University of Novi Sad - Univerzitet u Novom Sadu
Faculty of Agriculture - Poljoprivredni fakultet

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SEROPREVALENCE OF *NEOSPORA CANINUM* IN DOGS

LJILJANA PAVIČIĆ, VESNA LALOŠEVIĆ,
LJUBICA SPASOJEVIĆ-KOSIĆ, SAŠA LAUŠ, STANISLAV SIMIN[1]

SUMMARY: Neospora caninum is an apicomplexan parasitic protozoa that seriously impacts economic performance of diary and beef industries by causing abortion in cattle. Likewise, it is considered as a cause of severe neuromuscular disease in dogs around the world. Since canine neosporosis has not yet been properly investigated in Serbia,the aim of our work was to determine seroprevalence of N. caninum antibodies in a group of dogs from one region of Vojvodina (Serbia) and to evaluate the importance of the age as a possible risk factor for higher seropositivity to N.caninum in dogs. For this purpose, sera from 31 dog from territory of Krčedin and Vršac were examined using indirect fluorescent antibody test. All sera were screened at 1:50 dilution and positive samples were then titrated in two-fold dilution series to the respective endpoint (1:100). Our findings showed that 12,9% of tested dogs was positive to N. caninum antibodies, but no statistically important association between seroprevalence and the age of the dogs was discovered.

Key words: *Neospora caninum, dogs, seroprevalence, Vojvodina, age, risk factor.*

INTRODUCTION

Neospora caninum is an intracellular protozoan parasite of domestic and wild animals with worldwide distribution (Lyon, 2010). Until 1988 it was misdiagnosed as *Toxoplasma gondii*. Since then, *N. caninum* is considered as a major cause of reproductive failures in cattle, as well as the most common cause of infectious inflammatory myopathies and neurological diseases in dogs (Dubey et al., 2007). Beside woolfs and coyoties, dogs are definitive hosts of *N. caninum.* In dogs, ingestion of infected tissues of intermediate host (e.g. cattle) as well as transplacental transmission are dominant routs of acquiring the disease, although lactogenic infection has also been reported (Dubey et

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Short communication / *Kratko saopštenje*

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al.,2011, Dubey and Schares, 2011, Cavalcante et al., 2011). In infected puppies, disease tends to be more severe than in adult dogs. Clinical signs are usually noticed between 3 and 9 weeks of age and include ascending, often progressive and potentially lethal paralysis of the limbs and their rigid hyperextension (Lion, 2010, Reichel et al., 2007). In adult dogs, polymiositis and multifocal CNS signs are the most common clinical presentations, while ulcerative dermatitis is common in older and immunosuppresed dogs (La Perle et al., 2001, Reichel et al., 2007, Lion, 2010). Other possible manifestations of canine neosporosis are: myocarditis, hepatitis, pneumonia, dysphagia and abortion (Lion, 2010, Moura et al., 2011). Although the exact role of the dogs in the epidemiology of neosporosis has not yet been completely revealed, several epidemiological studies have indicated that the presence of dogs on farms may be an important risk factor for bovine neosporosis (Cedilo et al., 2008). This assumption has further led to recommendations for dog control on the farms as one of the preventive measures that should be implemented in farm management procedures in order to avoid infection in cattle (Reichel and Ellis, 2009, Dubey et al. 2007, Innes et al.,2002). Therefore, although the circulation routes of *N. caninum* between dogs and cattle require further investigation, presence of *N. caninum* antibodies in dogs should impose a reasonable doubt for the presence of bovine neosporosis in local herds. The aim of our study was a quick evaluation of seroprevalence of *N. caninum* in dogs from two regions of Vojvodina and investigation of possible connection between the seropositivity of the dogs and their age, since age is (together with gender, breed, diet, envirement and contact with other animals), according to some authors (Moura et al., 2011, Regidor-Cerillo et al., 2010, Collantes-Fernández et al. 2008, Paradies et al., 2007, Haddadzadeh et al., 2007), considered to be a risk factor for presence of *N. caninum* antibodies in dogs. To our knowledge, there have been no previous data concerning canine neosporosis in Serbia.

MATERIAL AND METHODS

A total of 31 dogs were included in this study, 10 of which were kennel dogs from Krčedin and 21 were the ambulatory patients from Vršac. All dogs were of a different breed, mostly hounds, of both gender, with age of dogs varying from 8 months to 11 years. Acquired anamnestic data and thorough clinical examination confirmed that there were no clinical signs of neosporosis in dogs at the moment of serum sampling.

A total of 31 serum samples were transported to the Laboratory of Parasitology of the Department of Veterinary Medicine, Faculty of Agriculture, Novi Sad, where they were tested for the presence of IgG antibodies against *N. caninum* using a standard method of indirect fluorescent antibody test (IFAT). For this purpose comerciall IFAT reagents (VMRD, USA) were utilised, using a 1:50 dilution as a cut-off value (screening test). All positive sera were further examined in a two-fold dilution series (1:100).

