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Original Article
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Investigation of some parasitic, bacterial and BVDV agents in dairy cows based on a past of abortions[1]

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ABSTRACT.- Aktürk F.S., Orta Y.S., Yaman S., Koçlu O., Atlı K., Öztürk D., Kant S. & Kale M. 2025. **Investigation of some parasitic, bacterial and BVDV agents in dairy cows based on a past of abortions.** *Pesquisa Veterinária Brasileira 45:e07591*, 2025. Department of Virology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Turkey. E-mail: drmkalex@yahoo.com

Blood samples were taken from 94 dairy cows based on a past of abortions (4th-7th months) in four different farms in Burdur province. The presence of *Chlamydophila abortus* (Antibody = Ab), *Coxiella burnetti* (Ab), *Sarcocystis* spp. (Ab), *Neospora caninum* (Ab) and bovine viral diarrhea virus (BVDV) (Antigen = Ag) were investigated in the collected blood samples. While seropositivity was determined for *C. abortus*, *C. burnetti* and *N. caninum* in the samples, BVDV was detected positive in the same samples. No samples were detected seropositive for *Sarcocystis* spp. (Ab). *N. caninum* showed the highest seropositivity in the samples tested, whereas *C. burnetti* had the lowest. When we looked at the mixed distribution of the factors in the positive animals, *C. abortus* + *N. caninum* was found to be the highest, and *C. burnetti* + *N. caninum* was the lowest. As a result of the study, it was determined that *N. caninum* was the most common abortion factor in cattle that had abortions between 4-7 months, and BVDV, *C. abortus* and *C. burnetti*, respectively, could also cause abortion. The high level of *N. caninum* indicates that canes and underground spring waters around the farms may be important reservoirs.

INDEX TERMS: Abort, dairy cow, bacteria, parasite, virus.

INTRODUCTION

Abortion is a serious reproductive problem in dairy cows around the world. Abortions cause significant economic loss, especially those performed late in pregnancy. Losses can be connected to the failure to replace lost calves, lower milk supply, treatment expenses, feeding animals, and early culling of profitable cattle (Abdelhadi et al. 2015). Based on some research, every abortion in a dairy cow causes losses ranging from $ 90 to $ 1,900 (Peter 2000, Eicker et al. 2003).

Abortion is the termination of a pregnancy when the ejected fetus is recognizable and non-viable, ranging in size from 45 to 260 days of gestation (Peter 2000). Risk factors for abortion in dairy cows include geography, management, genetics, environment, and viral factors. Brucellosis, leptospirosis, listeriosis, *Coxiella burnetti*, *Chlamydophila abortus*, bovine viral diarrhea virus (BVDV), *Neospora caninum*, *Sarcocystis* spp., mycotoxins, fungi, and yeasts are frequently found infectious causes of abortion in cattle (Hirsh 2004, Kabongo & Van Vuuren 2004, Ali & Khan 2006, Tooloei et al. 2008, Angelakis & Raoult 2010, Guatteo et al. 2011, Asmare et al. 2013, Parthiban et al. 2015, Shaapan 2016).

This study aimed to determine the presence of some bacterial, BVDV and parasitic infections in cows that aborted in the four-to-seven-month period under different dairy farming conditions and to determine the sources that may cause abortion under operating conditions.

MATERIALS AND METHODS

Ethical approval. This study was approved by the Animal Experiments Local Ethics Committee on Animal Use of the Burdur Mehmet Akif Ersoy University. The Committee determined that ethics committee permission was not necessary because the study was carried out for diagnostic purposes.

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Management and animals. Animals that were older than two years old and had abortions within four to seven months were used in four semi-intensive dairy cattle farms in Burdur province. Imported Holstein breed cows were bred on the farms. It was confirmed that no vaccination was administered to the animals before and during the study period. Rough and concentrated feed was used to meet the basic nutrients of dairy cows. It was determined that producers applied different rations according to the productivity levels of the animals. Within the scope of the research, it was determined that artificial insemination practices were carried out on the farms, but natural insemination was not performed. All businesses had dogs that were tied or free-ranging. Underground water resources for animal drinking water were common in farms.

