

The First Report of Seropositivity for *Neospora caninum* in Sheep from Turkey

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ABSTRACT

This study was aimed at determining the seroprevalence of *Neospora caninum* infection in sheep raised in Kars province and its vicinity in Turkey. Three-hundred-and-seventy-six sheep of varying ages and breeds, which were randomly selected from 5 foci located in the Kars region (Kars central district, and the Arpaçay, Kağızman, Selim and Susuz districts). The serum sample of each animal was tested for the presence of antibodies against *N. caninum* using the ELISA test. The test results demonstrated that, out of the 376 sheep included in the study, 8 (2.1%) were seropositive for antibodies against *N. caninum*. The highest seropositivity rate (8.9%) was detected in the central district of Kars province, while the second highest seropositivity rate (1.4%) was determined in the Susuz district. No anti-*Neospora caninum* antibodies were detected in the serum samples of the animals raised in the Arpaçay, Kağızman and Selim districts. The seroprevalence of neosporosis in sheep raised in the Kars region was determined to be 2.1%. This is the first report of seropositivity for *N. caninum* in sheep from the Kars region and Turkey.

Keywords: *Neospora caninum*; Seroprevalence; Sheep; Turkey; Kars

INTRODUCTION

Neospora caninum is a heteroxenous obligate intracellular parasite (1, 2). The definitive hosts of *N. caninum* are the dog, coyote and fox (3, 4). The intermediate hosts of the parasite are cattle, sheep, goats, deers, and horses. While *Neospora* causes neurological disorders in dogs and horses, it leads to abortions in cattle, sheep, goats and deers. Three infective forms exist in the life-cycle of *N. caninum*, namely, the tachyzoites, the bradyzoites found with in the tissue cysts, and the sporozoites found with in the oocysts (5). The definitive host becomes infected by ingesting food and water contaminated with sporulated oocysts, and dogs become infected by ingesting the cysts in the muscle tissue of the intermediate hosts or by ingesting food contaminated with

sporulated oocysts (3, 6, 7). Furthermore, aborted fetuses, placentae and uterine waste are the most common sources of infection for the definitive hosts of the parasite (5, 8, 9).

N. caninum infections are diagnosed on the basis of clinical findings, pathological findings and serological test results. As the infection has a sub-chronic course in sheep and presents with clinical findings resembling those of several other diseases, the diagnosis of neosporosis in sheep is difficult. For this reason, immunohistochemical methods (10), serological tests (11-13) and molecular biological techniques are used to diagnose the disease (14, 15). Among serological tests, ELISA has found common use in serological analyses, as it produces results within a short period of time (11, 16, 17).

Although there are many research reports related to infec-

tions of cattle and dogs, there is limited knowledge on sheep (18–23). In Turkey, studies were conducted on more cattle (24–28), but there is limited knowledge on dogs (18, 25, 29), goats (30–32), horses (33, 34) and wild boars (35). There is a dearth of studies on the presence of sheep neosporosis. Similarly there have been some serological studies (25, 27, 36) about dogs and cattle neosporosis in the Kars region, however there is little information about neosporosis in sheep. The aim of the study is determine seroprevalence in sheep with neosporosis in Turkey in the Kars region.

MATERIALS AND METHODS

Study Site and Animal Material

This study was conducted between January and May in 2013, in Kars province and its vicinity, in Turkey. The Kars region, located in north-eastern Turkey (43.05° E and 40.36° N), is mountainous and has a cold climate. In total, five foci were selected to represent the Kars region (Kars central district, and the Arpaçay, Kağızman, Selim and Susuz districts). Three-hundred-and-seventy-six sheep, varying from six months to five years of age and of the Akkaraman, Morkaraman and Tuj breeds, which were randomly selected from the 5 foci, constituted the material of the study. This study was approved by the Animal Ethical Committee of Mehmet Akif Ersoy University (30.04.2014/79).

Collection of Samples

Ten-ml blood samples were collected from the jugular vein of each of the 376 sheep into sterile vacuum tubes. The tubes were identified with unique protocol numbers and transported to the laboratory. The blood samples collected from the sheep were centrifuged at 3000 rpm for 10 minutes for the extraction of serum. The sera were transferred into 1.5-ml plastic tubes, labelled for identification, and stored at -20 °C in a deep freezer, until being tested.

