

# Prevalence of Anti-*Neospora caninum* Antibodies in Sheep in Nevşehir Province, Turkey

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## ABSTRACT

This study was carried out in order to investigate the presence of anti-*Neospora caninum* antibodies in sheep in the Nevşehir province in Turkey. Blood samples were taken from randomly selected 180 sheep, which were between 1 and 7 years of age and from different locations in Nevşehir province. All of the serum samples were ELISA tested for *N. caninum* antibodies. Fourteen (7.8%) of serum samples were found to be seropositive for *N. caninum* antibodies by using ELISA. According to the age, antibodies to *N. caninum* were found in six (7.7%) of 78 sheep at the age of 1-2 years of age, in four (7.3%) of 55 sheep at the age of 3-4 years of age, and in four (8.5%) of 47 sheep at the age of 5-7 years of age. Anti-*N. caninum* antibodies were detected in 13 (8.0%) of 162 ewes and 1 (5.6%) of 18 rams. Moreover, 34.8%, the highest seropositivity rate in this study was found in the central regions of Nevşehir, while the no antibodies were detected in Kozaklı, Acıgöl, Hacıbektaş and Derinkuyu regions. The seropositivity rates of *N. caninum* were not statistically significant with regard to age groups ( $P=0.342$ ), gender ( $P=0.261$ ) and in relation to the study centers ( $P=0.173$ ). This is the first serologic survey for *N. caninum* antibodies performed on sheep in Nevşehir province.

**Keywords:** ELISA; *Neospora caninum*; Nevşehir; Seroprevalence; Sheep; Ovine; Turkey.

## INTRODUCTION

*Neospora caninum* causes abortion and stillbirths in sheep, encephalitis in neonatal lambs and decrease in birth weight. In addition to the direct economic losses associated with abortion, some indirect losses such as treatment costs of infected animals, loss of meat and milk yield and disposal of aborted sheep are also important (1, 2).

*N. caninum* is an obligate intracellular protozoan parasite with a heteroxenous development. Animals such as cattle, sheep, goats, deer and horses, which are the intermediate hosts of *N. caninum* are infected with oocysts from dogs' faeces. In dogs, the definitive host, infection occurs when eating meat containing tissue cysts of intermediate host animals, as well as oocysts that they ingest from their own faeces (1, 3-5).

The biological cycle of neosporosis is similar to

*Toxoplasma gondii* and includes three infective stages: tachyzoite, bradyzoite and sporozoite. Tachyzoites and bradyzoites are found in the tissues of infected intermediate hosts and definitive hosts, while sporozoites are found in oocysts excreted by feces from definitive hosts (1, 6).

Studies to determine the presence of *N. caninum* in Turkey have been conducted especially on cattle. From these studies *N. caninum* seropositivity using ELISA was found to be 13.9% (7) in Central Anatolia, 8.0% in Thrace (8), 2.0% in Kars (9), 7.5% in Şanlıurfa (10), 7.0% in Eastern Anatolia (11), 9.2% in Sakarya (12), 7.0% in Kayseri (13) and 26.5% in Niğde (14).

In Turkey, the studies related to neosporosis have been reported on cattle, goat and horse, but there is a dearth of data on the seroprevalence of *N. caninum* in sheep and has only

been found in single study. In the latter study, neosporosis seropositivity was determined by ELISA in 8 (2.1%) of 376 sheep in Kars region (15).

Since the clinical signs of neosporosis in sheep are insufficient for diagnosis, the presence of *N. caninum* antibodies has been found useful using a number of serological assays. The aim of this study was to investigate sheep for neosporosis antibodies in Nevşehir province, where sheep breeding is intensive with a large dog population.

## MATERIALS AND METHODS

### Study Area

This study was conducted between February-August 2010, with 180 sheep which were randomly selected. The sheep were 1-7 years of age originating from seven different districts (Gülşehir, Nevşehir Central, Kozaklı, Acıgöl, Avanos, Hacıbektaş, Derinkuyu) in the Nevşehir province.

### Field Study

In this work, the different study sites were visited once a month for a period of the study period and blood samples were collected from the sheep. Blood samples were assigned protocol numbers and transported to the laboratory.

### Laboratory Analysis

Blood samples were centrifuged for 10 minutes at 3000 rpm and sera were obtained from each animal, placed in 1.5 ml microtubes, and kept in -20°C until serological examinations were performed by ELISA test.

### Enzyme Linked Immunosorbent Assay Test (ELISA)

Blood sera were examined by ELISA and seroprevalence were determined. The commercial competitive ELISA (cELISA) kit was obtained from the VMRD Inc., Veterinary Medical Research & Development, Pulman, WA, USA. The ELISA test was performed according to the procedure described by the manufacturer.

### Reading and calculation of results

Immediately after the addition of the stop solution, the microtiter plates were read on an ELISA reader (MR-96A, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China) at a wavelength of 620nm. The formula used for calculation of percent inhibition:

$$\% I=100 (1- (\text{Sample O.D.} \div \text{Negative Control O.D.}))$$

Using this formula where the test sample showed  $\geq 30\%$  inhibition, the result was considered positive. When the test sample showed  $<30\%$  inhibition, it was considered as negative.

### Statistical Analysis

The chi-square test was applied to compare the seropositivity rates in terms of gender, age groups and study centers.

