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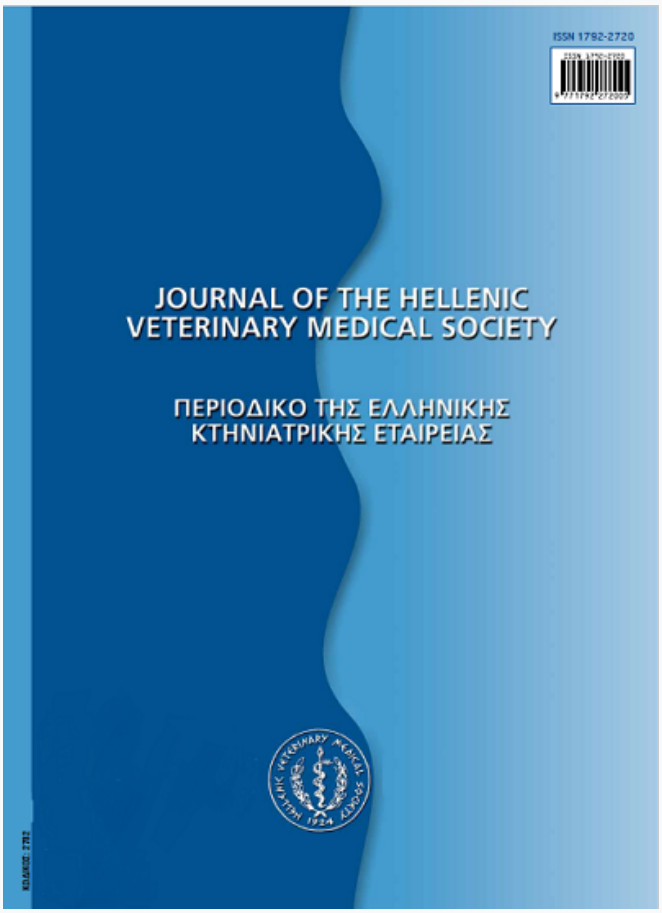

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Research article
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Seroprevalence of *Neospora caninum* in dairy cows in Belgrade city area, Serbia

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ABSTRACT. The protozoan parasite *Neospora caninum* is one of the most important abortifacient pathogen in cattle. Serological investigations are often used in order to estimate seroprevalence in herds. Aims of our study were to determine the seroprevalence among aborting and non-aborting dairy cattle in Belgrade city area as well as epidemiological factors that are important for *N. caninum* infection. Using commercial ELISA kit, we examined 188 sera. Out of 188, 142 samples originated from pregnant (non-aborting) cows from five farms, while 46 were from cows who had aborted. Overall seroprevalence was 25% (48/188). Seroprevalence was significantly higher ($p \leq 0.05$) in aborting than in non-aborting group of cows (37% and 21.1% respectively). At least one positive sample was detected on four (80%) out of five examined farms while seroprevalence among farms varied from 0 to 43.5%. On all examined farms crucial epizootiological factors (presence of dogs and low biosecurity measures) that favor the maintenance and spreading of the infection were identified. Our study revealed the presence of *N. caninum* antibodies in population of dairy cows in Belgrade city area. Infection is established in enzootic pattern on examined farms and high seroprevalence among aborting cows suggests that *N. caninum* could be important abortifacient pathogen.

Keywords: *Neospora caninum*, seroprevalence, dairy cow, Serbia

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INTRODUCTION

The protozoan parasite *Neospora caninum* is considered as a major cause of abortion in cattle (Dubey and Schares, 2006). *N. caninum* is a coccidian parasite with a wide host range (Dubey and Schares, 2011). In general, neosporosis is primarily disease of cattle which are intermediate host while dogs and related canids are definitive hosts of *N. caninum* (Dubey et al., 2007). The life cycle of *N. caninum* is typified by 3 infectious stages: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are found in the intermediate hosts and appear intracellularly. Dogs, as definitive host, got infected after ingestion of placentas and/or aborted fetuses containing tissue cysts. Infected dogs excrete unsporulated oocysts in feces (Dubey, 2003). Cattle can be infected through vertical or horizontal transmission. Vertical or trans-placental transmission from infected dams to their offsprings appears to be the major natural route of infection. Prenatally infected, but healthy calves remain persistently infected and can pass the infection to their own offsprings. Post-natal infection (horizontal transmission) occurs through ingestion of oocyst-contaminated fodder or drinking water (Innes et al., 2005; Conraths and Gottstein, 2007). Main feature of *N. caninum* infection in cattle is abortion although fetuses can be reabsorbed, mummified, stillborn, born alive with clinical signs or born clinically normal but persistently infected (Dubey and Schares, 2006). Cows of any age may abort starting from 3rd month of gestation to the term. Majority of neosporosis-induced abortions occur in 5-6 month of gestation (Dubey, 2003).

