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## Seroprevalence of *Neospora caninum* in dairy cattle herds in Central Anatolia, Turkey

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### ABSTRACT

A total of 3287 sera collected from cows in thirty-two herds in the eight provinces of Central Anatolia of Turkey were tested for presence of *Neospora caninum* antibodies by using commercial ELISA kit. The overall seroprevalence of *Neospora caninum* was 13.96% (95% CI 13.78 to 16.22). On a provincial basis, *N. caninum* infection was present in all provinces while cows sampled in Kirikkale had the highest rate ( $P < 0.05$ ). Antibodies to *N. caninum* were found in 34 (23.61%) of 144 aborted cows. The prevalence of *N. caninum* was significantly higher in the aborted cows than in non-aborted cows ( $P < 0.001$ ). However, the odds ratio was 1.97, suggesting an association between abortion and seropositivity. This is the first report on serological prevalence of *Neospora caninum* in cows in Central Anatolia, and neosporosis in dairy cattle seems to be widespread in some provinces of this region.

**Key words:** *Neospora caninum*, prevalence, cattle, Central Anatolia, Turkey

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### Introduction

The protozoan parasite *Neospora caninum* has become increasingly recognized as an important cause of abortion in dairy and beef cattle in the past decade. The parasite was described in litters of Boxer puppies in Norway in 1984 (BJERKAS et al., 1984) and recognized in 1988 (DUBEY et al., 1988). Domestic canids are the natural definitive host for *Neospora caninum*. Infected dogs excrete oocysts in their faeces, which may then be ingested by intermediate hosts such as cattle, sheep, goats, deer and horses (DUBEY, 2003).

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There is no direct transmission between cattle. However, the parasite is maintained by congenital transmission and is a major factor in providing persistence of *Neospora* to their offspring. Abortion due to neosporosis may occur over several generations. There is no known effective and economic treatment for bovine neosporosis (DUBEY and LINDSAY, 1996; DUBEY, 2003). However, vaccination of cattle with inactivated *N. caninum* tachyzoites was reported to prevent cattle from abortions (INNES et al., 2002). To date, numerous sporadic and endemic cases have been reported almost worldwide (BUXTON et al., 1997; CAMPERO et al., 1998; DIJKSTRA et al., 2001; PEREZ et al., 1998; WALDNER et al., 2001). In Turkey, prevalence of neosporosis in cows was determined as 8.02% in Thrace (BIYIKOGLU et al., 2005); 7.5% in Sanliurfa (SEVGILI et al., 2005); 9.2% in Sakarya (ONCEL and BIYIKOGLU, 2003). Neosporosis was also reported in dogs in Turkey (COSKUN et al., 2000).

The seropositivity rate of *N. caninum* in cows in Central Anatolia has not been known to date. The objective of this study was to evaluate the significance of neosporosis in cows in this region of Turkey

### Materials and methods

Blood samples were collected from 3287 cows in 8 provinces (32 herds) in Central Anatolia, Turkey (Fig. 1) over a full one-year period (January to December 1998). All of the herds had previous history of abortion, but the reasons for that had not been determined. All animals were lactating cows randomly selected from among adult female cattle. Jugular vein blood was collected in vacutainer tubes and was transferred to the Central Veterinary Control and Research Institute. After centrifugation at 3000 rpm  $\times$  15 min, sera were separated and stored at -20 °C until analysis.

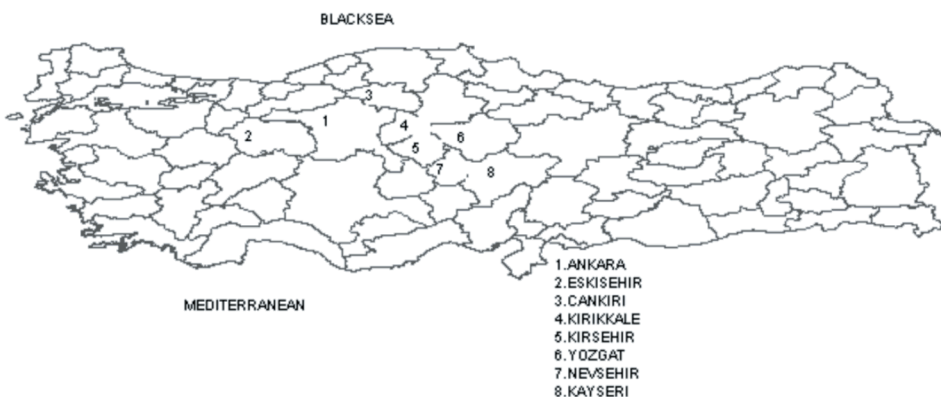


Fig. 1. Map of Turkey, showing sampling provinces.

