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RESEARCH ARTICLE

The İncidence of Neospora Caninum in Dairy Cows with Abortion and İnfertility Problems in Aksaray Providence

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ABSTRACT

The contribution of animal husbandry to the national economy cannot be ignored. The causes of infertility are various and complex. Economic losses are increasing in dairy cattle breeding due to infectious and non-infectious causes. The percentage of abortion due to infectious agents is not fully known, but infectious agents lie in about 90% of the cases whose etiology can be determined. *Neospora caninum* is considered to be one of the most important abortion factors of cattle. Although, this protozoa is always ignored. It causes abortion in cows. In this study, it was aimed to detect *N. caninum* from the blood serum obtained from 137 dairy cattle brought to Aksaray University Faculty of Veterinary Medicine between 2017-2019 and suffering from abortion and infertility problems. For serological diagnosis, ELISA (Enzyme-Linked ImmunoSorbent Assay) test was performed.
As a result, *N. caninum* agent was detected in 37.22 % (51/137) of the samples that belong to cattle with abortion and infertility problems. It was concluded that economic loss due to infertility in dairy cattle and protozoa, which are one of the infection factors, should be given importance.
Keywords: Abortion, Dairy Cow, Infertility, *Neospora caninum*.

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Aksaray İlinde Abortus ve İnfertilite Problemi Olan Süt İneklerinde *Neospora Caninum* İnsidansı

ÖZ

Hayvancılığın ülke ekonomisine katkısı yadsınamaz. İnfertilitenin nedenleri çeşitli ve karmaşıktır. Süt sığırcılığında bulaşıcı ve bulaşıcı olmayan nedenlerle ekonomik kayıplar artmaktadır. Enfeksiyöz ajanlara bağlı abortların yüzdesi tam olarak bilinmemekle birlikte, etiyolojisi belirlenebilen olguların yaklaşık %90'ında enfeksiyöz ajanlar bulunmaktadır. *Neospora caninum* sığırların en önemli abortus faktörlerinden biri olarak kabul edilmektedir. Bununla birlikte, bu protozoa her zaman göz ardı edilir. İneklerde abortusa, köpeklerde ise ölümcül sinir-kas hastalıklarına neden olur. Bu çalışmada, Aksaray Üniversitesi Veteriner Fakültesi'ne 2017-2019 yılları arasında getirilen abort ve infertilite sorunu yaşayan 137 süt sığırından elde edilen kan serumundan *N. caninum'un* saptanması amaçlanmıştır. Serolojik tanı için ELISA (Enzyme-Linked ImmunoSorbent Assay) testi yapıldı. Sonuç olarak abortus ve infertilite sorunu olan sığırlara ait örneklerin %37,22'sinde (51/137) *N. caninum* etkeni tespit edilmiştir. Süt sığırlarında infertiliteye bağlı ekonomik kayıplara ve enfeksiyon faktörlerinden biri olan protozoalara önem verilmesi gerektiği sonucuna varıldı.
Anahtar Kelimeler: Abortus, İnfertilite, *Neospora caninum*, Süt İneği

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INTRODUCTION

The causes of fertility loss are diverse and complex. Problems that cause low productivity and infertility in dairy cattle farms are grouped under four main headings. These are as follows: functional disorders, infectious disorders, congenital-acquired structure anomalies and care-nutrition disorders. The causes of abortion in cows may be classified as non-infectious and infectious factors. Non-infectious causes are; hereditary or chromosomal disorders, nutritional problems, toxic conditions, and hormonal imbalances. Infectious causes are reported as bacterial, fungal and protozoal agents. The percentage of abortion due to infectious agents is not known, but infectious agents underlie approximately 90% of the cases whose etiology can be determined. While bacterial agents such as *Brucella, Campylobacter, Leptospira, Chlamydia, Salmonella, Listeria* cause bacterial infertility and abortions in cows; viruses such as Bovine viral diarrhea virus (BVDV) and protozoa such as *Trichomonas, Neospora, Toxoplasma, Sarcocystis* species cause infertility and abortions (Yoo, 2010). Neospora caninum, which is a protozoa agent, is one of the infectious causes that has been increasing in importance in cow reproduction in recent years. Infestation occurs by ingestion of food contaminated with an infected dog or fox feces containing the eggs (oocysts) of *N. caninum*. This protozoa, causes high abortion rates in cows. Dogs become infected when they consume infected cattle abortion material or placenta. Eggs are then produced in the dog's digestive tract and shed in the feces, remaining stable in the environment for about six or eight months. Eggs can be present in fresh or conserved forages such as grass, silage, hay and any other feeds. *Neospora caninum* is considered as one of the most important abortion factors of cattle. Neosporosis has been recognized one of the most important reproductive diseases in cattle all over the world. This protozoa, which has a widespread transmission feature, 90% of the cows in herds can be infected (Barber and Trees, 1996; Wouda, 2000; Dubey, 2003; Dubey et al., 2007; Dubey and Schares, 2011; Khan et al., 2020).

