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## Βιβλιογραφική αναφορά:

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## A study of *Neospora caninum* antibody seroprevalence in dairy cows in Turkey

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**ABSTRACT.** *Neospora caninum* is a intracellular protozoan parasite and is one of the major causes of repeated abortions, foetal malformations, pre-term deliveries, stillbirth and possible loss of milk yield in livestock. The presence of specific antibodies against *N. caninum* in the blood serum of dairy cows is investigated in the present study. A total of 184 blood serum samples of dairy cows were examined in Bursa province in the Marmara Region. *N. caninum* antibodies were measured using an indirect enzyme-linked immunosorbent assay (ELISA) (The Svanovir Neospora-Ab ELISA). From the total sample, antibodies to *N. caninum* were detected in 62 of the 184 examined cows (33.3%) and neurological findings were seen in a calf.

**Keywords:** *Neospora caninum*, ELISA, dairy cows, abort, Turkey, Bursa.

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## INTRODUCTION

Many factors affect livestock production and productivity. The economic performance of dairy activity is directly related to the reproductive rates of the herd (Lamy et al., 2012). *Neospora caninum* is considered one of the major causes of repeated abortions in livestock. Neosporosis is a parasitic disease caused by *N. caninum*, a cyst-forming coccidian species and an obligate intracellular parasite. *N. caninum* is considered one of the major causes of repeated abortions, foetal malformations, pre-term deliveries, stillbirth and possible loss of milk yield in livestock, thus generating severe economic losses (Bartova et al., 2015; Dubey and Lindsay, 1996; 1-5). Dogs were the first discovered definitive hosts of this parasite. Other natural definitive hosts are coyotes, dingo and grey wolves (Dubey and Schares, 2011-12). The transmission of parasite is both vertical and horizontal. But the vertical transmission is more frequent than the horizontal. Cows may remain infected with *N. caninum* for their whole life and transmit the infection over several generations (Davison et al., 1999-31). The pregnant cow may show no outward signs but when the parasite passes to the unborn calf it can cause abortion. And also some calves survive the gestation period and born neurological signs or still born, others may look healthy but are infected with the parasite.

Serological prevalences of *N. caninum* in cattle worldwide have been reported by Dubey and Schares, 2011-12. In Europe, seroprevalence of *N. caninum* were detected 1%, in Germany, 15.2% in Greece, 0.7% in Norway, 55.9% in Romania, 22.5% in Spain, 20.1% in Slovakia, 2.8% in Sweden, 10.77% in Turkey and 12.9% in the United Kingdom (Dubey and Schares, 2011-12). During the 16 year period 2000-2016, several studies focusing on the detection of *N. caninum* antibodies in dogs and cattle in Turkey (Büyükköglu et al., 2003; Vural et al., 2006; Ocal et al., 2014; Adanır et al., 2015; Karatepe and Karatepe, 2016).

The aim of the present study was to determine the

positivity to *N. caninum* and the influence of the infection on the occurrence of abortions and mothers of calves with neurological signs in dairy farm in Bursa in Turkey.

## MATERIALS AND METHODS

Cattle of various ages, breeding systems and localities in Bursa, Turkey. Bursa is located near the Sea of Marmara, right opposite of Istanbul and in the Cities place category with the GPS coordinates of  $40^{\circ} 11' 35.8728''$  N and  $29^{\circ} 4' 27.1272''$  E. Blood samples were collected from 186 adult dairy cows (Holstein), aged 3-5 years in Bursa province, located in northwestern Turkey. Blood samples were collected through vein puncture from each animal in tubes without anticoagulant, and to obtain the serum, blood samples were centrifuged. The serum samples were stored at  $-20^{\circ}\text{C}$  until analysis. *N. caninum* antibodies were measured using an indirect enzyme-linked immunosorbent assay (ELISA) (The Svanovir Neospora-Ab ELISA). Abort and embryonic death records of positive animals were examined retrospectively.

## RESULTS

From the total sample, antibodies to *N. caninum* were detected in 62 of the 186 examined cows (33.3%, 95% CI 17.2%-49.4%). Retrospective records of 62 animals with positive titration of *N. caninum* were examined (table 1,2). In the clinical course of the calf with neurological signs, it was observed that the suction reflex was strong and there was a spastic paralysis in the hind legs starting from the pelvic muscles. In the hind limbs, there was a hyperextension which was not corrected by hand and the head was slightly tilted to the left and there were tremors. No pathology was found in the general clinical course of its mother. As a result of clinical and serological findings, congenital neosporosis was diagnosed in the calf. The calf died as a result of progressive paralysis within 13 days. The operational veterinarian was asked to send the brain, lung and heart tissues from the necropsy for histopathological evaluation. However, histopathological evaluation was not performed due to deterioration.

