

Seroprevalence of *Neospora caninum* in Cattle in the Province of Şanlıurfa*

Murat SEVGİLİ, Mehtap Gül ALTAŞ

Department of Parasitology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa - TURKEY

Oktay KESKİN

Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa - TURKEY

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Abstract: Blood samples were collected from 305 cows in the province of Şanlıurfa for determining the seroprevalence of *Neospora caninum*. Serum samples were analyzed for antibodies against *N. caninum* antigen using a commercially competitive inhibition (CI) ELISA. Antibodies to *N. caninum* were found in 23 of the 305 (7.5%) cow sera based on ELISA test results. With regard to seropositivity, no significant difference was observed among the origin, animal breed, and age ($P > 0.05$).

Key Words: *Neospora caninum*, cattle, ELISA

Şanlıurfa Yöresinde Sığırlarda *Neospora caninum*'un Seroprevalansı

Özet: Şanlıurfa yöresinde sığırlarda *Neospora caninum*'un seroprevalansını belirlemek için 305 sığırdan kan örnekleri toplandı. Serum örneklerinin analizi için ticari kompetatif inhibisyon (CI) ELISA testi kullanıldı. ELISA test sonuçlarına göre 305 sığırın 23 (% 7,5)'ünde *N. caninum* antikoruna bulundu. Seropozitiflik açısından örnek alınan odaklar, hayvan ırkları ve yaşları arasında önemli bir fark ($P > 0,05$) bulunmadı.

Anahtar Sözcükler: *Neospora caninum*, sığır, ELISA

Introduction

Neospora caninum is a coccidian protozoan parasite that induces abortion and neonatal mortality in cattle, sheep, goats and horses in many countries (1-6). Until 1988, the disease was often misdiagnosed as toxoplasmosis because of the close structural and biological similarities between *N. caninum* and *Toxoplasma gondii* (1-5).

Neosporosis is recognized as one of the most common and important causes of sporadic and endemic bovine abortion in dairy and beef cattle worldwide (7-15). The economic impact of *Neospora*-induced abortions depends on direct costs and the value of fetuses lost. Indirect costs include those associated with establishing the diagnosis, rebreeding cows that aborted and possible loss of milk yield (2,6,16-18).

Abortion is the sole clinical symptom observed in cattle due to neosporosis. For this reason, clinical diagnosis is difficult. Thus, serological tests are necessary for an exact diagnosis. Several serological tests, including the enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody technique (IFAT), the direct agglutination test (DAT), and immunoblots (IB), can be used to detect anti-*N. caninum* antibodies (1-5). Many serological studies have been conducted on bovine neosporosis (9,10,19-31). The authors are unaware of any research article on the seroprevalence of bovine neosporosis in Turkey.

The objective of the present study was to determine the seroprevalence of *N. caninum* in cows in and around Şanlıurfa.

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Materials and Methods

Three hundred five cows were used as material. The animals were randomly selected. The owners were questioned about animal management and age, and the information obtained was recorded. Blood samples were collected from 305 Holstein, Swiss Brown or hybrid cows in farms in the central Şanlıurfa, Siverek, Birecik, Viranşehir, and Akçakale districts. In the present study the majority of the cattle were raised on small-scale farms (4-19 per farm). Of these, 110 were more than 5 years old, and 195 were between 2 and 4 years old.

Serum samples were stored at -20 °C until laboratory testing. They were analyzed for antibodies to *N. caninum* using a modification of a monoclonal antibody-based, competitive inhibition ELISA (CI-ELISA), which is commercially available (Veterinary Medical Research and Development, Pullman, WA, USA). The test has a sensitivity of 97.6%, and specificity of 98.6%. The kit was used according to the manufacturer’s instructions. The plates were read in a ELISA microplate reader (Molecular Devices VERSAmix) at a wavelength of 620 nm. The optical density (OD) of the CI-ELISA was read on an automatic plate reader and the OD readings of the test samples were expressed to give the % inhibition by the following formula:

$$\% \text{ Inhibition} = 100 - [(\text{Sample OD} \times 100) / (\text{Mean Negative Control OD})]$$

Samples with an inhibition of 30% or greater were considered seropositive according to the manufacturer’s recommendation (VMRD).

A chi-square test of independence was used to analyze associations between infection by *N. caninum* and other factors studied in the present study. For statistical analysis, the STATCALC of the EPI INFO V.6 computer program was used.

Results

Results obtained from the cow sera in and around Şanlıurfa using ELISA are given in the Table.

Antibodies to *N. caninum* were found in 23 of the 305 (7.5%) cow sera based on ELISA results. Among the 195 cows in the 2-4 age group, 17 (8.7%) were seropositive, whereas among the 110 cows above 5 years old, 6 (5.4%) were seropositive. Among the 98 Holstein cows, 8 (8.1%) were seropositive. Of the 90 Swiss Brown cows, 6 (6.6%) were seropositive, and of the 117 hybrid cows, 9 (7.6%) were seropositive.

