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Seroprevalence and potential risk factors of *Neospora caninum* infection in dairy cattle in Croatia

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ABSTRACT

Neospora caninum, a cyst forming coccidian parasite, is an important cause of abortion in cattle worldwide. The present work aims to update knowledge of the presence of *Neospora* in cattle from Croatia. The survey was carried out on dairy cows in the Bjelovar-Bilogora County in northwest Croatia (2652 km²). The cows were selected by simple randomization from county dairy producer records. Cows were tested for the presence of *N. caninum* antibodies with the Neospora-Ab iscom enzyme-linked immunoassay kit (SVANOVIR). *N. caninum* antibodies were detected in 23 from 395 (5.6%, confidence interval (CI); CI 95%; 3.5 to 8.1) lactating cows. Epidemiological studies of potential risk factors, such as the presence of a dog, cat, swine, free range poultry and management practices, such as silage processing and pasture practising showed that none of these factors was associated with *N. caninum* antibodies. Seropositivity of cows was associated to abortion (odds ratio (OR); OR = 8.307, P = 0.002) and to purchasing of replacement animals from other sources (OR = 5.27, P = 0.0002). A strong correlation was also found between antibodies, history of abortion and origin (imported animals) of heifers or cows (OR = 5.52; P = 0.001).

Key words: *Neospora caninum*, cattle, abortion, risk factors, Croatia

Introduction

Neospora caninum is a protozoan parasite, which has been reported to cause abortions in cattle worldwide (DUBEY and LINDSAY, 1996; DUBEY, 2003). Subclinical infections have been also identified as a cause of decreased milk production and reduced

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feedlot-calf performance (THURMOND and HIETALA, 1997; BARLING et al., 2000). The economical losses to the beef and dairy industries are considerable (PARE et al., 1997; WALDNER et al., 1998). To estimate the seroprevalence of *N. caninum*, various studies were undertaken in different European countries, such as Belgium (DE MEERSCHMAN et al., 2002), Denmark (JENSEN et al., 1999), England and Wales (DAVISON et al., 1999a; DAVISON et al., 1999b), France (OULD-AMROUCHE et al., 1999), Germany (SCHARES et al., 2003), Italy (OTRANTO et al., 2003), Northern Ireland (MCNAMEE et al., 1996), Portugal (CANADA et al., 2004), Spain (MAINAR-JAIME et al., 1999; QUINTANILLA-GOZALO et al., 1999), Sweden (BJORKMAN et al., 2000), Switzerland (GOTTSTEIN et al., 1999) and the Netherlands (WOUDA et al., 1999). It has to be noted that the recent supranational survey regarding the seroprevalence in cattle in Germany, the Netherlands, Spain and Sweden showed that prevalence varied greatly between the different countries. The prevalence among single reactors for dairy herds varied from 16% in Sweden up to 76% in the Netherlands (BARTELS et al., 2006).

In contrast to the well defined status of the disease in most European countries, the risk of this disease has not been sufficiently recognized in the region of Southeast Europe.

Little is known about the prevalence of *N. caninum* in cattle in Croatia. Bovine neosporosis, according to seroprevalence data, was first reported in dairy cattle (BECK et al., 2006), and in sheep (MARTINKOVIĆ et al., 2006). So far some research has been conducted to define the importance of management factors. BARTELS et al. (1999) reported that exposure of cattle to poultry, feeding on mouldy corn silage, the use of communal pasture for young stock and other factors were positively associated with abortions. OULD-AMROUCHE et al. (1999) demonstrated that the exposure of cattle to rabbits and/or ducks, tethered housing and water ponds were all positively associated. The association between *Neospora* abortion and canids is still uncertain. The exact role of canids in the epidemiology of bovine neosporosis remains unclear. PARE et al. (1998) and few Dutch scientists (BARTELS et al., 1999; MOEN et al., 1998; WOUDA et al., 1999; DIJKSTRA, 2002) supported the positive association between the prevalence of neosporosis and the presence of dogs. The same was not demonstrated by OULD-AMROUCHE et al. (1999) and ROMERO et al. (2002). The present study was carried out to investigate the serological prevalence of *N. caninum* infection in dairy cattle and to characterize possible risk factors with *N. caninum* infection and reproductive disorders.

Materials and methods

The survey was carried out in the Bjelovar-Bilogora County in North-western Croatia (2652 km²), at an altitude mainly ranging from 120 to 150 m above sea level (a.s.l.), over a period of one month (October, 2004). The area included 7,814 registered milk producers with a highly variable number of dairy cows on their farms. In order to ensure a

95% confidence interval and maximum accepted error of 5%, using the worse acceptable prevalence of 50%, 395 blood samples (+5% for no responding producers) were drawn by simple randomization from county dairy producer records which gave an 5% absolute error for the sample size (Win Episcopo 2.0). From selected milk producers one lactating cow was randomly selected among adult female cattle on their farms. Jugular vein blood was collected in plain vacutainer tubes and was transported to the laboratory. After centrifugation at 1000 g for 15 min, serum was removed and stored at -20 °C until analysis.

