Seroprevalence of *Neospora caninum* Antibodies in Cattle in Eastern Turkey

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ABSTRACT

Neosporosis is a disease caused by an intracellular protozoon *Neospora caninum* and is one of the most important causes of bovine abortion. This study was undertaken to detect *N. caninum* seroprevalance in Erzurum, the largest province of Eastern Turkey using Enzyme Linked Immunosorbent Assay (ELISA). A total of 385 cattle sera including 47 previously aborted cow sera of different ages and breed were collected between June and December 2009. The anti-*N.caninum* antibodies were tested by competitive ELISA kits. As a result, 41 of 385 (10.65%) cattle were found to be seropositive while this rate was 21.27% (10/47) in aborted cows.

Keywords: Cattle, Eastern Turkey, ELISA, *Neospora caninum*

INTRODUCTION

Neospora caninum, an important cause of parasitic abortion in cattle, was first observed in 1984 in a Norwegian dog showing symptoms of encephalomyelitis and myositis (1). Dogs are the definitive host for the biological life-cycle of the parasite, while cattle, sheep, deer, and horses act as intermediate hosts. It has been reported that the disease spreads among cattle by horizontal and vertical means. It has also been reported that horizontal infection in cattle occurs through intake of food and water contaminated with oocysts spread from fecal material of definitive hosts. Similarly, transplacental infection occurs during pregnancy, inducing abortion (2, 3). Neosporosis is not considered to be passed during mating. In cows, the only clinical observation is abortion earlier on during pregnancy, although cows may present stillborn or live calves after full term. The prominent clinical sign observed in this species is central nervous system disorders (4).

It has been reported that transmissions in carnivores occur due to intake of cystic forms of the parasite through infected tissues. Aborted fetuses, placental and uterine material are the most common source of infection for dogs (3, 5, 6). Cattle neosporosis was first reported as epidemic abortion in a dairy cattle farm in 1987 in Mexico (7). *N. caninum* was reported to be the cause of majority of abortion cases throughout the world, particularly during recent years (8).

Hobson *et al.* (9) and Romero *et al.* (10) showed that *N. caninum* causes abortion in cattle, as well as reducing meat and milk yield and therefore leading to substantial economic losses.

The disease is generally diagnosed in cattle clinically or serologically, by pathological findings, immuno-histochemical methods, tissue culture and molecular biological techniques. To detect antibodies specific for *N. caninum* tachyzoites, antigens are examined using serologic tests, such as ELISA and IFAT (11-15).

Several surveys have been conducted in different regions of Turkey to determine the prevalence of neosporosis in cattle using various techniques. The seropositivity of *N.caninum* in cattle has been found between 5 to 33% in the central Anatolian Region of Turkey (16), 7% in the Elazig, Malatya,

Mus and Bingol cities (17), 7% in the Kayseri region (18), 2% in the Kars region (19).

Seroprevalence of neosporosis was found to be 56.9% in Argentina (20), 12.5% in Wales (21), and 15.5% in Poland (22).

The study objective were to determine *N. caninum* seroprevalence in cattle, an important cause of abortion in the Erzurum region, using a commercially competitive ELISA (c-ELISA) kit.

MATERIALS AND METHODS

Sample collection

Blood serum samples were collected from grazing cattle of Erzurum and its adjacent districts. Each village of the districts was considered to be an epidemiological unit. Blood samples of 385 cattle in total were collected from various age groups in representative villages around Erzurum and districts using a randomized sampling method. In view of information provided by cattle owners, 47 of the sample group had previous abortion history. Sample size was calculated with a confidence interval of 95% considering the expected prevalence of the disease is 10±3%.

Blood samples of 5 ml were collected from vena jugularis of cattle into vacuum serum tubes, and then transferred to the laboratory. Serum as separated by centrifugation and the samples stored at -20°C until tested.

Serologic examination

Commercially competitive ELISA kit (c-ELISA; VMRD Inc., USA) was employed to test *N. caninum* seroprevalence. The test was carried out in the Parasitology Laboratory of Erzurum Veterinary Control and Research Institute. Serum was processed as recommended by the manufacturer and read at 630 nm wavelength in an ELISA reader (ELX 800 UV, Universal Microplate Reader, Bio-Single Instruments, Inc). Samples causing ≥30% inhibition were regarded as positive. Samples causing <30% inhibition were considered as negative.

The formula used for calculating percentage inhibition: %I = 100 - [(Sample OD/Mean Negative OD) × 100].

Chi-square (X^2) test was used for statistical analyses of the results. Statistical significance was considered at $p<0.05$.

RESULTS

Fourty one of 385 cattle serum samples (10.65%) showed antibodies against *N. caninum*, whereas 344 (89.35%) were seronegative using ELISA. Distribution of seropositivity by study centers is presented in detail in Table 1. Based on the study results, the highest seroprevalence was observed in Koprukoy (46.66%), while no antibodies were found in cattle serum obtained from districts Narman, Uzundere, Hinis, Cat, Tekman, Askale and Pazaryolu. There was a statistically significant difference between districts ($P<0.01$).