Infected cattle are three to seven times more likely to abort compared to uninfected cattle. Infected heifers are at the highest risk during the first pregnancy (Innes et al., 2005). Cows with *N. caninum* antibodies are more likely to abort than seronegative cows while up to 95% of live born calves from seropositive dams will be congenitally infected and clinically normal (Dubey, 2003; Dubey and Schares, 2006).

Many countries, such as Turkey (Kurtede and Ural, 2009; Kacar et al., 2012) Romania (Mitrea et al., 2012), China (Wang et al., 2010), Poland (Wisniewski et al., 2002), Netherland (Wouda et al., 1999), France (Ould-Amrouche et al., 1999), Brasil (Corbellini et al., 2002) and Hungary (Hornok et al., 2006) reported about *N.caninum*-associated abortions and seroprevalence in aborting as well as non-aborting cows. There are several reports regarding seroprevalence (Gavrilović et al., 2006; Klun et al., 2008; Vidić et al., 2011; Pavičić et al., 2011; Savović et al., 2012; Kuruca et al., 2013; Klun, 2014) and detection of *N. caninum* genome by molecular methods (Cvetojević et al., 2013, 2015; Klun, 2014) in Serbia.

The aims of our study were to determine the seroprevalence of *N. caninum* in dairy cattle as well as to identify epizootiological factors important for introduction, maintenance and spreading of *N. caninum* infection in examined farms in Belgrade city area, Serbia.

MATERIAL AND METHODS

Samples

We examined 188 serum samples from cows. This group consisted of 46 cows who had aborted and 142 pregnant (non-aborting) dairy cows. Serum samples from cows who had aborted were selected randomly from archive of samples that were submitted for diagnostic investigations of abortions during 2015 and 2016 from the Belgrade city area. For each of those cows, month of gestation in which abortion was detected was available and used for further evaluation of results. Pregnant cows included in this study originated from 5 farms (designated as A, B, C, D and E) from Belgrade city area. All pregnant cows were between fourth and seventh month of gestation in the moment of blood sampling. Farms included in our study were medium to large, breeding between 200 and 2500 black and white Holstein Friesian cows. A questionnaire form was completed in each farm with following data: number of cows on farm, abortion rate in previous year (sporadic: <3%; low: 3-5%), data regarding previously confirmed *N. caninum*-associated abortions (confirmed or not confirmed), presence of dogs on farm (present or absent), availability of fodder to dogs (available or not available), procedure for removing placentas after delivery and/or aborted fetuses in case of abortion (collecting and disposal to special container or leaving in the maternity barn and dispose with manure).

Serological analyses

Blood samples were collected by tail vein venipuncture. Blood was left at room temperature until the separation of serum. The presence of *N. caninum* antibodies

were determined using commercial enzyme-linked immunosorbent assay (ELISA) kit (Neospora caninum Antibody Test Kit, Idexx, Switzerland) according to manufacturer's instructions.

Statistical analyses

An odds ratio (OR) was used to measure the association between an exposure of cows to *N. caninum* and abortion as an outcome.

RESULTS

The overall seroprevalence of *N. caninum* was 25% (48/188). Antibodies were detected in 18 (37%) out of 46 cows who had aborted and in 30 (21.1%) out of 142 pregnant cows. These results showed significant difference (p=0.016) between cows that have been infected and healthy ones meaning that seropositive cows were 2.4 times more likely to abort than seronegative (OR = 2.4; 95% CL: 1.17-4.91). In positive aborting cows, abortions were detected from the third to the seventh month of gestation (mean 4.7 months). At least one positive sample was detected on four (80%) out of five examined farms while seroprevelence among farms varied from 0 to 43.5% (Table 1.). Abortion rate was low on two farms (farm A and C) and sporadic on three farms (farms B, D and E). Previous *N. caninum*-associated abortions were confirmed on three (B, D and E) out of five farms. To dogs, kept on all farms, fodder was easily accessible. Only on one farm (farm C), placentas and/or aborted fetuses were collected to special container while on other farms afterbirth material were left in maternity barn and later were disposed with manure.

Table I.

DISCUSSION

Researches from different countries report about various seroprevalences of *N. caninum* depending on category (aborting or non-aborting cows). Kacar et al. (2012) detected 7.4% (11/148) seropositivity among cows that had had abortion in region of Kars while Kurtede and Ural (2009) reported that *N. caninum* seroprevalence in Turkey varied from 2 to 13.96% depending on the region of the country. Mitrea et al. (2012) detected high seroprevalence (41.7%) among randomly sampled dairy cows on farms in southern Romania. Results of seroprevalence in non-aborting cows in our study was similar to prevalence in Poland (20.7%) (Wisniewski et al., 2002). Seroprevalence in our group of aborting cows was lower comparing to results from the Netherland where Wouda et al. (1999) found seroprevalence from 17 to 87% in herds with history of *N. caninum* abortion. Ould-Amrouche et al. (1999) reported on seroprevalence of 5.6% (107/1924) in Normandy in France. Much lower seroprevalence (2.5%) comparing to our study was reported from northeast Hungary (Hornok et al., 2006).