**Table 1:** Result of 2nd and 3rd pregnancies of 49\* dairy cattle which developed abortion or early embryonic death at the 1st pregnancies

	A	B	C	Total number of dairy cattle
1st pregnancy	25	24	13	62
2nd pregnancy	5	6	38	49*
3. pregnancy	0	2	47	49*

**A:** Number of early embryonic death **B:** Number of abortions formed in 4-6 months of pregnancy **C:** Number of normal pregnancy

**Table 2:** Results of 2nd, 3rd and 4th pregnancy of 13\* cows with no problems in first pregnancy

	<b>A</b>	<b>B</b>	<b>C</b>	Total number of dairy cattle
1st pregnancy	25	24	<b>13*</b>	62
2nd pregnancy	3	3	<b>7</b>	<b>13*</b>
3rd pregnancy	0	4	3	7**
4th pregnancy	0	2	5***	7**

**A:** Number of early embryonic death **B:** Number of abortions formed in 4-6 months of pregnancy **C:** Number of normal pregnancy **D:** Number of calves with neurological signs

\*\*Number of cattle with no problems in 1st and 2nd pregnancy

\*\*\*Neurological findings were found in the calf of one of the cows

## DISCUSSION

Neosporosis has aroused an immense interest mainly due to repeated abortions in cows and a negative effect on their breeding economy. The majority of *N. caninum* infections appear to have a chronic course, probably causing life-long persistence of the parasite in tissues of infected animals (Dubey, 1996; Ricardo Vilas Boas, 2015). Abortion, occurring during the middle of gestation, is the primary clinical sign of the infection in cattle. There was a significant association between seropositivity for *N. caninum* and occurrences of abortions among dairy cows, thus reinforcing the role of *N. caninum* as a major cause of reproductive disorders (Dubey et al 2006; bruhn, 2013; ghalmi, 2012). It is important to note that the reproductive problems described on 36 farms (57.14%) were occurrences of abortions, while on 26 (41.26%), they consisted of birth of weak calves, among which some evolving to death. Abortion is the main clinical manifestation of bovine neosporosis in dairy cattle, and depending on the stage of pregnancy, this can lead to abortion or the birth of a healthy but chronically infected calf. Most abortions occur in the early second trimester, but they may occur throughout gestation (Dubey et al., 2006). Bursa province, where the study is conducted, is important for dairy cattle farms. Therefore, abortions are economically important. In this study, serological screening confirmed the influence of *N. caninum* on the occurrence of abortions in selected dairy herd and abortion were occurred between 5th and 8th months of gestation.

Transplacental transmission during pregnancy can occur in pregnant cows infected with *N. caninum*. Congenitally infected fetus birth or abortion is closely related to the immunity of pregnant cow, placenta and fetus (Klauck et al., 2016). Some studies regarding neosporosis have shown that fetal infection decreases with the rise of the number of gestations, and consequently with the animal's age, mainly be-

cause animals acquire immunity against the parasite, so, the animals may not show high levels of antibody against *N. caninum*. This may be thought to be related to increased immunity (Romero et al., 2002; Dijkstra et al., 2003; Williams et al., 2009). Abort is the most common site reflection. However, congenitally infected calves with low calving syndrome have low chance of survival and may have neural pathological clinical implications. Sometimes, as a result of congenital encephalomyelitis, calves with paresis and dysphoria may occur in the hind legs and neurological reflections may occur after weeks of birth (Dubey and Schares, 2011; Klauck et al., 2016). In this study, there was a calf with neurological signs. Our results were parallel to the other studies and suggest that abortion and / or early embryonic deaths in animals infected with *N. caninum* are much higher in heifers, and that subsequent pregnancies may end up in some infected animals normally.

During the past decade, *N. caninum* infection has emerged as an important reproductive disease in cattle throughout the world. Surveys in several countries from three continents have identified *N. caninum* infection as the major diagnosed cause of bovine abortion (anderson et al.). In Europe, antibodies against *N. caninum* have been detected in 2.8-60 % cattle. There are considerable differences among countries, within countries, and between regions, with the highest in Turkey- 60 % (Kul et al., 2009) and the lowest in Sweden- 2.8% (Loobuyck et al., 2009). In Turkey, Aktaş M et al (2005) found that out of 513 cattle in the Eastern Anatolia Region, 36 (7.01 %) were found to be seropositive by cELISA. In another study, in the Marmara Region, rate of seropositivity was detected to be 8.4% (32/366) (Oncel et al., 2003; Bıyıkoglu et al., 2003). In the present study, *N. caninum* antibodies were found in 33.3% cows post-abortion in the Marmara Region, Bursa province. This solution was showed that *N. caninum* infection is one of the major

causes of repeated abortions in Turkey. The results of the above studies carried out in different regions of Turkey is in line with this results (Vural et al., 2006; Kaya et al., 2011; Celik et al., 2013; Ocal et al., 2014; Karatepe and Karatepe, 2016).

## CONCLUSION

In conclusion, both results obtained in this study and the other results in Turkey, as in many countries in Europe have also revealed the presence of serolog-

ically *N. caninum* in cattle in Turkey. The risk of *N. caninum* seems to be important for dairy cows with cases of abortion and prevention should be increased by checked *N. caninum* regularly in the future studies. In addition, more detailed research on the pathogenesis and consequences of vertical transmission is required.

## CONFLICT OF INTEREST

None declared.

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