

Short Communication / Kısa Bilimsel Çalışma

Serodiagnosis of bovine neosporosis with immunocomb assay in Ankara region

Arif KURTDEDE<sup>1</sup>, Şükrü KÜPLÜLÜ<sup>2</sup>, Kerem URAL<sup>1</sup>, C. Çağrı CINGI<sup>1</sup>, Murat GÜZEL<sup>3</sup>, M. Çağrı KARAKURUM<sup>1</sup>, A. Evren HAYDARDEDEOĞLU<sup>1</sup>

<sup>1</sup>Department of Internal Medicine, <sup>2</sup>Department of Gynaecology and Obstetrics, Faculty of Veterinary Medicine, University of Ankara; <sup>3</sup>Department of Internal Medicine, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay

**Summary:** Serodiagnosis of bovine neosporosis in 60 cows in Ankara was evaluated by a commercially produced solid phased immunoassay principle, immunocomb. Serum samples were taken from aborted (n=12) and non-aborted (n=48) cows in three different locations of Ankara. Six out of 12 aborted cows (50%) were seropositive by immunocomb (IC) assay indicated that neosporosis may be an important cause of abortions in this region. In conclusion, as a practical note, the IC assay was very simple, easy and quick to use for serological testing and screening of neosporosis both for large and small volume of herds.

Key words: Neosporosis, immunocomb, cattle, Turkey.

Ankara yöresinde sığır neosporozisinin immunocomb testi ile serolojik tanısı

**Özet:** Ankara'da 60 sığırda neosporozis'in serodiyagnozu ticari olarak üretilmiş katı fazlı immunoassay prensibi ile çalışan immunocomb (IC) ile belirlendi. Serum örnekleri Ankara'nın üç farklı bölgesinde abort yapmış (n=12) ve abort yapmamış (n=48) ineklerden alındı. Abort yapmış 12 inekten 6'sında (% 50) IC ile belirlenen seropozitivite neosporozis'in bu bölgedeki abortların önemli bir nedeni olabileceğini gösterdi. Sonuç olarak pratikte, IC'nin oldukça basit, kolay ve kısa sürede seri serolojik kontroller için ve gerek büyük gerekse küçük sürülerde neosporozis'in monitorizasyonu için kullanılabilmesi kanısına varıldı.

Anahtar sözcükler: Neosporozis, immunocomb, sığır, Türkiye.

*Neospora caninum* (*N. caninum*) infection, recognized as one of the most important cause of abortion in dairy cattle, has been reported as the cause of abortion and therefore neonatal mortality (8). Economic loss associated with *N. caninum* infection is because of increased prevalence of abortions and decreased milk and meat production in infected animals (3,13).

The objective of the present study was to survey aborted and non-aborted cattle in Ankara to determine the serodiagnosis of *N. caninum* by immunocomb.

In the present study the serodiagnosis of *N.caninum* in 60 cows in three locations of Ankara were tested by commercially available Neospora Ab test kit (Bovine Neospora Ab test kit; Immunocomb).

**Study site and blood sampling**

The present study was conducted in Ankara. Convenience samples were collected from cows in three locations.

Blood samples were obtained from 60 cows over a period of 4 months. Fourtyeight of the 60 cows had no history of abortion while the remaining 12 were aborted previously. Information on the aborted cows (n=12) is

given in Table 1. Samples from non-aborting cows were randomly selected.

Table 1. The presence of antibodies to *N.caninum* on the aborted cows (n=12) in Ankara.

Tablo 1. Ankara'da abort yapan sığırlarda (n=12) *N. caninum* antikorlarının varlığı.

No	Age (years)	Abortion time (during gestation months)	No of abortions recorded	<i>N.caninum</i> antibody titer (detected within immunocomb)
1.	6	8	First	-
2.	5	6	First	-
3.	4	4	First	High
4.	6	4	Second	High
5.	2	5.5	First	-
6.	4	5	First	Medium
7.	3	5	First	Medium
8.	4	8	First	High
9.	5	4	First	-
10.	2	6	First	Medium
11.	5	2	First	-
12.	5	6	First	-

### Immunocomb analysis

The IC assay was performed according to the manufacturer's instructions, including the color development, and the evaluation and interpretation of the results, independently. Before reading and interpretation of the results the immunocomb was air dried and aligned with the calibrated color combscale. Positive and negative control sera, provided by the manufacturer (Biogal), were used in each test and the color resulting from the positive control sample (C+) was taken into comparison within the calibrated color combscale. Finally the most appropriate color was selected and the titre was evaluated for each sample. Samples with an identical or higher color intensity than the positive control (C+) were considered to have positive reaction for Neospora (High positive, positive, medium positive) (Table 1).

Of the 60 cows tested by the IC system 6 cows (10%) were seropositive to *N.caninum*. Seropositive cows (n=6) were found on two locations and all of them were aborted (Table 1). *Neospora caninum* antibodies were not detected in the non-aborted cows (n=48).