The serum samples were screened for specific antibodies to *Neospora caninum* with commercially available diagnostic kit (IDEXX Lab. Inc. Westbrook, Maine, USA) using X check software program. All control tests were performed in duplicate. The diluent, wash solution, dilution buffer, and anti-bovine IgG horseradish peroxidase conjugate and substrate were provided by IDEXX. The optical density (OD) values of the wells were read with ELISA reader (Titertek Multiskan Plus MK II), at a wavelength of 650 nm. The presence and absence of antibody to *Neospora caninum* were determined by sample to positive (S/P) ratio for each sample. Samples with an S/P ratio greater than 0.5 were designated as positives.

The prevalence found in seropositive cows in the provinces was compared using Chi-square test (Analytical Software Package, Statistix version 1.0, 1996).

## Results

Antibodies to *Neospora caninum* were detected in 13.96% (95 per cent CI 13.78 to 16.22) of 3287 cows. Seropositive cows (n = 459) were found in 28 (87.5%) herds in 8 provinces. Sera obtained from 4 herds were all negative (Table I). From the 3287 cows sampled, 144 had a previous record of abortion. Of these, 34 were seropositive and 110 were seronegative. The prevalence of *N. caninum* was significantly higher ( $P < 0.001$ ) in the aborted group than in the non-aborted group. Seropositive cows were approximately two times more likely to abort than seronegative cows (odds ratio = 1.97 CI: 1.33, 2.95)

Table 1. Number of herds according to seropositivity rate groups

Seropositivity rate	0	0-10%	10-20%	20-30%	30-40%	40%<
N° of herd (%)	4 (12.5)	14 (43.75)	9 (28.12)	2 (6.25)	-	3 (9.37)
Overall seropositivity (%)	4 (12.5)	28 (87.5)				

The rate of seropositivity in Kirikkale province was higher ( $P < 0.05$ ) compared to findings in other provinces in Central Anatolia (Table 2). The lowest prevalence (5.1%) was recorded among cows from Nevsehir. The statistical differences of seropositive cows present in each province are shown in Table 2.

Table 3 shows the distribution of seropositive cows in the different age groups. Among the 1563 cows in the 2-4 age group, 214 (13.69%) were seropositive, whereas among the 1734 cows above 5 years old, 245 (14.12%) were seropositive. There was no statistical difference between these two groups ( $P > 0.05$ ). Seropositivity was the highest (22.42%) among the 272 cows in the 6-year-old age group.

Table 2. Seroprevalence of *N. caninum* in non-aborted and aborted cows in eight provinces

Province	Non-aborted cows (%)	Aborted cows (%)*	Seropositivity rate in each province (%)**
Ankara	42/426 (9.85)	4/27 (14.81)	46/453 (10.15) <sup>cd</sup>
Cankiri	28/410 (6.82)	1/8 (12.5)	29/418 (6.93) <sup>de</sup>
Nevsehir	18/381 (4.72)	2/11 (18.18)	20/392 (5.10) <sup>e</sup>
Kirsehir	70/384 (18.22)	10/25 (40)	80/409 (19.55) <sup>b</sup>
Kayseri	42/407 (10.31)	4/18 (22.22)	46/425 (10.82) <sup>c</sup>
Kirikkale	133/407 (32.67)	9/27 (33.33)	142/434 (32.72) <sup>a</sup>
Eskisehir	21/381 (5.51)	0/6 (0)	21/387 (5.43) <sup>e</sup>
Yozgat	71 /347 (20.46)	4/22 (18.18)	75/369 (20.32) <sup>b</sup>
Total	425/3143 (13.52)	34/144 (23.61)	459/3287 (13.96)

\*There was significant difference between aborted and non-aborted groups (P< 0.001).

\*\*Among provinces, different superscripts indicate significant difference (P<0.05).

Table 3. Seropositivity related to age of cows

Age groups*	2-4 age group			Above 5 years of age				Total
	≤2	3	4	5	6	7	≤8	
No of sera tested	67	204	1292	1156	272	264	32	3287
No of positives(%)	12 (17.91)	38 (18.62)	164 (12.69)	154 (13.32)	61 (22.42)	30 (11.36)	0	459
No of sera tested in age groups	1563			1724				3287
No of positives in age groups (%)	214 (13.69)			245 (14.21)				459 (13.96)

\*There was no significant difference between two age groups (P>0.05).