Blood samples. Blood samples were taken from the jugular vein of the animals into 10 ml anticoagulant-free tubes. These samples were brought to the laboratory via a cold chain and centrifuged at 3000 rpm for 30 minutes. The serum samples obtained were stored at -30 °C until testing. Additionally, blood samples were taken into K3 EDTA tubes to detect BVDV. These blood samples were centrifuged at 3000 rpm for 30 minutes, and the leukocytes obtained as a result of the process were taken with the help of a Pasteur pipette and transferred to a different Eppendorf tube. The transferred leukocyte sample was washed with PBS (phosphate buffer solution) and centrifuged at the same speed and minute. The upper supernatant was decanted at the end of the process, and the washed leukocyte samples were stored at -80 °C until the test application.

***Coxiella burnetti* (ELISA-Ab).** *C. burnetii* commercial ELISA kit (PrioCheck™Ruminant Q Fever Ab Plate Kit, Thermo Fisher Scientific, Belgium) was used to detect *C. burnetii* antibodies (Ab) in blood samples. The test was performed according to the procedure specified by the commercial company.

***Chlamydophila abortus* (ELISA-Ab).** A commercial ELISA kit (ID Screen® *Chlamydophila abortus* indirect Multi-species, IDvet, France) was used to detect *C. abortus* antibodies in blood samples. The test was performed according to the procedure specified by the commercial company.

***Sarcocystis* spp. (ELISA-Ab).** Bovine *Sarcocystis* spp. in blood samples. A qualitative commercial ELISA kit (Sunlong Biotech Co., Ltd., Catalog Number: SL0229Bo, China) was used to detect the presence of antibodies. The test was performed according to the procedure specified by the commercial company.

***Neospora caninum* (ELISA-Ab).** A qualitative commercial ELISA kit (Sunlong Biotech Co., Ltd, Catalog Number: SL0201Bo, China) was used to detect the presence of bovine *N. caninum* antibodies in blood samples. The test was performed according to the procedure specified by the commercial company.

BVDV (ELISA-Ag). A qualitative commercial ELISA kit (Sunlong Biotech Co., Ltd, Catalog Number: SL0138Bo, China) was used to detect the presence of BVDV antigen (Ag) in blood leukocytes. The test was performed according to the procedure specified by the commercial company.

Statistical analysis. SPSS statistical program was used to analyze the study results. Numerical data obtained for viral, bacterial and parasitic factors were evaluated with descriptive analyses.

RESULTS

In the general distribution of infection among animals, *Neospora caninum* positivity was determined at the highest rate (n = 51, 54.25%) and *Coxiella burnetti* positivity at the lowest rate (23, 24.46%) (Table 1).

Considering the mixed distribution of the factors in the positive animals, the highest rate of positivity was found in *Chlamydophila abortus* + *N. caninum* (n = 9, 9.57%) and the lowest rate in *C. burnetti* + *N. caninum* (2, 2.13%) coinfection (Table 2).

Distribution of the results obtained by farms is as follows: in Farm-I, the highest seropositivity was 75% (12/16) for *N. caninum*, the lowest seropositivity was 12.5% (2/16) for *C. burnetti*, in Farm-II, the highest seropositivity was *N. caninum* at 42.85% (18/42), the lowest seropositivity was *C. abortus* at 33.33% (14/42), in Farm-III, the highest seropositivity was *N. caninum* at 71.42% (10/14), the lowest positivity was for both *C. abortus* and BVDV at 35.71% (5/14) and in Farm-IV, the highest positivity was determined to be BVDV at 59.09% (13/22), and the lowest positivity was determined to be *C. burnetti* at 9.09% (2/22) (Table 3).