Neosporosis has emerged as an important disease of cattle within the primary, and only sign observed in adult cows, clinical sign of abortion especially occurring during the middle of gestation (1,7). Cows of any age may have abortion from 3 months of gestation to term, mostly at 5-6 months of gestation (1). In agreement with the latter report, affected cows in the present study had the abortion time of 4 (no=3 and no=4), 5 (no=6 and no=7), 6 (no=10) and 8 (no=8) months of gestation respectively.

Two major route has been reported for the transmission of *N. caninum*, either by horizontal postnatal infection through a definitive host, probably a canid, or by vertically from an infected dam to the foetus (1,9). At recently although canids status of being definitive hosts for *N. caninum* remains unclear (7), several epidemiological studies suggested dogs' role in the transmission of neosporosis (4,11,14).

Susceptible hosts, although only have been reported experimentally in mice, may be infected by ingesting food and water contaminated with *N. caninum* oocysts by dog feces (10). Thus in the present study as being informed by the owners, the fodder and drinking water in the resting field of the cows could be contaminated with *N.caninum* oocysts by the neighbouring dogs.

In a previous seroepidemiological study in dogs and cattle, 36 dogs out of 152 (23.6 %), from dairy farms with a known prevalence of *N. caninum* antibodies in the cattle, were seropositive to *N. caninum*, suggesting the relationship between *N. caninum* infection of farm dogs and cattle (14). Similar observation has been reported by Dijkstra and others (6) in a previous study hypothesizing

that a newly introduced dog infected with *N. caninum* from already infected cattle, transmitted the infection to other cattle within shedding of oocysts.

In the diagnosis of bovine neospora infection a veterinary diagnostic laboratory assistance is required. At present two major types of serological testing, indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA), have been widely used. However there are a variety of different procedures in order to carry out these tests, in which most of them are based on the usage of culture-derived tachyzoites of *N. caninum* bovine or canine isolates (1). Careful consideration must be given to use and evaluation of serologic results, particularly in order to establish appropriate cut-of titers for each test. A definitive cut-off titer for serodiagnostic survey has not been settled for bovine neosporosis because of lack of certainty of serological diagnosis for chronically infected cattle and therefore the limited knowledge from noninfected cattle (1,7).

In Tekirdağ and Kırklareli, Trakya region in Turkey, a seroprevalence study using an ELISA in cattle showed that 7 out of 124 (5.6%) and 15 out of 150 (10 %) animals in Tekirdağ and Kırklareli respectively, had antibodies against *N. caninum* (5). In another seroprevalence study in Sanliurfa province, southeast part of Turkey, 23 cattle (7.5%) out of 305 were found to be positive against *N. caninum* antibodies (12).

In recent years, a commercially available immunocomb (IC) system (Immunocomb bovine Neospora antibody test kit; Biogal) has been designed and developed for the determination of cow serum IgG antibody titers for *N. caninum*. This assay is based on a solid phased immunoassay principle, that is attached within purified *N. caninum* antigen. The present test is very simple, reliable and quick, that the results can be obtained in 40 minutes. The rapid results in kit format, that the test provides, is appropriate for laboratory and field use, especially for serological screening of herds (2).

IC system allows producers for testing animals against neosporosis before breeding selection and/or prior to purchasing as for indicating the infection status. As an example of where the IC system used was a 150 cow dairy herd that Dr. Landmann, from Tick Fever Research Centre-Queensland Australia, recently dealt with, where as 24% of the animals were infected with *N. caninum*. In regard to the latter researcher, the IC system is a valuable tool that is helpful for the exclusion of neosporosis and he recommend this assay in various countries (2).

The observation that 6 out of 12 aborted cows (50%) in Ankara were seropositive by immunocomb (IC)

system indicated that neosporosis may be an important cause of abortions in this region. Although neosporosis has been recently reported in Turkey, these data give a preliminary indication of the prevalence of infection in the cattle population in Ankara, Turkey. Further studies are necessary to investigate the seroprevalence in other regions, and the possible association between seropositive animals and bovine abortions.

Finally, as a practical note, especially for the serological testing and for screening of both the large and small volume of herds, the IC assay was very simple, quick and reliable. However reading of the IC assay results is done by eye, which may cause misinterpretations, it is always necessary to evaluate the results by two or more independent well qualified operatives. Furthermore larger, long-term investigations are necessary to evaluate immunucomb in comparison to other tests (e.i. ELISA, IFAT) for the diagnosis of neosporosis in Ankara, Turkey.

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### Address for correspondence

Prof. Dr. Arif Kurtdede  
Ankara Üniversitesi  
Veteriner Fakültesi  
İç Hastalıkları ABD,  
Dışkapı-Ankara 06110 Türkiye