Whether Neosporosis is a problem for humans is still a question to be answered. Hence infected dogs and cattle may be major risk factors for transmission of the infection to humans especially in farm and slaughterhouse workers. Although the presence of protein structures or specific antibodies of the causative agent has been demonstrated in humans, there is no evidence that it can lead to abortion or other clinical findings. The other questions that are eagerly awaited about Neosporosis are; the reactivation mechanism of the protozoa during pregnancy, abortion mechanism, protozoa immunology, and drug treatment options. (Dubey, 2007; McCann et al., 2008).

Neospora caninum is an important cause of infectious abortion, infertility, early fetal death, stillbirth, decrease in milk yield-reproductive performance, earlier culling, decrease in post-weaning growth and feed efficiency in cattle world-wide (VanLeeuwen et al., 2010). Infection is common. Serological surveys suggesting that from 5-66 % of cows are seropositive, and the protozoa is passed by vertical transmission from cattle to calf with no signs of the disease. There is an increase in abortions world-wide, especially in European countries. In the examinations, it is determined that it is of *Neospora* origin. Seropositivity in dairy cows has been reported as 7-33% as a result of studies conducted in different regions in Turkey, which acts as a bridge between Europe and Asia (Kul et al., 2009; Dubey and Schares, 2011; Yıldız. and Gökpınar, 2017; Erol et al., 2019).

This suggests that the causative agent may cause diseases that cause significant economic losses in dairy cows. *N. caninum* is estimated to cause cattle producers losses exceeding a billion dollars each year world-wide. The annual economic loss in dairy, and beef cattle in Australia is estimated to be 85 million dollars, and 25 million dollars, respectively. In New Zealand, this loss is 17.8 million dollars annually in the dairy industry. In a study conducted in the USA, it was determined that there is a loss of approximately 1 kg per day in milk production in cows carrying *N. caninum* antibodies. In the Netherlands, the loss per farm is estimated at 249 €, on a country basis of 19 million €, and in Switzerland the average annual loss is 9.7 million €. It is estimated that up to 42% of cows abort due to Neospora caninum and the economic damage is directly related to the value of the fetus (Häsler et al., 2006; Dubey et al., 2007).

Several laboratory methods are now available for the detection of *N. caninum* infection in animals. Serologic examinations are proposed to detect *N. caninum* antibodies. Among serologic methods, ELISA technique is adequately reliable in terms of definingspecific antibodies titers to *N. caninum*. Furthermore, it has greater applicability for epidemiologic researchs (Dubey et al., 2007; Guido et al., 2016).

In this study, it was aimed to detect *N. caninum* from the blood serum obtained from 137 dairy cattle brought to Aksaray University Faculty of Veterinary Medicine between 2017-2019 and suffering from abortion and infertility problems. Thus, the presence of Neosporosis which is becoming increasingly important as a cause of abortion in dairy cows in the world, and in Turkey, will be determined in Aksaray. The study was planned with the thought that it would constitute an important source of data for the country's livestock and future studies.

MATERIAL and METHOD

1. Blood Serum Samples

In the study, blood serums obtained from a total of 137 dairy cattle with abortion and infertility problems brought to Aksaray University Veterinary Faculty for diagnosis between 2017 and 2019 were taken for study. Blood serums were centrifuged at 3000 rpm for 5 minutes, and stored. The resulting supernatant was inactivated at 56°C for 30 minutes and stored at -20°C until use.

2. Test Kit and ELISA Reader

A commercially available ELISA assay kit (IDEXX Neospora Ab Test, Cat No. 99-09566) was used in the study. The OD results obtained as a result of the tests performed with the ELISA kit were evaluated with the Biotek ELx800 ELISA reader.

Method

Double ELISA (Enzyme-Linked Immuno Sorbent Assay) test was performed on the samples whose serums were taken for serological examination. Serum samples were applied with the commercial ELISA kit for the presence of IgG against *N. caninum* as specified by the company and the results were obtained.

It was examined with a spectrophotometer at a wavelength of 630 nm. Serum samples causing ≤ 30% inhibition were considered positive. All blood serums were studied in duplicate.

In the study, 2 groups were formed in the light of the information collected from the sick animals and/or samples that came to the veterinary faculty (Tables 1 and 2). Group formation was based on whether the animals gave birth and how many times they gave birth. According to the information in the inventory, it was observed that the animals in group one had repeat breeder complaints. In Group 2, it was determined that there was an abortion problem.