There was no statistically significant relationship between seroprevalence and breed and age groups (P > 0.05).

Discussion

Several serologic tests including ELISA, IFAT, DAT and IB can be used to detect *N. caninum* (1-6,8,28). The ability of a test to distinguish infected from noninfected individuals is often described by its diagnostic sensitivity and specificity (28). All the serological tests mentioned above are useful for identifying sera with moderate to high levels of anti-*Neospora* antibodies (8,9). At present,

Table. *Neospora caninum* seropositivity in the province of Şanlıurfa with regard to age, breed and origin.

	AGE		BREED			ORIGIN				
	2-4 age group	Above 5 years of age	Holstein	Swiss Brown	Hybrid	Central	Siverek	Viranşehir	Akçakale	Birecik
The number of animals tested	195	110	98	90	117	66	63	62	56	58
The number of seropositive animals (%)	17 (8.7)	6 (5.4)	8 (8.1)	6 (6.6)	9 (7.6)	6 (9.0)	5 (7.9)	5 (8.0)	4 (7.1)	3 (5.1)
The number of seronegative animals (%)	178 (91.2)	104 (94.5)	90 (91.8)	84 (93.3)	108 (92.3)	60 (90.9)	58 (92.0)	57 (91.9)	52 (92.8)	55 (94.8)

the 2 major types of serological tests most commonly employed for the diagnosis of *Neospora* infection are IFAT and ELISA (28,29). ELISA also enables more rapid analysis of samples than IFAT or IB, and so is extremely useful in the large-scale screening of cattle herds. In this study, sera were analyzed for antibodies to *N. caninum* using a commercial modification of a monoclonal antibody-based competitive inhibition ELISA kit, which has a high sensitivity (97.6%) and specificity (98.6%) to *N. caninum* antibodies.

By means of ELISA, *Neospora* antibodies were found between 5.6% and 59% in investigations conducted in different countries. Seropositivity rates were found to be 36.8%, 5.6%, 6.8%, 30.6%, 24%, 27.3% and 15.5% in Spain, France, Germany, northern Spain, the northwestern United States, England and Poland, respectively (7,15,19-21,25,26). Using IFAT, *Neospora* antibodies were found to be 12.6%, 59%, 50.7% and 24.3% in cows in Ireland, Scotland, northern Spain and England, respectively (22-25). In the current study, antibodies to *N. caninum* were found in 23 (7.5%) of the 305 sera tested using CI-ELISA.

The relationship between age and seroprevalence in bovine neosporosis is speculative. Jensen et al. (14) suggested that seroprevalence increases with age. In contrast, Sanderson et al. (21) reported that cows below 3 years of age had higher CI-ELISA inhibition percentage values than cows above 6 years of age. They also suggested that infected cows can infect fetuses, and if these calves have not been reinfected, antibody titers decline over time, resulting in an apparent decrease in seroprevalence with cow age. Thurmond and Hietala (16) reported that it should be taken into consideration that postnatal transmission could have been masked if older infected cows were culled because of abortion or low milk production. However, Davison et al. (30) found no

relationship between age and seroprevalence. Likewise there was no significant association between age and seroprevalence in the present study.

Neospora infections associated with abortion and congenital infections have been reported in both dairy and beef cattle, but there are more reports attributing significant numbers of abortions in dairy cattle (1-6,27). Quintanilla-Gozalo et al. (7) reported that the herd seroprevalence of *N. caninum* was higher in dairy than in beef herds and an important degree of association was observed between infection and herd production. The same authors observed that herd seroprevalence in dairy and beef cattle could be mainly due to different management practices between the 2 types of cattle and not to different breed susceptibilities. In this study, of the 98 Holstein cows, 8 (8.1%) were seropositive, of the 90 Swiss Brown cows, 6 (6.6%) were seropositive, and of the 117 hybrid cows, 9 (7.6%) were seropositive. Thus, no relationship was noted between serologic status and breed. Similarly, other authors have found no significant association between breed and *N. caninum* (5,31).

The geographical distribution of prevalence may be due to climatological differences between geographical areas. Increased rainfall and mild to high temperatures might account for differences in oocyst survival and subsequently cow exposure, or may reflect the density distribution of other unrecognized definitive hosts (21). In the present study, no relationship was noted between seroprevalence and individual cow origin. We think that this may have resulted from similar climatologic and management factors in sera collected in different regions.

In conclusion, antibodies to *N. caninum* were 7.5% in cows from Şanlıurfa. Further studies are necessary to investigate the seroprevalence of neosporosis in other regions, and the relationship between seropositive status and bovine abortions in Turkey.

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