Seroprevalence of *Neospora caninum* in Cattle in Nigde Province, Turkey

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ABSTRACT

This study was carried out in order to investigate the presence of *Neospora caninum* antibodies in local cattle breeds in the Nigde province. The animals used in this study consisted of cattle of at least one year of age slaughtered at a local abattoir in Nigde province of Turkey. Blood samples were obtained from 264 cattle (207 male, 57 female) and serum samples were tested for antibodies to *N. caninum* using commercial competitive ELISA (c-ELISA) (VMRD, Pulman, WA, USA). According to the serological examination, 70 (26.51%) of 264 cattle were seropositive for *N. caninum*. Anti-*N. caninum* antibodies were detected in 63 (30.43%) of 207 male cattle and 7 (12.28%) of 57 female cattle. There was a statistically significant differences in seropositivity between genders ($p < 0.05$). This is the first serologic survey for *N. caninum* antibodies performed on cattle in Nigde province.

Keywords: *Neospora caninum*; Neosporosis; Enzyme-Linked Immunosorbent Assay (ELISA); Seroprevalence; Cattle; Nigde; Turkey.

INTRODUCTION

Neospora caninum is an obligate intracellular protozoan parasite, closely related to *Toxoplasma gondii*. Neosporosis is a major cause of abortion in cattle in many countries (1, 2). *N. caninum* has a heteroxenous life cycle. Dogs are both intermediate and definitive host for *N. caninum*, and intermediate hosts such as cattle, sheep, goats and horse becomes contaminated with oocysts in infected dog feces (3, 4). Dogs become infected with contaminated meat containing tissue cysts of intermediate host animals and at the same time they may infect other dogs with oocysts in their feces (5).

There are three infectious stages of the parasite: tachyzoites, bradyzoites, and sporozoites. Tachyzoites and bradyzoites occur in tissues of infected hosts (intermediate and definitive) whereas sporozoites are present in oocysts that are excreted in the feces of the definitive host. *Neospora caninum* can be transmitted horizontally by ingestion of infected tissues or by ingestion of food or drinking water contaminated oocysts, or it can be transmitted vertically from an infected dam to her fetus during pregnancy (4, 5).

Neosporosis especially causes abortion and neonatal mortality in cattle. Most neosporosis-induced abortions occur at 5-6 month gestation. Fetuses may die *in utero*, be resorbed, mummified, autolyzed, stillborn, born alive with clinical signs, or born clinically normal but chronically infected. Neosporosis-induced abortions occur all year-round (1-3, 6).

In Turkey the seroprevalence of *N. caninum* in cattle have been reported in various studies (7, 8) and the prevalence of *N. caninum* was determined to be 2-37.7%. The aim of the present study was to determine the prevalence of antibodies to *N. caninum* in the cattle in the Nigde province of Turkey.

MATERIALS AND METHODS

Study Area

This study was performed on local cattle breeds of Nigde province, situated in central-Turkey (with an altitude of

1240 m, 37˚58′ N longitude-34˚41′ E latitude), where neosporosis has not previously recognized. The province has a continental climate with warm and dry summers and cold and snowy winters. Annual average precipitation is 348.8 mm, average temperature is 11.1˚C and average relative humidity is 55%.

Sampling of Cattle and Blood Collection

This study was carried out to detect the seroprevalence of *Neospora caninum* on cattle of at least one year of age slaughtered at a local abattoir of Nigde. A total of 264 cattle which consisted of 57 female and 207 male were selected randomly. Blood samples were collected from the jugular vein of these cattle into sterile tubes. Serum samples were obtained by centrifugation at room temperature (25˚C), at 4,000 rpm for 10 minutes and were stored at -20˚C until analyzed.

Serologic Examination

The serum samples were tested for antibodies to *N. caninum* using commercial competitive ELISA (c-ELISA) (VMRD, Pulman, WA, USA). The test was performed according to the procedure described by the manufacturer and the results were assessed at a wavelength of 630nm using an ELISA reader (spectrophotometer). The formula used for calculation of percent inhibition:

% I = 100 - (Sample O.D. x 100) ÷ (Mean Negative Control O.D.)

Where the test sample showed ≥ 30% inhibition, it was considered as positive. Where a test sample showed < 30% inhibition, it was considered as negative.

Statistical Analysis

In this study, chi-square test was used to compare the seropositivity rates in terms of gender. Statistical significance was considered at $p < 0.05$.

RESULTS

According to the serological examination, 70 (26.51%) out of 264 cattle were seropositive for *N. caninum* (Table 1). Anti-*N. caninum* antibodies were detected in 63 (30.43%) of the 207 male cattle and 7 (12.28%) of the 57 female cattle. There was a statistically significant differences in seropositivity between genders with a higher proportion of male cattle being infected compared to females ($p < 0.05$).