Epidemiological data on age, breed, herd abortion history, number of cows, heifers and calves in the herd; number of dogs, cats and pigs on the farm, poultry management on the farm, pasture, silage processing, and cow replacing management were recorded in a questionnaire filled out by the participating veterinarians. Data from the questionnaire were compiled in a database.

Anti-*Neospora* antibodies were detected using a commercially available iscom ELISA Kit (SVANOVIR) following the manufacturer's instructions. The results were expressed as the percentage positivity (PP) of the positive control sera: below twenty PP indicates a negative result and a test result of above twenty indicates a positive result.

Multivariate logistic regression models were used to identify risk factors for *N. caninum* seropositivity. Each model was applied at the individual cow level using epidemiological data as independent variables and serological status as a dependent variable. All statistical analysis was performed using STATA 7.0.

Results

Among 395 dairy cows, *N. caninum* antibodies were detected in 23 cases, with overall seroprevalence of 5.8% (CI 95%; 3.5 to 8.1). The average number of adult cows per farm was 6.59 (C.I. 95%; 6.1-7.1), with average number of 5.19 heifers (C.I. 95%; 3.55-6.82) and 2.23 calves (C.I. 95%; 1.52-2.93). The average number of dogs on farm was 1.28 (C.I. 95%; 1.20-1.36) and cats 1.81 (C.I. 95%; 1.60-2.03). The mean number of pigs on farm was 8.90 (C.I. 95%; 6.98-10.81). A range of potential risk factors such as: presence of dog, cat, swine and free range poultry on farm was checked for potential statistical association. Furthermore, management practices such as silage processing and pasture practice were also checked for association. Statistical analysis shows that none of the above mentioned factors is associated to the *N. caninum* seropositivity. Univariate logistic regression shows that seropositivity of cows to *N. caninum* is associated to abortion (OR 8.307, P = 0.002) and to purchasing replacement animals from other sources (OR = 5.27, P = 0.0002). The multivariate logistic model revealed the existence of an association between the positive serological result and the history of abortions in the herd combined with the importation of heifers or cows into the herd (OR = 5.52; P = 0.001).

Table 1. Number of cattle, prevalence (%) of *N. caninum* antibodies in sampled animals and general characteristics of herd, odds ratio (OR) for seropositive results, 95% confidence interval (CI) and P-value

Variable	N	Seroprevalence (%)	OR	CI	P-value
Age (years)					
0-5	17	11.76	1		0.45
6-10	139	4.32	0.34	0.06-1.82	
>11	239	6.28	0.50	0.10-2.40	
Breed					
Simmental	357	5.88	1		0.88
Holstein	38	5.26	0.89	0.20-3.94	
Number of cows					
0-5	214	5.61	1		0.73
6-10	133	6.77	1.22	0.50-2.98	
11-15	20	10.00	1.87	0.39-9.01	
16-20	19	0			
>20	9	0			
Abortion history					
No	325	3.08	1		0.002
Yes	70	18.57	8.307	2.19-31.48	
Importation of cows/heifers					
No	248	2.42	1		0.0002
Yes	147	11.58	5.27	2.03-13.7	

The Table 1 presents the seroprevalence for *N. caninum* antibodies by collected epidemiological data on sampled animals and general characteristics of herd.

Discussion

Our study confirmed for the first time the presence of antibodies against *Neospora caninum* in cattle in the Republic of Croatia. In the Bjelovar-Bilogora County, where the research was carried out, antibodies were determined in 23 dairy cows with complete prevalence of 5.8% (CI 95%; 3.5 to 8.1). Values similar to this were detected in a survey conducted in the Czech Republic (VACLAVEK et al., 2003) with the prevalence of 5.83% and in a study carried out in France where antibodies were determined in 5.6% animals according to OULD-AMROUCHE et al. (1999). Apart from in European countries, almost

the same prevalence of 5.5% was found in Thailand (KYAW et al., 2004) and in Japan 5.7% (KOIWAI et al., 2006).