Table 1. Distribution rates of infection in animals

Agents	Positive (%)	Negative (%)
Chlamydophila abortus (Ab)	37 (39.36)	57 (60.64)
Coxiella burnetti (Ab)	23 (24.46)	71 (75.54)
Sarcocystis spp. (Ab)	-	94 (100)
Neospora caninum (Ab)	51 (54.25)	43 (45.75)
BVDV (Ag)	38 (40.42)	56 (59.58)

Ab = Antibody, Ag = antigen, BVDV = bovine viral diarrhea virus.

Table 2. Distribution of factors in positively detected animals

Agents	n	%
Chlamydophila abortus (Ab)	6	6.38
C. abortus (Ab) + *Neospora caninum* (Ab)	9	9.57
C. abortus (Ab) + BVDV (Ag)	5	5.32
C. abortus (Ab) + *Coxiella burnetti* (Ab) + *N. caninum* (Ab)	3	3.19
C. abortus (Ab) + *C. burnetti* (Ab) + BVDV (Ag)	4	4.25
C. abortus (Ab) + *N. caninum* (Ab) + BVDV (Ag)	5	5.32
C. abortus (Ab) + *C. burnetti* (Ab) + *N. caninum* (Ab) + BVDV (Ag)	5	5.32
C. burnetti (Ab)	4	4.25
C. burnetti (Ab) + *N. caninum* (Ab)	2	2.13
C. burnetti (Ab) + BVDV (Ag)	5	5.32
N. caninum (Ab)	22	23.40
N. caninum(Ab) + BVDV (Ag)	5	5.32
BVDV (Ag)	9	9.57

Ab = Antibody, Ag = antigen, BVDV = bovine viral diarrhea virus.

Table 3. Positivity levels of factors identified in farms*

Farm	*Chlamydophila abortus*[¥]	*Coxiella burnetti*[¥]	*Neospora caninum*[¥]	BVDV[£]
I	8 (50%)	2 (12.50%)	12 (75%)	3 (18.75%)
II	14 (33.33%)	17 (40.47%)	18 (42.85%)	17 (40.47%)
III	5 (35.71%)	2 (14.28%)	10 (71.42%)	5 (35.71%)
IV	10 (45.45%)	2 (9.09%)	11 (50%)	13 (59.09%)

* More than one factor has been detected together in cattle in farms; [¥] Factor-specific antibody positive results in cattle; [£] Bovine viral diarrhea virus (BVDV) antigen positive results.

DISCUSSION

Abortions have a significant negative impact on reproductive efficiency, leading to substantial economic losses for the cattle industry (De Vries 2006). There are infectious and non-infectious factors among the causes of abortions in cattle. Infectious agents such as viruses, bacteria, protozoa, and fungi can be responsible for abortions in cattle (Hovingh 2009). These pathogens can lead to extensive economic losses, highlighting the need for control measures to prevent infection or disease (Givens & Marley 2008).

Chlamydophila abortus is an agent that particularly affects ruminant animals (cattle, sheep, goats) and non-mammalian hosts, causing abortions and fetal losses, leading to economic losses (Borel et al. 2006, Blumer et al. 2011). In continuing pregnancies of cattle, *C. abortus* infection typically occurs between the 6th and 8th months of gestation, sometimes resulting in weak and premature calves, especially in heifers experiencing their first pregnancy (Macías-Rioseco et al. 2020, Struthers et al. 2021). The serological prevalence of *C. abortus* can vary significantly between regions within and across different countries. (Softic et al. 2018, Adesiyun et al. 2020). In the presented study, the seroprevalence of infectious *C. abortus* infection among animals was determined to be 39.36% (n = 37). The seroprevalence of *C. abortus* in cattle varies between 0.83% and 51.3% worldwide (Yin et al. 2014, Derdour et al. 2017). On the other hand, some studies did not detect antibodies in dairy cattle herds with a history of abortion (Öztürk et al. 2012). The variability in the prevalence of *C. abortus* between our study and others can be attributed to various factors. Among these differences, there may be variations in the virulence of chlamydial strains and potential distinctions in innate immunity among animals. Additionally, factors such as frequent exposure of study animals to infected or carrier animals, unrestricted movement of diseased cattle from contaminated regions, nutritional deficiencies, suboptimal management practices, grazing strategies, cattle breeds, variations in the size of examined samples, types of serological tests used, and the efficacy of these tests (sensitivity and specificity), as well as the geographical location of the study, could contribute to this variation.