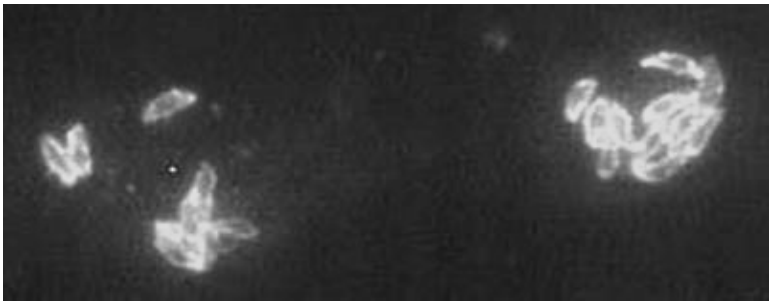
„Nikon“ fluorescence microscope and „Leica“ digital camera were used for visual examination of the asseyd sera and recordance of the positive ones, respectively.

RESULTS AND DISCUSSION

N. caninum antibodies were detected in 12,90% (4/31, 95% CI: 3,6% - 29,8%) of samples, which matches the results of other authors (Reichel et al., 2007, Lyon, 2010

) who reported seroprevalences ranging from 0% to 20% in clinically healthy dogs. Moreover, our findings appear quite similar to those from municipalities of Lages and Balneário Camboriú (Santa Catarina State, Brasil) where Moura et al. (2011) determined seroprevalence of 12,3%. On the other hand, in their study titers of positive sera ranged from 1:50 to 1:800, while ours were on a low, cut off level, of 1:50.

An example of positive IFAT, recorded during our research, is given in a picture below (Picture 1).



Picture 1. Indirect fluorescent antibody test (VMRD, USA). *Neospora caninum,* fluorescence of the tachizoites in reaction with positive dog sera
Slika 1. Indirektni imunofluorescentni test (VMRD, USA). Neospora caninum, fluorescencija tahizoita sa pozitivnim serumom psa

Regarding the age of the dogs, no statistical differences were observed between the occurrence of anti-*N.caninum* antibodies (p = 1.000, 2-sided Fisher's exact test) in dogs from the two studied age groups (Table 1).

Table 1. Seroprevalences according to the age of the dogs (N=31)
Tabela 1. Seroprevalenca u odnosu na starost pasa (N=31)

Dog age groups *Starosne grupe pasa*	No. of dogs in the group *Br. pasa u grupi*	No. of seropositive dogs Br. seropozitivnih pasa	Prevalence *Prevalenca*
Dogs ≤ 2 years old	16	2	12,5 %
Dogs > 2 years old	15	2	13,3 %

This finding is consistent with findings of researchers from Turkey (Çoŝkun et al., 2000), Brasil (Romanelli et al., 2007) and Poland (Goździk et al., 2011), despite differences between sample sizes and formulation of the age groups. For example, we have decided to divide our dogs in two groups, one consisting of dogs ≤ 2 years old and another containing dogs > 2 years old, in order to get uniform distribution within the groups which seemed necessary for statistical processing of such small sample. In Poland, however, large size of the sample (257 dogs) allowed Goździk et al. (2011) to compare seroprevalences between five different age groups (under 1year old, 1 to 5, 5 to 10 and dogs over 10 years old).

On the other hand, results of our study directly oppose to those of Moura et al. (2011), Regidor-Cerillo et al. (2010), Collantes-Fernández et al. (2008), Paradies et al. (2007) and Haddadzadeh et al. (2007) who all detected an increase in seroprevalence in dogs > 1 year old, which they explained by domination of postnatal over congenital infection of dogs with *N. caninum*, suggesting a higher exposure of adult animals to the sources of infection.

The small size of the sample was, once again, the main determinant of our choice of statistical test (Fisher's instead of $\chi 2$ test) used in this study.

CONCLUSION

This study demonstrated that *N. caninum* is present in dog population from the territory of Krčedin and Vršac, with prevalence of at least 12,90% (4/31, 95% CI: 3,6% - 29,8%). Furthermore, we find that age of the dog should not be of importance as a risk factor for the presence of *N. caninum* antibodies.

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SEROPREVALENCA *NEOSPORA CANINUM* KOD PASA

LJILJANA PAVIČIĆ, VESNA LALOŠEVIĆ

Izvod

Neospora caninum je parazitska protozoa iz kola *Apicomplexa* koja pričinjava ozbiljne ekonomske gubitke mlečnoj industriji i proizvodnji tovnih grla izazivajući abortuse kod goveda. Takođe, ovaj parazit se smatra uzročnikom ozbiljne neuromuskularne bolesti kod pasa širom sveta. Pošto neosporoza pasa u Srbiji još uvek nije adekvatno ispitana, cilj našeg rada je bilo utvrđivanje prevalence antitela na *N. caninum* unutar grupe pasa iz jednog regiona Vojvodine (Srbija) i ocena uticaja starosti pasa, kao faktora rizika, na visinu seroprevalence. U ovu svrhu, upotrebom testa indirektne imunofluorescencije ispitani su serumi 31-og psa sa teritorije Krčedina i Vršca. Svi serumi su pregledani pri razređenju 1:50 ("screening test"), a pozitivni uzorci su zatim titrirani ponovo, u dvostrukom razređenju (do 1:100). Antitela na *N. caninum* utvrđena su kod 12,9% ispitanih pasa, ali statistički značajna povezanost između starosti pasa i visine seroprevalence nije pronađena.

Ključne reči: *Neospora caninum*, psi, seroprevalenca, Vojvodina, starost, faktor rizika.

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