One of the most important factors associated with the occurrence of *N. caninum* and related abortions is the presence of dogs and wild canids on the farms. After the assured recognition that dogs are a final host (MCALLISTER et al., 1998; LINDSAY et al., 1999), it was obvious that the presence of dogs on farms can have an important role in the horizontal spread of *N. caninum* through oocysts excreted in the environment. However, the presence of dogs on farms and their impact in transmission is still a controversial question. PARE et al. (1998) clearly show that the presence and the number of dogs are in positive correlation with seropositivity and frequent abortions. The same opinion has been supported by other researchers (MOEN et al., 1998; BARTELS et al., 1999; WOUDA et al., 1999; DIJKSTRA et al., 2002). Alongside the results obtained by logistic regression in this research we were not able to connect the presence and number of dogs on farms with seropositivity in cattle whereby we could join the results obtained by KYAW et al. (2004), PITEL et al. (2001), and ROMERO et al. (2002). BARLING et al. (2000) consider that the role of dogs living near to cattle could even be protective; because they prevent the contact of stray dogs with the cattle and by this protective behaviour they reduce the exposure of cattle to infective oocysts.

In Netherland, BARTELS et al. (1999) noticed that the exposure of cattle to poultry, feeding with corn silage and at the same time holding juvenile livestock in shared pastures leads to a higher number of seropositive animals. OULD-AMROUCHE et al. (1999) proved that the exposure of cattle to rabbits and/or ducks, holding in boarded pens and exposure to artificially created lakes or pools is in positive correlation with seropositivity to *N. caninum*. We did not discover factors which could demonstrate that the presence of these management factors are associated with the occurrence of *N. caninum*, which is in agreement with other authors (PARE et al., 1998; DAVISON et al., 1999a; ROMERO et al., 2002). The presence of cats, pig, poultry and the way of holding or feeding (silage or pasture) the cattle were not statistically noticeable factors in our study, thus it was not possible to connect these risk factors with seropositivity in different herds.

From all factors considered in this study, the statistically most important is abortion of seropositive cows (OR = 8.32; P = 0.002). The same results have been described and confirmed in California (THURMOND and HIETALA, 1997), New Zealand (PFEIFFER et al., 2002), Great Britain (DAVISON et al., 1999) and the Netherlands (WOUDA et al., 1999) where the risk of abortion increased 7.4-fold, 4.2-fold, 3.5- fold and 3-fold respectively. Another important fact found in this epidemiological investigation was the replacement and introduction of new cows to the herd (OR = 5.27; P = 0.0002) which indicates the importance of neosporosis and their mutual correlation. Also, the correlation of serologically positive cows and abortions in combination with the introduction of new

cows in herd (OR = 5.52; P = 0.001) was confirmed by multivariant logistic regression. These facts could indicate that importing animals from other European countries without any serological checking assisted in introducing and spreading neosporosis from farm to farm.

The number of cattle in herds, breed constitution and age of cattle had no influence on the prevalence of antibodies against *N. caninum*.

Conclusion

Apart from infectious diseases which cause abortion in cattle, protozoan parasite *N. caninum* was recognized as one of the leading causes of abortion, causing major economical losses. For the first time we have proved here the presence of sera antibodies against *N. caninum* in the Republic of Croatia, which surely points to the presence of this protozoon. Seropositivity is correlated with the introduction of new animals to the herd and, since a high number of cattle from the European Union have been imported into Croatia, it is possible that these animals have contributed to the appearance and the spread of the disease. Due to its importance, neosporosis must be considered as a possible cause of abortion, and it is also necessary to determine the spread throughout Croatia as well as the influence of dogs and other wild canids in the spread of the disease. Since in this study we did not notice any correlation with the presence of dogs on farms, it is to be assumed that vertical transmission is the primary mode of transmission.

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SAŽETAK

Neospora caninum, cistotvorna kokcidijska, značajan je uzročnik pobačaja u goveda u čitavom svijetu. U radu su prikazani novi podaci o prisutnosti protutijela za neosporu u goveda u Hrvatskoj. Istraživanje je provedeno na mlječnim kravama na području Bjelovarsko-bilogorske županije (2652 km²). Životinje su odabrane metodom slučajnoga odabira iz registra proizvođača. Krave su bile pretražene na prisutnost protutijela za *N. caninum* imunoenzimnim testom (SVANOVIR). Protutijela su bila dokazana u 23 od 395 pretraženih krava u laktaciji (5,6%, interval povjerenja (CI) 95%: 3,5 do 8,1). Epidemiološko istraživanje s ciljem određivanja mogućih rizičnih čimbenika kao što su prisutnost pasa, mačaka, svinja, slobodno držane peradi te menadžment farme koji uključuje pripremu i skladištenje silaže te načine ispaše pokazala su da niti jedan od prije navedenih čimbenika nije povezan s prisutnošću protutijela protiv *N. caninum*. Seropozitivnost krava bila je povezana s pobačajima (odds (OR) = 8,307, P = 0,002) i s uvođenjem u stado životinja iz drugih stada (OR = 5,27, P = 0,0002). Također je dokazana snažna povezanost između seropozitivnosti, pobačaja i podrijetla (uvezene životinje) junica ili krava (OR = 5,52; P = 0,001)

Ključne riječi: *Neospora caninum*, goveda, pobačaj, rizični čimbenici, Hrvatska