Coxiella burnetti infections are generally asymptomatic; however, they may be associated with reproductive disorders such as abortion, stillbirths, repeated estrus cycles, low birth weight in animals, and metritis (Tulu et al. 2018). Abortion events typically occur in the later stages of pregnancy, ranging from three to 80%, with unspecified characteristic clinical signs of *C. burnetii* infection (Angelakis & Raoult 2010). In this study, the seroprevalence of *C. burnetti* was determined as 24.26% (n = 23). *C. burnetii* has been detected on all five continents, displaying a broad prevalence across various species. The apparent prevalence is slightly higher in cattle (20.0 to 37.7%) compared to sheep and goats (around 15 to 25%) (Guatteo et al. 2011). A significant factor associated with low rates in herds is temperature, as fewer abortion events occur between the months of November and December. However, this trend gradually increases from January to February, subsequently decreasing again in March (Cantas et al. 2011). Previous studies suggest that tick species play a crucial role in spreading bacteria, especially in wild animals and are considered an important factor in transmitting the infection to domestic animals (Psaroulaki et al. 2006).

Sarcocystosis is an illness affecting birds and mammals caused by various species of single-celled coccidian protozoa within the sarcocystis group. Abortions are usually sporadic but may occur in the form of “storms.” Carnivores, both wild and domestic, spread sarcocystis, and cattle become infected through the consumption of feed or forages contaminated with carnivore feces. Abortions can take place at any stage of gestation (Kaltungo & Musa 2013). When pregnant mammals ingest sporocysts, they may experience abortions or give birth to stillborn fetuses. Definitive hosts typically do not exhibit any clinical signs of sarcocystosis (Fayer 2004). While 10-100% seropositivity was determined in sarcocyte screening studies in cattle brought to the slaughterhouse (Dubey et al*.* 1989, *Silva et al. 2002, Obijiaku et al. 2013),* it was reported that the presence of 0.50% sarcocytes was detected in 595 cases of aborted dairy cattle in the California Veterinary Diagnostic Laboratory System between 1987 and 1989 (Jamaluddin et al. 1996). In our study, no seropositive sample was detected for *Sarcocytes* spp.

Neosporosis is a common infection in cattle and a leading cause of abortions worldwide (Anderson et al. 2000). This protozoal parasite can persist as a chronic infection within the cow and be vertically transmitted to the fetus through the placenta during pregnancy (Schares et al. 1998). Most neosporosis-induced abortions typically occur around the 5th to 6th month of gestation (Dubey & Lindsay 2006). Cattle can also acquire *Neospora caninum* infection through horizontal (postnatal) transmission by ingesting oocysts shed in the feces of definitive hosts like dogs and coyotes (Dubey & Schares 2006, Gay 2006). The serological prevalence in cattle varies depending on factors such as country, region, the type of serological test used, and the cutoff level employed to determine exposure. In some dairy farms, up to 87% of cows may test seropositive (Dubey 2003). In the present study, the seroprevalence of *N. caninum* was 54.25% (n = 51). Some studies obtained similar results (Morales et al. 2001, Youssefi et al. 2010) or studies that obtained lower seroprevalence in previous studies conducted on cattle herds with a history of abortion (Derdour et al. 2017).

