Prevalence of Neospora caninum in cows with stillbirth and abortion

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Summary: The aim of this study was to determine the prevalence of *Neospora caninum* in cows with stillbirth and abortion. For this aim a total of 134 sera was collected and divided into two groups under abortion and stillborn which were tested for antibodies against *N. caninum* using a commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit (VMRD, USA). Abortion group was divided into 90-150, 150-210, 210-260 day groups. Cows expulsing their fetus after 260 days of gestation were accepted as stillbirth group. Antibodies against *N. caninum* were detected as 47 out of 134 (35.07%) sera. Mean prevalences of abortion and stillbirth group were 24.44% and 56.81%, respectively. In the abortion group, prevalences were detected as 23.07% for 90-150 day group, 18.51% for 150-210 day group and 29.72% for 210-260 day group.

Keywords: Abortion, Neospora caninum, prevalence, stillbirth

Ölü doğum ve abort yapan ineklerde Neospora caninum prevalansı

Özet: Bu çalışma abort ve ölü doğum yapan ineklerde *Neospora caninum*'un prevalansını belirlemek amacıyla yapıldı. Bu amaçla iki gruba (abort ve ölü doğum) ayrılmış toplam 134 serum, ticari kompetatif ELISA kiti (c-ELISA) ile *N. caninum*'a karşı oluşmuş antikorlar yönünden test edildi. Abort yapan grup 90-150, 150-210, 210-260 gün olmak üzere 3 gruba ayrıldı. Gebeliğin 260. gününden sonra yavru atan inekler ölü doğum grubu olarak kabul edildi. 134 serumun 47'sinde (%35.07) *N. caninum*'a karşı antikorlar tespit edildi. Abort ve ölü doğum gruplarının ortalama prevalansı sırayla %24.44 ve %56.81 olarak belirlendi. Abort grubunun prevalansları ise 90-150 gün için %23.07, 150-210 gün için %18.51 ve 210-260 gün grubu için %29.72 olarak tespit edildi.

Anahtar sözcükler: Abort, Neospora caninum, ölü doğum, prevalans

Introduction

Neospora caninum is a coccidian parasite and an important cause of abortion in cattle worldwide. It was first identified in dogs with encephalomyelitis and myositis in 1984 and was described as a new genus and species in 1988. Domestic dog is definitive host while cattle, sheep, goats, horses, deer are intermediate hosts. Dogs can acquire infection by ingestion of infected tissues; and intermediate hosts can be infected either by horizontal postnatal infection or by vertical transmission during pregnancy. There is no cow to cow transmission (ANDERSON et al., 2000; DUBEY, 2003).

In *Neospora* infection, cows can abort; fetuses may die in utero, be resorbed, mummified, autolyzed, stillborn, born alive with clinical sings or born normally but persistently infected. Moreover, infection may cause premature culling, diminished milk production and repeat breeder problems in herds (DUBEY, 2006; SIMSEK et al., 2008; THURMOND and HIETALA, 1996, 1997).

Abortion is defined as fetal death and expulsion between 42 and 260 days of gestation. If death occurs at 1-2 month of gestation, it is usually termed as "early embryonic death" whereas a calf that is born death between 260 days and full term defined as "stillbirth" (HOVINGH, 2009; is PETER, 2009). In Neospora infection, abortion can occur both in dairy and beef cattle at any stage of pregnancy. However, most abortion occurs during the fourth to sixth month of gestation. Aborted cows can abort again (ANDERSON et al., 2000; DUBEY, 2003). Neospora caninum is a major cause of abortion in cattle in many countries. Several studies indicate that 12 to 42% of aborted fetuses from dairy cattle are infected with N. caninum. In some dairies up to 87% of cows found as seropositive. Economic loss is estimated in millions of dollars resulting from abortion, stillbirth, rebreeding, reduced milk production and premature culling (DUBEY, 2003).

In Turkey seroprevalence of N. caninum in cows was determined as 13.96% (459/3287) in Central Anatolia (VURAL et al., 2006), 8.02% (22/274) in Thrace (BIYIKOGLU et al., 2003), 9.2% (10/92)in Sakarya (ONCEL and BIYIKOGLU, 2003), 7.5% (23/305) in Sanliurfa (SEVGILI and ALTAS, 2005), 7% (13/186) in Kayseri (ICA et al., 2006) and 7.01% (36/513) in Eastern Anatolia (AKTAS et al., 2005). Seroprevalence of N. caninum was found significantly higher in dairy cows with repeat breeder (13.48%, 12/89) than healthy pregnant (3.19%, 3/94) (SIMSEK et al., 2008). In aborted cows, seroprevalence was determined to be 23.61% (34/144) in Central Anatolia (VURAL et al., 2006), 3.12% (1/32) in Eastern Anatolia (AKTAS et al., 2005), and 33.3% (3/9) in Kayseri (ICA et al., 2006).