Enzyme-linked Immunosorbent Assay (ELISA)

A commercial ELISA kit (Che kit *Neospora caninum* Antibody ELISA test kit, IDEXX Laboratories, Berne, Switzerland) was used to detect the presence of anti-*N. caninum* antibodies in the serum samples. The ELISA test was performed in accordance with the instructions of the manufacturer of the kit. The absorbance of the colour change, resulting from the reaction, was read at a wave length of 450

nm using an automated ELISA reader (Molecular devices SPECTRAMax PLUS-384, Sunnyvale, California, USA), and the measured values were recorded.

Interpretation of the Test Results

The test results were calculated as percentage (%) inhibition values, using the formula below:

$$\% \text{ Inhibition} = \left[\frac{(\text{Optic Density (OD) of the Sample} - \text{OD of the Negative Control})}{(\text{OD of the Positive Control} - \text{OD of the Negative Control})} \times 100 \right]$$

According to the calculations made using this formula, if the percentage inhibition value of the tested sample was <30%, the result was considered to be negative. If the resulting inhibition varied between 30–39%, the result was considered to be uncertain, and if the inhibition was found to be >40%, the test result was considered to be positive.

The statistical evaluation of the seropositivity rates determined for the sheep with respect to the different age groups, breeds and study sites (foci) was made using the SPSS 10.1 statistical software package and with the chi-square (χ^2) test. *P* values smaller than 0.05 ($P < 0.05$) were considered to be statistically significant.

RESULTS

Out of the 376 sheep tested by ELISA in the Kars region, 8 (2.13%) were determined to be seropositive for antibodies against *N. caninum*. The comparison of the 5 foci, where the study was carried out, showed that the highest seropositivity rate was in the central district of Kars province (8.86%), followed by the Susuz district (1.35%). No antibodies were detected in the serum samples of the sheep raised in the Arpaçay, Kağızman and Selim districts (Table 1). Differences between the seropositivity rates for *N. caninum* with respect to the study sites were found to be statistically significant ($P < 0.05$).

When evaluated for the different age groups, the highest seropositivity rate was detected in sheep aged 1 to 3 years (2.60%) and the lowest seropositivity rate was determined in sheep older than 3 years of age (0.96%), (Table 2).

When evaluated for the different sheep breeds, the highest seropositivity rate was detected in the Tuj sheep (8.86%), while seropositivity was not detected in any of the Akkaraman sheep (Table 3). The seropositivity rate of the Tuj

Table 1. The seroprevalence of *N. caninum* in sheep with respect to the different foci

Study Site (Focus)	Number of tested animals	Number of seropositive animals	Number of suspect animals	Seropositivity (%)
Kars Central District	79	7	1	8,86 ^b
Arpaçay	70	0	0	0
Kağızman	72	0	0	0
Selim	81	0	0	0
Susuz	74	1	0	1.35 ^a
Total	376	8	1	2.13

a, b: Differences between the study sites shown with different superscripts are statistically significant ($P < 0.05$).

Table 2. The seroprevalence of *N. caninum* in sheep with respect to the different age groups

Age Group	Number of tested animals	Number of seropositive animals	Number of suspect animals	Seropositivity (%)
<1	118	3	1	2.54
1-3	154	4	0	2.60
3>	104	1	0	0.96
Total	376	8	1	2.13

$P > 0.05$

Table 3. The seroprevalence of *N. caninum* in sheep with respect to the different breeds

Breed	Number of tested animals	Number of seropositive animals	Number of suspect animals	Seropositivity (%)
Akkaraman	70	0	0	0
Morkaraman	227	1	0	0.44 ^a
Tuj	79	7	1	8.86 ^b
Total	376	8	1	2.13

a, b: Differences between the breeds shown with different superscripts are statistically significant ($P < 0.05$).

breed was significantly higher than that of the Morkaraman and Akkaraman breeds ($P < 0.05$).