## Discussion

Neosporosis has been reported in many countries (KIM et al., 2002; CABAJ et al., 2000; BUXTON et al., 1997; CAMPERO et al., 1998; PEREZ et al., 1998; DIJKSTRA et al., 2001; WALDNER et al., 2001) with different prevalence rates since the disease was recognized in 1988. The prevalence of 13.96% for neosporosis found in this study is higher than that reported for cattle in Canada (9%), Korea (4.1%), but is lower than reported in Poland (15.6%) (WALDNER et al., 2001; KIM et al., 2002; CABAJ et al., 2000). On a provincial basis, the prevalence of neosporosis in cows in Ankara, Kirsehir, Kayseri, Kirikkale and Yozgat provinces in Central Anatolia is higher than those reported previously in the other provinces

of Turkey (BIYIKOGLU et al., 2005; SEVGILI et al., 2005; ONCEL and BIYIKOGLU, 2003). However, all results should be evaluated carefully, because the rates have been obtained by various diagnostic methods under different climatic and geographical conditions.

The cows that were examined in this study belonged to small-scale farms and any small incidence of the disease could affect the farmers' economic situation, although abortion cases are sporadic in Central Anatolia. In the present study, the prevalence of *N. caninum* was higher in the aborted group than in the non-aborted group ( $P < 0.001$ ). Studies (WALDNER et al., 1998; PARE et al., 1998) have indicated that there is a correlation between *N. caninum* seropositivity and abortion rate. THURMOND and HIETALA (1997) reported that the risk of abortion decreased with each subsequent pregnancy and also found that cattle infected at the time of abortion outbreak and their congenitally infected offspring are at increased risk of subsequent foetal loss. The herds in Central Anatolia that showed different prevalence rate of neosporosis have a potential risk of abortion in the future. Recent work (AKCA et al., 2005) in the Kars province of Turkey has suggested that *N. caninum* was introduced to the region by imported cattle and therefore the risk of vertical transmission to fetuses was important. In the present study, a high rate of *N. caninum* was found in some herds, especially in Kirikkale, Yozgat and Kirsehir provinces, which have a large population of imported cattle.

Dogs are a natural definitive host for *Neospora caninum* (MCALLISTER et al., 1998; LINDSAY et al., 1999). Many authors (PARE et al., 1998; LINDSAY et al., 1999) have shown that the presence of farm dogs is a risk factor for *N. caninum*-associated abortion in cattle. Infectious material, like aborted fetuses, dead calves and foetal membranes could be a major source of infection to dogs. The high seroprevalence of neosporosis might be related to the presence of many dogs in the farms sampled in Kirikkale, Yozgat and Kirsehir provinces.

The studies have not shown an association between serological status and cow age (WALDNER et al., 1998; DAVISON et al., 1999). But SANDERSON et al. (2000) determined that seropositivity in cows under three-year old was higher than in cows above six-year old. The results of the present study showed that there was no association between seroprevalence and age ( $P > 0.05$ ).

*N. caninum* is an important cause of reproductive failure in dairy herds. Culling seropositive cows and their offspring has been recommended for the control of neosporosis in herds with a low prevalence of infection. Valuable seropositive cows can be used as breeding stock by embryo transfer to seronegative recipients (DUBEY, 2003). The results of this study confirm the presence of *Neospora caninum* antibodies in cows in Central Anatolia. Finally, we emphasize that further studies are needed to clarify the impact of neosporosis on the animal industry and losses due to clinical neosporosis in livestock in Turkey.

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**SAŽETAK**

Ukupno je sakupljeno 3287 uzoraka seruma krava podrijetlom iz trideset dva stada na području šest pokrajina Središnje Anatolije. Svi su uzorci pretraženi na prisutnost protutijela za vrstu *Neospora caninum* primjenom komercijalnih ELISA kompleta. Ukupna seroprevalencija iznosila je 13,96% (95% CI 13,78 do 16,22). Prisutnost protutijela zabilježena je u krava podrijetlom iz svih pokrajina. Najveća seroprevalencija zabilježena je u krava na području Kirikkale ( $P<0,05$ ). Protutijela za vrstu *N. caninum* potvrđena su u 34 (23,61%) od 144 krave koje su prethodno pobacile. Istraživanjem je uočeno da je seroprevalencija značajno veća u krava koje su pobacile u odnosu na one koje nisu pobacile ( $P<0,001$ ). Odnos je iznosio 1,97 što upućuje na povezanost između pojave pobačaja i seropozitivnosti.

**Ključne riječi:** *Neospora caninum*, prevalencija, govedo, središnja Anatolija, Turska

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