The aim of this study was to determine the prevalence of *N. caninum* in cows at different stages of gestation.

Material and Method

For this purpose a total of 134 cow sera that sent to Central Veterinary Control and Research Institute, Parasitology Laboratory without age and breed information were collected by farm veterinarians. Sera samples were stored at -20°C until tested. Based on the definition abortion and stillbirth, sera were divided into two groups as abortion and stillbirth. Abortion group was further divided into 90-150, 150-210, 210-260 day groups. Antibodies to N. caninum were detected by using a commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit (VMRD, USA). The test was done following the instructions of manufacturer. The mean optical density (OD) at 630 nm was determined for all wells using a microplate reader (ELx 800 UV, Universal Microplate Reader, Bio-Tec Instruments, Inc). The percent inhibition for each test sample was determined using the below mentioned formula:

Inhibition (%) = 100-	Sample O.D. × 100	
	Mean negative control O.D.	

The samples with values of $\geq 30\%$ inhibition were regarded as positive and those with the values < 30% inhibition were regarded as negative.

Findings

The overall prevalence of *N. caninum* was 35.07% (47/134). Mean prevalences of abortion and stillbirth group were 24.44% and 56.81% respectively. In abortion group, prevalences were 23.07% for 90-150 day group, 18.51% for 150-210 day group and 29.72% for 210-260 day group. Results of the study are also summarized in tables (Table1 and 2).

Table 1. Total prevalence of *N. caninum* in abortion and stillbirth group.

	Abortion	Stillbirth	Total
The number of animal tested	90	44	134
The number of in- fected animals	22	25	47
Prevalence (%)	24.44	56.81	35.07

Table 2. Prevalence of *N. caninum* in different stages of gestation.

Days	Abortion/ Stillbirth	Infected	Prevalence (%)
90-150	26	6	23.07
150-210	27	5	18.51
210-260	37	11	29.72
> 260	44	25	56.81

Discussion and Conclusion

In this study, the prevalence of *N. caninum* was determined in different stages of gestation and the overall prevalence was found as 35.07%. This result was obviously higher than the prevalence findings of randomly selected cows (AKTAS et al., 2005; ICA et al., 2006; VURAL et al., 2006). Higher prevalences were accumulated in stillbirth (56.81%) and 210-260 day groups (29.72%) while lower prevalences were detected in 150-210 (18.51%) and 90-150 (23.07%) day groups. Although most abortion occurs during the fourth to sixth months (ANDERSON et al., 2000), this study indicated that abortion and stillbirth ratios were

higher after 210 days. Nevertheless, high seroprevalences were observed at every stages of gestation in cows with abortion and stillbirth. Except Eastern Anatolia, results showed that seroprevalences increase in aborted cows (AKTAS et al., 2005; ICA et al., 2006; VURAL et al., 2006).

Seroprevalence data about dog neosporosis are limited in Turkey (COSKUN, 2000; YILDIZ et al., 2007). Coskun et al. (2000), found 10% seropositivity in 150 dogs by indirect fluorescent antibody test (IFAT) in two provinces of Turkey. Yıldız et al. (2007), examined 117 dogs with IFAT in Kırıkkale province and reported 26.5% seropositivity. High or low prevalences in aborted cows may be associated with the prevalence ratios of *N. caninum* in dogs.

There are only three studies about aborted cows however there is no seroprevalence data related with abortion and stillbirth dates. These studies are especially focused on the general seroprevalence of the parasite (AKTAS et al., 2005; ICA et al., 2006; VURAL et al., 2006). Our study is the first detailed study about the seroprevalence of *N. caninum* at different stages of gestation in Turkey. We think that further studies are required to determine the prevalence of *N. caninum* in definitive and intermediate hosts for a clear understanding of disease's epidemiology. Additionally, for attaching necessary importance to the disease, economic effects of *N. caninum* should be surveyed in detail.

In conclusion, current study illustrated that *N*. *caninum* may cause abortion in any stage of pregnancy and has a high prevalence in cows with stillbirth.

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