Seroprevalence of *N. caninum* obtained from our study is higher than previously reported from other researches in Serbia. Savović et al. (2012) detected overall seroprevalence of 17.3% (18.8% in aborting and 16.7% in non-aborting cows) in Vojvodina province. In south Banat region, Gavrilović et al. (2013) detected *N. caninum* antibodies in 4.6% (23/500) cows while among 27 aborting cows they found 7 (26%) positive. Kuruca et al. (2013) obtained overall seroprevalence of 15.4% (55/356) and among them 12.2% (9/74) in cows with reproductive disorders and 16.3% (46/356) in reproductively healthy cows. Vidić et al. (2011) found that 3.7% (5/132) of cows in

Farm	Number of cows on the farm	Abortion rate	Previously confirmed *N. caninum*-associated abortions	Presence of dogs	Availability of fodder to the dogs	Removing of placentas and/or aborted fetuses	Examined/positive samples
A	250	low	not confirmed	present	available	leave in barn	20/4 (20%)
B	1100	sporadic	confirmed	present	available	leave in barn	39/17 (43.5%)
C	200	low	not confirmed	present	available	special container	20/0 (0%)
D	1000	sporadic	confirmed	present	available	leave in barn	25/6 (24%)
E	2500	sporadic	confirmed	present	available	leave in barn	38/8 (21%)

Vojvodina province had *N. caninum* specific antibodies. In two different investigations, Klun et al. (2008, 2014) performed serological examinations from samples from all regions of Serbia. In pilot study, Klun et al. (2008) detected seroprevalence of 8.6% from 350 cows. In more detailed study, Klun (2014) found that 108 (7.6%) out of 1496 cows were seropositive to *N. caninum*. Despite global and regional differences in seroprevalence, *N. caninum* is globally widespread. However, results from different investigations should be interpreted with caution because of many variables that can influence on the obtained results (number of examined animals, animals included in study – aborting or non-aborting, diagnostic test used, different husbandry systems, regional specificity etc).

Specific antibodies may persist longlife but fluctuate, sometimes even below detection limit of serological tests (Dubey and Schares, 2006). As a confirmation of its significant fluctuation, Sager et al. (2001) reported, in repetitive serological investigations (at 3–12 months interval), decrease of the overall *N. caninum*-seroprevalence from 17 to 12%. According to Innes et al. (2005), serum antibody response to *N. caninum* in pregnant cattle fluctuates throughout gestation. Therefore, in order to increase the possibility to detect seropositive cows, we sampled cows between the fourth and the seventh month of gestation when rise of antibody levels is expected. Despite the fact that seropositive cows are three to seven times more likely to abort (Innes et al., 2005), an important question in disease understanding why not all infected cows abort has raised? To better understand mechanism of parasite-associated abortions and control of the disease, it would be of uttermost importance to define the markers related to the diagnosis and more importantly to the risk of abortion in the infected cow (Almeira and Lopez-Gatius, 2015).

Possible reasons for high seroprevalence obtained in our study could be low biosecurity which was common characteristic for all farms included in our study. Dogs on farms and accessibility to fodder along with lack of infrastructure and procedures for disposal of afterbirth and/or abortion material (placentas, aborted fetuses) contribute to maintaining and spreading *N. caninum* throughout the farm. Interestingly, only one farm (farm C) in our study did not have any positive cow. Although, dogs were also present on this farm as well as had access to fodder, on that farm special attention was given to disposal of afterbirth and/or abortion material. It could be assumed that good management of disposal of afterbirth and/or abortion material could prevent and interrupt parasite life cycle on the farm. Nevertheless, the most important preventive measure is avoiding dogs, which are definitive hosts and source of this infection, to come into either direct or indirect contact with cows. Neosporosis-associated abortions in bovine herds may have an epizootic or enzootic pattern (Dubey and Schares, 2006). Annual abortion rates on examined farms are rather stable. Therefore, we can conclude that *N. caninum*-associated abortions on those farms would be expressed through enzootic pattern.

CONCLUSIONS

In conclusion, our study revealed the high seroprevalence of *N. caninum* antibodies in population of dairy cows in Belgrade city area. Based on epizootiological data from examined farms, infection is established in enzootic pattern. High seroprevalence among aborting cows suggests that *N. caninum* could be important abortifacient pathogen in examined region. Improving biosecurity on farms is the first precondition for implementation of possible programs for control or eradication of the disease.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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