Table 1. Distribution of seropositivity according to epidemiology units.

Origin (district)	Animals (No.)	Positives (No.)	Percentage (%)
Narman, Uzundere, Hinis, Cat, Tekman, Askale, Pazaryolu	129	0	0
Olur	16	1	6.25
Karacoban	8	1	12.50
Pasinler	21	1	4.76
Karayazi	27	4	14.81
Koprukoy	15	7	46.66
Aziziye	24	4	16.66
Tortum	18	1	5.55
Horasan	29	11	37.93
Ispir	29	3	10.34
Oltu	25	3	12.00
Yakutiye	15	2	13.33
Palandoken	11	2	18.18
Senkaya	18	1	5.55
Total	385	41	10.65

$p<0.01$

The same study also observed seropositivity in 10 of 47 cows (21.27%), which previously had aborted. Seropositivity results for aborting and non-aborting cattle are shown in Table 2. There was a statistically significant difference between the rate of seropositivity among aborting animals (21.27%) and that of non-aborting ones (9.17%); ($P<0.01$).

Evaluating the results with respect to age, seropositivity was observed in 13.04% of cattle younger than a year, in 9.43% of cattle of 1 to 3 ages, and in 10.67% over 3 years of age. The correlation between age groups and percent of pos-

Table 2. Seropositivity rates of aborted and non-aborted cattle

	Animals (No.)	Positives (No.)	Percentage (%)
Aborted	47	10	21.27
Not Aborted	338	31	9.17
Total	385	41	10.65

$p<0.01$

Table 3. The distribution of seropositivity according to age groups

Age (year)	Animals (No.)	Positives (No.)	Percentage (%)
≤1	23	3	13.04
1≤3	53	5	9.43
>3	309	33	10.67
Total	385	41	10.65

p<0.01

itivity is illustrated in Table 3, and the difference observed among age groups was found to be statistically significant (P<0.01).

DISCUSSION

Neospora caninum is an important protozoon leading to serious loss in yield, such as abortion, stillborns, postnatal breed death, infertility and low milk yield (23). Neosporosis was determined first in 1984 in Norway in a dog, and then found in cattle in 1987 in Mexico during an epidemic abortion (1, 8, 24). Serologic tests including IFAT and ELISA are commonly used to determine antibody titers specific to *N. caninum*. *Neospora caninum* tachyzoites obtained from tissue cultures are employed as antigens in such serologic tests. ELISA was reported to be more specific than IFAT (23) and therefore, the ELISA was used during the study.

Seroprevalence of neosporosis has been determined in many countries. Seropositivity in dairy farms was found to be 31% in Spain (25); 49% in Portugal (26); 30% in Australia (27); 59% in Mexico (28) and 16% in Poland (29), while seroprevalence was determined in beef cattle at a rate of 4% in Korea (30); 9% in Canada (31) and 12% in Mexico (32). Various serologic studies were carried out in different regions of Turkey and it has been observed that seroprevalence rate varied from 2 to 32.72% (16-19, 33).

In this study, 385 blood samples were collected from the villages considered as epidemiological units in 20 districts of Erzurum. The study performed with c-ELISA revealed that 41 cattle had antibodies formed against *N. caninum* versus 344 cattle showing seronegativity. When comparing our results with other studies in various provinces, it was observed that seropositivity was higher than findings in Elazig, Kayseri, and Kars. The fact that this study revealed higher results than other provinces can be attributed to various factors, such as presence of a dog almost in every residence and feeding the dogs with aborted fetuses and fetal membranes, based on the information provided by cattle owners.

Neospora caninum has been reported to lead to neonatal death and abortion in cattle (6, 23, 34). A study conducted in Kayseri (18) reported that 3 of 9 aborting cows (33.3%) were seropositive, whereas Aktas *et al*. (17), showed that the seropositivity rate among aborting cows was 3.12%. In our study, 47 of the serum samples originated from cows with previous abortion history. Serological findings in our study resulted in 10 of 47 aborting cows (21.27%) showing anti-*N.caninum* antibodies.

There are various possible explanations for the association between seropositivity in neosporosis and the age of the animal. Some studies have reported a correlation between age and infection (12, 35), while others suggest that no such correlation exists (36, 37). Sevgili *et al*. (24) reported that the seropositivity rate was 8.7% among the age group of 2-4 years versus 5.4% in cattle of 5 years or more in their study carried out using ELISA on cattle of the Sanliurfa region. Our study separated samples into 3 groups based on age, and found a significant difference in seropositivity between the age groups. Similar results were also obtained from the study by Sevgili *et al*. (24).

Breaking the biological cycle is considered an important combat measure to prevent recurring abortions due to neosporosis, which leads to substantial economic losses. Dogs, the final host of the parasite, are present in almost every house living on cattle ranches in Erzurum. Therefore, proper disposal of dog feces should be followed, and dogs should not be allowed exposure to stalls and pastures. It is also important to emphasize that contaminated aborted material should not be allowed to be consumed by dogs, but disposed through burial, as the ingestion of aborted material by dogs is an important means of transmitting the disease.

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