Group 1 (n=35); animals that have never given birth (nulliparous), Group 2 (n=102); defined as the group of animals that primipar, and/or multipar. According to these criteria, a total of 137 blood serum samples formed the first group.

The second group was divided into 4 groups (Lactation I-II-III and IV) according to their lactation status (Table 2). The blood sample numbers in the four groups were 27, 38, 24, and 13, respectively. In this way, homogeneity was tried to be achieved between the groups according to the number of births per animal.

RESULTS

In the results obtained in the study, *N. caninum* agent was detected in 37.22% (51/137) of the total samples. Their distribution is given in tables the below (Tables 3 and 4).

Accordingly, in table 3; No causative agents were detected in group 1. In group 2, the causative agent was detected in 50% (51/102). This determination gives us information about the distribution of *N. caninum* in the herd. There is a direct ratio between the increasing age of the animal and the contamination.

In table 4, according to the lactation status (I, II, III, and IV, respectively) of the agent detected from the samples in group 2, 7.41% (n=2/27); 60.53% (n=23/38); 70.83% (n=17/24) and 69.03% (n=9/13) were detected.

Table 1. Animal groups.

Groups	Maternal status	n
1	Nullipar	35
2	Primipar and Multipar	102

Table 2. Distribution of animals in group 2 according to their lactation status.

Lactation status	n
I	27
II	38
III	24
IV and more	13

Table 3. Distribution of sera with antibodies to *N. caninum* by groups.

Groups	*N. caninum*
1	0 % (0/35)
2	50 % (51/102)

Table 4. Distribution of the presence of antibodies detected in animals in Group 2.

Lactation status	n
I	7.41 % (2/27)
II	60.53 % (23/38)
III	70.83 % (17/24)
IV and more	69.03 % (9/13)

DISCUSSION

Neospora caninum has been recognized world-wide, first all as a disease affecting only dogs, then as an important cause of infection abortions in cattle. It is a protozoa that was first recognized as a cause of abortion in infected cows (Barber and Trees, 1996; Dubey, 2003).

Neosporosis is responsible for abortion and neonatal deaths in cattle (Barber and Trees, 1996; Dubey, 2003; Ocal et al., 2014). Therefore; this suggests that the causative agent may cause diseases that cause significant economic losses in dairy cows. *N. caninum* is estimated to cause cattle producers world-wide losses exceeding a billion dollars each year (Reichel et al., 2013). The increase in abortions originating from *Neospora caninum* draws attention in European countries and of course in Turkey, which acts as a bridge between Europe and Asia (Akca et al., 2005; Vural et al., 2006; Ica et al., 2006; Kul et al., 2009; Kurtdede and Ural, 2009;Ocal et al., 2014).

Seropositivity in dairy cows in our country varies between 7% and 33%. In *N. caninum* screening studies conducted in cattle from different regions in Turkey: 5.5% in Kırıkkale region (n: 200; Yıldız and Gokpınar, 2017), 7.2% in Kars region (n: 1100; Mor and Akca, 2012), 5-33% in Central Anatolia region, 21.03% in Afyonkarahisar region (n:485; Celik et al., 2013), and 23.4% in Kırşehir Çiçekdağı region (n:116; Yıldız et al., 2017) are reported as seropositive. Some researchers have detected a seropositivity rate of 32.35% (n:986) in the field surveys they have conducted in different provinces (Erol et al., 2019). Acıcı et al., (2019) reported that they detected 49.9% *N.caninum* DNA in their study on waste fetuses. Although 37.2% (51/137) seropositive results detected serologically in our study are higher than in some regions mentioned above, it is consistent with the rate found in studies conducted especially in Turkey.

The gradual increase in Neosporiasis in Turkey is also noteworthy. In our study, *N. caninum* was detected in Aksaray province. The presence of the agent was found to be 50% (n:51/102) in group 2 in aborted dairy cows. In this study, it was concluded that the high seropositive rate was due to the detection of the agent on the blood serums brought with the complaint of abortion. Since other reported studies are in the form of field scanning, it is seen that the seropositivity does not exceed 30% (Celik et al., 2013; Erol et al., 2019).

As a result, the presence of *N. caninum* in dairy cows was detected in Aksaray province. It was concluded that the protozoa should be found, and the studies should be more comprehensive, and concentrated throughout the province. It was concluded that animal owners and veterinarians should be informed about this infection and the study should be expanded more comprehensively. We believe that these results will be a source for future studies. It was concluded that *N. caninum* infection may also be responsible for abortion and economic losses in dairy farms in Aksaray province.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Permisson: This study was made from blood samples brought to Aksaray University Veterinary Faculty Virology Department and is not subject to HADYEK's permission in accordance with Article 8 (k) of the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees. In addition, the authors declared that they comply with the Research and Publication Ethics.

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