained similar to those previously reported in the study area by Mitrea et al. (2012) (40.3%) and very close with those recently recorded in Italy (21.1%) and Poland (20.1%) (Manca et al., 2022; Kowalczyk et al., 2016), which have been carried out in several cattle farms.

Different seroprevalence rates recorded between the categories of animals with reproductive disorders and those with reproductive capacity are common to studies conducted worldwide and underline the impact of the disease on the infected herds (Köse et al., 2021).

The increased seroprevalence rate recorded in cows with a history of abortion corresponds to previous data reported in a dairy herd in Spain (36.8%) (Pabón et al., 2007), dairy cattle farms from central and northwestern parts of Romania (40.9%) (Gavrea et al., 2011) and in a dairy cattle farm in South-Eastern Romania (Ilfov County, 66.6%) (Mitrea et al., 2012).

In addition, the increased seroprevalence found in cows with repeated breeding syndrome supports the results of previous research conducted in Turkey (Canatan et al., 2014), Greece (Lefkaditis et al., 2020), and Italy (Villa et al., 2022), which demonstrated the adverse effects of *N. caninum* infection on the reproductive capacity of animals. According to the studies cited, the number of inseminations per conception was higher among seropositive animals compared to seronegative animals.

In most countries, there are no national control programmes for neosporosis, and the epidemiological data are obtained from scientific studies. Therefore, the disease is very likely to be underestimated, and thus there are difficulties in implementing eradication measures.

There is well known that infection with *N. caninum* in fresh cows and pregnant cows is an indicator that the disease can be transmitted to subsequent generations. By passing of parasite from cow to calf, without clinical signs it is maintaining the subclinical infection within the herd and subsequently poses high risks for reproductive failure (Dubey et al., 2017).

The dairy cattle industry is seriously impacted on the economic profit, since dairy production dependent on the farm reproductive efficiency (Saraz et al., 2017). Numerous studies carried worldwide, including in Europe, have suggested significant economic losses associated with *N. caninum* infection in cattle, resulting in reproductive failure (Reichel et al., 2013; Liu et al., 2020). Therefore, control of neosporosis as well of other reproductive diseases will contribute on the animal health and welfare, reproductive efficiency, and the economic profit of the farm (Mitrea, 2002; Gilbert, 2016).

CONCLUSIONS

The present study provides updated data about the disease's presence in the studied farm and its persistent nature, suggesting that infection with *N. caninum* may be the primary cause of reported reproductive disorders. The high seroprevalence rates obtained in each category of analyzed animals emphasize the impact of the disease on the affected animals. Considering these, the monitoring of neosporosis in a limited area over time could contribute to a better understanding of the epidemiology of infection.

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