## RESULTS

It was determined that 14 (7.8%) of 180 sheep in Nevşehir province were seropositive for *N. caninum* antibodies by ELISA. The seropositivity of neosporosis in sheep by age was found to be 7.7% (6/78) for sheep of 1-2 years of age, 7.3% (4/55) for sheep of 3-4 years of age and 8.5% (4/47) for sheep between 5-7 years of age (Table 1). We found no significant difference in seropositivity rates for *N. caninum* among the age groups ( $P=0.342$ ).

In terms of gender, seropositivity was detected in 13 (8.0%) of 162 sheep, while seropositivity was detected in only 1 (5.6%) of 18 rams (Table 2). There was also no statistically significant difference in seropositivity between males and female sheep ( $P=0.261$ ).

The highest seropositivity rate of *N. caninum* was found to be 34.8% in central region of Nevşehir, followed by the Avanos (16.0%) and Gülşehir (5.3%). No seropositivity in sheep examined was detected in Kozaklı, Acıgöl, Hacıbektaş and Derinkuyu (Table 3). There was no statistically significant difference ( $P=0.173$ ) between the study centers for *N. caninum*, although the seropositivity in central region of Nevşehir was notably higher than those in other study sites.

## DISCUSSION

Seroprevalence of sheep to neosporosis has been reported in several countries of the world. *N. caninum* seropositivity was detected as 0.4% in England and Wales by ELISA and IFAT (16); 10.3% in Switzerland by IFAT; (17), 9.2% in Brazil by IFAT (18); 2.0% in Italy by ELISA (19), 0.6% in New Zealand by ELISA and IFAT (20), 17.1% in Brazil by IFAT (21), 56.6% in Jordan by ELISA (22), 8.1% in Brazil by IFAT (23), 12.2% in Iraq by ELISA (24), 27.7% in Pakistan by ELISA (25), 16.8% in Greece by ELISA (26), 3.0% in Argentina by IFAT (27), 5.5% in Mexican by ELISA (28), 34.3% in Spain by ELISA (29), 1.1% in aborted sheep and 1.7% in healthy

**Table 1:** The seroprevalence of *Neospora caninum* in the sheep in terms of age

Age	Number of tested sheep	Number of seropositivity sera	Seropositivity (%)
1-2	78	6	7.7
3-4	55	4	7.3
5-7	47	4	8.5
Total	180	14	7.8

(P=0.342)

**Table 2:** The seropositivity of *Neospora caninum* in sheep with ELISA in terms of gender

Gender	Number of tested sheep	Number of seropositivity sera	Seropositivity (%)
Female	162	13	8.0
Male	18	1	5.6
Total	180	14	7.8

(P=0.261)

**Table 3:** The seropositivity of *Neospora caninum* in the sheep in terms of study centers

Study centers	Months	Number of serum samples	Number of positive samples	Seropositivity (%)
Gülşehir	February	38	2	5.3
Nevşehir Central	March	23	8	34.8
Kozaklı	April	34	0	-
Acıgöl	May	30	0	-
Avanos	June	25	4	16.0
Hacıbektaş	July	11	0	-
Derinkuyu	August	19	0	-
Total		180	14	7.8

(P=0.173)

sheep in Iran by ELISA (30), 16.3% in Brazil by IFAT (31) and 19.3% in Italy by ELISA (32). Filho *et al.* (33) determined the incidence of infection as 62.2% and the rate of vertical transmission was 15.4% by ELISA and IFAT in Brazil.

In this study, *N. caninum* seropositivity was determined to be 7.8% by ELISA in sheep in the Nevşehir province. These differences in seropositivity rates between countries may be related to the effect of vertical or horizontal transmission on the occurrence of infection, the serological method used, the region where the study was performed, the breeding method, the breed and number of sheep examined.

The studies relating to neosporosis in Turkey have been conducted mostly on cattle, goat and horse. However, there

has been a limited number of studies on the parasite in sheep and only one study on the seroprevalence of *N. caninum* in sheep in Kars province (15). In Kars, Gökçe *et al.*, (15) detected the seropositivity as 2.1% of 376 sheep by using ELISA. *N. caninum* seropositivity of 7.8% detected in present study carried out by ELISA in sheep in Nevşehir province was found to be higher than that of other serological studies conducted in Turkey (15). This may be associated with difference in presence of the definitive host dogs, the different breeds of sheep used and the different geographic region.

Gökçe *et al.* (15) stated that they found the highest seroprevalence rate in sheep between 1-3 years of age (2.6%) and the lowest seroprevalence rate in sheep older than 3 years (0.9%). In their study they found no statistical difference between the age groups. In our study, *N. caninum* positivity was found to be 7.7% for sheep between 1-2 years of age, 7.3% between 3-4 years of age, 8.5% between 5-7 years of age. However no statistical significant difference were found between the age groups (P=0.342).

The present study, as was also reported in their studies (15, 18), also did not found differences between age groups. The relationship between age and neosporosis indicates that *N. caninum* has homogeneous distribution in all age groups and this state may be related to vertical transmission of the parasite.

In the present study, although we found no differences in the *N. caninum* seroprevalence rates with regard to mean study locations among sheep (P=0.173), *N. caninum* antibodies in central region of Nevşehir were more common than those in other study sites. The difference was probably due to the breeding conditions and the population of the definitive host, dogs.

In conclusion, this study is the first to demonstrate the presence of *N. caninum* antibodies in the sheep in Nevşehir province suggesting that the seropositivity rate of 7.8% posed a risk for the infection of additional sheep. For this reason and in order to determine the economic losses such as abortion, weak or stillbirths caused by neosporosis in sheep, and to create the necessary protection and control programs, it is critical to make additional comprehensive molecular and serological studies including for dogs, the definitive host.

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