Cattle are commonly infected with BVDV, and pregnant animals that are vulnerable are likely to become infected fatally. The outcome of fetal infections varies depending on factors such as the timing of the infection, the biotype, and other properties of the virus. Confirming that BVDV infection is the cause of abortion is difficult since the virus can infect the fetus without causing abortion, and the infection causes a variety of fetal pathologies (Anderson 2007). Infections in the first trimester can lead to infertility, embryonic death, fetal resorption, mummification, or abortion. However, infections with non-cytopathic BVDV between 18 and 125 days of gestation may result in persistently infected live calves. Transient fetal infections, which are defined by the emergence of a fetal immune response, the synthesis of certain fetal antibodies, and the removal of the virus, are frequently the outcome of fetal infections that occur after about four months of gestation. On the other hand, later gestational infections can also result in abortions. Congenital defects can also be present in term calves born as a result of mid-gestational infections, which occur between 100 and 150 days of gestation (Grooms 2004). Virus isolation, serology, immunohistochemistry, PCR, and antigen capture ELISA approaches have been utilized to control this infection by screening for persistently infected animals (Anderson 2007). In the presented study, BVDV was determined to be 40.42% in the general distribution of infection among animals. It has been reported that the presence of BVDV has been detected in less than 10% of aborted animals (Meyling et al. 1990). In a previous study conducted in Burdur province on dairy cattle with abortion problems, the BVDV rate was determined as 2.2% (Öztürk et al. 2012). According to data obtained in this study, a high presence of BVDV was detected in cattle with cases of abortion in Burdur province, Turkey.

When we looked at the mixed distribution of the factors in the positive animals in our study, the highest rate of *C. abortus* + *N. caninum* (n = 9, 9.57%) and the lowest rate of *C. burnetti* + *N. caninum* (2, 2.13%) infections were found. *N. caninum* (29%) and *C. burnetti* (6%) were detected in pathological, microbiological and serological analyses in fetuses and placentas obtained from abortion cases of dairy cattle in Uruguay. *N. caninum* was also detected in two fetuses where BVDV was detected (Macías-Rioseco et al. 2020). It has been reported that there is a synergy between N. caninum and other viral agents (BHV-1 and BVDV), immunosuppressive agents and genetic factors in reproductive disorders in cattle (Björkman et al. 1996). As a matter of fact, Santos et al. (2005) determined that Leptospira spp., BHV-1 and BVDV were also potential abortive agents in cattle with a past of abortion and in cattle with N. caninum antibodies. Barr et al. (1991) reported that sensitivity to N. caninum infection increased in maternal viral infections (BHV-1/BVDV).

It was determined that *N. caninum* levels were high in three of the businesses and BVDV was found to be quite high in one. In the examinations carried out in semi-intensive farms, it was observed that dogs roamed freely around the animal living areas and came into contact with feed (hay, organic feed) and water bowls. It was reported that annual antiparasitic drug applications were not made to the dogs in the farms, and groundwater (no disinfestation, chlorination) was given to the animals. Information was received from the animal owner that most of the animals in the farms where BVDV infection was seen at a high rate were imported from abroad and that the farm was not vaccinated against BVDV. It was seen that the businesses included in the study were far from residential centers and close to forest areas. It has also been reported that there are problems such as the lack of forage crop production, inadequate and unsuitable barns, lack of labor force, high costs and lack of cultivated land in dairy cattle farms developed in Burdur province (Yılmaz & Ata 2016).

CONCLUSION

As a result, it was determined that *Neospora caninum*, followed by bovine viral diarrhea virus (BVDV), was the most important cause of waste in semi-intensive dairy farming in dairy cattle over two years old that aborted in the 4-7 month period in the Burdur region. We estimate that allowing dogs to roam freely in dairy cattle habitats, not giving antiparasitic drugs to dogs, and providing groundwater to animals have the largest share of abortion cases in these farms. An increase in infection may have also been caused by seasonal factors, inadequate physical conditions, and a lack of vaccination in the herds used in the study.

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Data availability statement.- The data that support the findings of this study are openly available in personnel archives. The authors confirm that the data supporting the findings of this study are available within the article.

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