DISCUSSION

The sero-diagnosis of neosporosis in cattle, dogs, sheep, goats and horses has been investigated in many countries across the world. Research has also been carried out on neosporosis in cattle, goats, horses, dogs and wild boars in different regions of Turkey, including the Kars region (24-36). However, to the authors' knowledge, ovine neosporosis has not been

investigated before either in the Kars region or in any other part of Turkey. Therefore, the present study is the first report of neosporosis in sheep from Turkey.

Many researchers have been carried out worldwide on ovine neosporosis, and the study with the closest proximity to Turkey was conducted in the Mosul province of Iraq and demonstrated a seroprevalence of 12.2% for *N. caninum* in sheep (37). Seropositivity for neosporosis in sheep populations was reported as 1.70% in Iran (23) and as 27.7% in Pakistan (38). In a study conducted in Britain and Wales, out of 660 sheep presenting with abortion, only 3 (0.45%) were determined to be seropositive using the direct ELISA method and immunofluorescence antibody test (IFAT), and it was suggested that the exposure of sheep to *N. caninum* in their natural environment occurred rarely (11). Seropositivity rates in sheep were reported as 1.8% in the Rio Grande do Norte region of Brazil, based on IFAT results (39) and as 3% in the Humid Pampa region of Argentina (40). Furthermore, seropositivity was determined to be 2% in Italy (41) and 0.62% in New Zealand (42). In the present study, only 8 out of 376 sheep (2.13%) having been determined to be seropositive, is in parallel with the findings of some literature reports, but on the other hand indicates a seropositivity rate lower than that reported in some other studies. The differences observed between these studies were attributed to these researches having been conducted in different countries, as well as to the differences in the type of serological test applied, the numbers and breeds of the tested sheep, and the numbers of the definitive hosts, namely, the dogs.

When evaluated for the five different foci, the highest seropositivity rate was determined in the central district of Kars province (8.86%), while no antibodies were detected in the serum samples of the sheep raised in the Arpaçay, Kağızman and Selim districts. The difference observed between the study foci for seropositivity was considered to arise from the possible contamination of the grazing area or feed, or from the number of infected dogs roaming in the site. In previous research conducted in the Kars region with an aim to determine the seropositivity rates of cattle for *N. caninum*, seropositivity was ascertained as 2% by Akça *et al.* (36) and as 7.2% by Mor and Akça (25). The seropositivity results obtained for sheep in the present study are similar to those reported by Akça *et al.* (36) and lower than those reported by Mor and Akça (25).

In a study carried out in cattle raised in the Kars region, Akça *et al.* (36) determined that, the seropositivity rates

detected in cattle of the Simmental breed were higher than the seropositivity rates detected in the local cattle breeds. Similarly, in their research on the seroprevalence of *N. caninum* in sheep and goats, Nasir *et al.* (38) demonstrated that seropositivity rates were significantly higher in short and thin-tailed sheep. These results suggest that, in some animal breeds, neosporosis may occur at higher rates. In the present study, 7 out of the 79 Tuj sheep (8.86%), and only 1 out of the 227 Morkaraman sheep (0.44%) were found to be seropositive, while all of the 70 Akkaraman sheep were confirmed to be seronegative for *N. caninum*. Accordingly, the seropositivity rate of the Tuj sheep being significantly higher than that of the other sheep breeds included in the study suggests that the Tuj breed could be more susceptible to neosporosis.

While some researchers have reported age to be influential on seroprevalence (21), some other researchers have indicated age not to have such an effect (13, 30). Abo-Shehada and Abu-Shehada 2010 (43) suggested that seroprevalence increases with age, owing to an increased rate of ingestion of sporulated oocysts, as a result of the horizontal transmission of the infection, with age. In the present study, the evaluation made for the different age groups demonstrated the highest seropositivity rate to have occurred in 1-to 3-year-old sheep, yet no statistically significant difference was observed between the different age groups for seropositivity ($P>0.05$).

In conclusion, the seroprevalence of neosporosis in sheep raised in the Kars region was determined to be 2.13%. This result demonstrates, for the first time, the presence of *N. caninum* in sheep from the Kars region, and from Turkey, on the basis of serological analysis. Further research is required to determine the prevalence of *N. caninum* in Turkey in different regions of the country.

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