Table 1: The seropositivity of Neospora caninum in cattle with ELISA according to gender

Gender	Number of Sera Examined	Number of seropositivity sera	Seropositivity (%)
Female	57	7	12.28
Male	207	63	30.43
TOTAL	264	70	26.51

DISCUSSION

Neospora caninum is one of the most important causes of bovine abortion and neonatal mortalities (1, 2). Studies have shown that seropositive animals are up to 3.5 times more likely to abort than seronegative herd-mates (9). Economic loss associated with *N. caninum* infection is not only due to increased prevalence of abortions, but also to decreased milk and meat production in infected cattle (10). Due to clinically insufficient symptoms various serological tests (ELISA, IFAT and DAT) have been used to detect antibodies to *N. caninum* and identify infected animals (2, 3). *Neospora caninum* infections have been reported from most parts of the world and the worldwide seroprevalences of *N. caninum* in cattle are summarized in Table 2.

When the *N. caninum* seroprevalence results of the study are compared with the results of the studies conducted around the world, *N. caninum* prevalence rate of this study was lower than Argentina (11), Spain in dairy cattle (12), Portugal (13), Mexico (14), in Georgia (15) and Romania (16). On the other hand, seroprevalence result of our study was higher than the results obtained in Southern Vietnam (17), in Wales and England (9), in Spain in beef cattle (12), in Brazil (18), in Northern Alberta (19), in Korea (20), in Texas (15), in Northwest Iran (21) and in South Australia (22). The frequency differs depending on the geographical location, breeding conditions and type of serological tests.

In Turkey, seropositivity has been reported with ELISA as 9.2% in Sakarya (23), 7.01% in Eastern Anatolian region (24), 13.96% in Central Anatolia (25), 8.02% in Thrace (26), 2%, 7.2% in Kars (7, 27), 7.5% in Şanlıurfa (28), 7% in Kayseri (29), 8.19% in Elazığ (30), 4.88% in Van (31), 10.65% in Eastern Turkey (32), 21.03% in Afyonkarahisar (33), 37.7% in Kırıkkale, Aksaray, Burdur (8), 35.07% in Central Veterinary Control and Research Institute (34), 10.77% in Kırıkkale, İzmir, Tokat (35) and 5.3% in Burdur (36). Furthermore, seroprevalence of *N. caninum* in cattle has been determined as 10% by using Immunocomb (IC)

Table 2: Select reports of prevalence of Neospora caninum in cattle

Country	Prevalence %	Test	Author	Reference
Argentina	88.8 (in aborting cattle)	IFAT	Campero *et al.*, 1998	11
Southern Vietnam	5.5 (in dairy cattle)	ELISA	Huong *et al.*, 1998	17
Brazil	14.09 (in dairy cattle)	IFAT	Gondim *et al.*, 1999	18
Spain	17.9 (in beef cattle) 36.8 (in dairy cattle)	ELISA	Quintanilla-Gozalo *et al.*, 1999	12
Wales and England	12.5 (in dairy cattle)	ELISA	Davison *et al.*, 1999	9
Portugal	49 (in dairy cattle)	ELISA	Thompson *et al.*, 2001	13
Northern Alberta	9 (in beef cattle)	ELISA	Waldner *et al.*, 2001	19
Mexico	59 (in dairy cattle)	ELISA	García-Vázquez *et al.*, 2002	14
Korea	4.1 (in beef cattle)	IFAT	Kim *et al.*, 2002	20
Georgia and Texas	32.1% (in dairy cattle) 10.3% (in dairy cattle)	WESTERN BLOT	Ortega *et al.*, 2007	15
Romania	34.6 (in dairy cattle)	ELISA	Gavrea *et al.*, 2011	16
Northwest Iran	10.5 (in dairy cattle)	ELISA	Nematollahi *et al.*, 2011	21
South Australia	2.5 (in beef cattle) 3.8 (in dairy cattle)	ELISA	Nasir *et al.*, 2012	22

assay in Ankara (37) and 19% by IFAT in Western Turkey (38).

In the present study, seroprevalence of *N. caninum* was found to be 26.51% in cattle from Nigde in Turkey. The rate of seroprevalence of this study has been shown to resemble results of research conducted by Celik *et al.* (33). However, the seroprevalence results of our study are higher than the results of studies carried out in cattle from different parts of Turkey (7, 23-32, 35-38) while it is lower than the results obtained by Öcal *et al.* (8) and Piskin and Utuk (34). The different prevalence rates of disease may be associated with the different geographical locations, management practices and type of the serological tests used.

In this study, the cattle were classified according to gender and *N. caninum* antibodies in the male cattle were more prevalent than those in the female cattle. The differences between the genders for the presence of antibody may be due to factors such as age of the animals and different breeding conditions.

From a prophylactic point of view, the removal of all potentially infected tissues, such as aborted fetuses and placentas from the environment, that might serve as a source of infection for susceptible hosts is advisable. In addition, fecal contamination of feed and water sources by potential host animals should be minimized (3-5).

This is the first serologic survey for *N. caninum* performed on cattle in Nigde province which detected a seroprevalence of *N. caninum* as 26.51%. Therefore, neosporosis should be taken into consideration in abortion cases and neonatal deaths. In addition, further investigations, including molecular techniques are required in order to determine the importance of this parasite as a cause of clinical disease in dogs which are definitive hosts and of abortion in cattle in Nigde province.

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