

Study of the serological prevalence of Neospora caninum in healthy and aborted cows in Aq Ghala

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Article	Abstract
<p>Received: 26th November 2021 Received in revised form: 10th December 2021 Accepted: 13th December</p> <p>Keywords: Neospora, Neosporosis, Dairy cow, caninum, Abortion</p>	<p>The causative agent of neosporosis is a protozoan called <i>Neospora caninum</i>, a branch of the <i>epicomplex</i>. This Parasite can infect various species of domestic and wild animals. It is found in dogs, cattle, sheep, and goats in nature. It is noteworthy, however, that dogs are known to be the ultimate host of this protozoan, and other warm-blooded animals are the intermediate and random hosts of the Parasite, which become infected by eating oocytes excreted in dog feces. Therefore, considering the critical role of this Parasite in abortion and the significant damages caused by it, this study aimed to investigate the serological prevalence of <i>Neospora caninum</i> in healthy dairy cows and aborted in Aq Qala in Golestan province. In this study, 90 dairy cows from semi-industrial farms in Aq Qala and its suburbs were sampled in the winter of 2021. The sampling of the study population was blood sampling from the tail vein. The samples were stored at -20 ° C until testing. During sampling, the animal's characteristics, such as calving belly (in three categories of several calves less than two bellies, between two to five bellies and more than five bellies), will be recorded. The ELISA method performed a serological examination of infection caused by <i>Neospora caninum</i>. The results were analyzed in three groups using the chi-square method using SPSS software version 20. The results obtained in the present study indicated that 45.6% of the samples were infected with <i>Neospora caninum</i>. Also, the frequency of repeat breeder was 44.4%, abortion was 38.9%, and healthy was 16.7%.</p> <p>It was also found that there is a substantial difference between the factors of repeat breeder, abortion and healthy, in positive and negative samples ($p < 0.05$) and the number of positive samples between cows with a history of abortion and repeat breeder more than Cows are healthy. On the other hand, there was no significant difference between the repeat breeder, abortion and healthy samples ($p < 0.05$). Therefore, due to the high prevalence of <i>Neospora caninum</i> infection in Aq Qala city of Golestan province, the use of health instructions to control this complication should be considered.</p>

Introduction

Neospora caninum was first identified in 1984 in dogs in Norway. Neosporosis was first reported in 1989 in New Mexico dairy cows with a history of miscarriage. Research following the observation of neosporosis in California has shown that abortions caused by *Neospora caninum* in dairy cows are not a newly emerging infection [1]. The evolution of *Neospora caninum* consists of three stages: tachyzoite, tissue cyst, and oocyst. The dog, as the final host, excretes non-sporulated oocytes along with the feces, which are converted to spore form in the environment, then sporulated oocytes or spores form with water and food by intermediate hosts such as cattle, sheep, goats, horses, and other animals are consumed [2]. The two stages of asexual division of tachyzoite and bradyzoite occur

in the intermediate host body. After the spore oocysts enter the middle host body, the sporozoites enter the macrophages in the released intestine because they multiply rapidly to give them tachyzoite. They say, then the macrophage cell is torn, and the tachyzoites attack the cells of other organs but are primarily found in nerve cells [3].

Neospora caninum has a dual host cycle; the dog is the ultimate host and acts as an intermediate host. The final host in which the Parasite's sexual reproduction has been unknown for many years. It was thought to be a predatory animal until, in 1998, researchers found oocysts in the feces of dogs that ate mice infected with *Neospora caninum*. The oocytes expelled by the final host are oval. They are 10-11 microns in diameter and were sprayed in the outside environment for three days, and two sporocysts are formed in each oocyst; each sporocyst contains four sporozoites [4-8]. Cattle, sheep, goat, deer and horse are the intermediary hosts. Solid antibodies have been detected in the serum of buffalo, coyotes, red and grey foxes, and camel, as well as mice, rats, fox, cat, and rabbit that have been experimentally infected. The Parasite is found mainly in cattle and horses [9].

One study showed that humans could be exposed to infection, but no evidence that the disease was zoonotic. Inhumane primates have been experimentally infected and have transmitted the infection to their offspring. Adult non-pregnant cows show no signs of the disease if they become infected with *Neospora caninum*. Dairy and broiler cows may abort their embryos, whether recently infected or congenitally infected. Abortion may occur in recurrent pregnancies. Cows may have abortions at any age from the third to the last month of pregnancy. Most miscarriages happen in the fifth to sixth months of pregnancy. More cases do not have these antibodies, which is valid for dairy cows and broilers [10, 11]. In a seroepidemiological study of *Neospora caninum* infection in cows of Sanandaj city, the rate of parasite infection was associated with increasing age. The infection rate in suckler cows was much higher than that of non-suckler cows. The rate of infection in farms with dogs (46.8%) compared to farms without dogs (7.43%) showed a significant difference [12]. In a study on the detection of *Neospora caninum* in aborted embryos in cattle farms in Arak city by PCR method, it was concluded that there was a significant relationship between neosporosis infection and the number of abortions performed in the studied cows [13].

Method

In this study, 90 dairy cows were sampled from semi-industrial farms in Aq Qala and its suburbs in the winter of 2021. The sampling of the study population was blood sampling from the tail vein. The samples were stored at - 20 ° C until testing. At the time of sampling, the animal's characteristics, such as calving abdomen (in three categories of number of calves less than two bellies, between two to five bellies and more than five bellies), were recorded.

Serological examination

The ELISA method performed a serological examination of infection caused by *Neospora caninum*. In this ELISA, the P38 antigen was further evaluated, and the cut-off was determined. This study used PBS-T solution (containing 20% horse serum) and blocking solution (diluted 1:50) to cut serum samples. The diluted serum was incubated in ELISA plates containing *Neospora caninum* antigen blocked by horse serum for one hour. Next, wash with PBS-T solution and incubate with Anti-dog IgG conjugate bound to Horseradish Peroxidase enzyme. After rinsing again with PBS-T solution and distilled water, substrate solution and chromogen (hydrogen peroxide and tetramethyl benzene) Was added. After incubation, the reaction was stopped with 2 N sulfuric acid. The test results were read at 450 nm. The results were analyzed in three groups of abortion by chi-square method using SPSS software version 20.

Results

The frequency of calving status of the studied cows is shown in Table 1.

Table 1 Frequency of calving status of the studied cows

Group	Abundance	Number
Less Than Two Bellies	33.3	30
Between Two And Five Bellies	33.3	30
More Than 5 Bellies	33.3	30

The frequency of positive and negative samples in the studied cows is shown in Table 2. The results obtained in the present study indicate that 45.6% of the samples were infected with *Neospora caninum*. Table 3 showed that the frequency of repeat breeder was 44.4%, abortion was 38.9%, and healthy was 16.7%.

Table 2 Frequency of positive and negative samples in the studied cows

	Abundance	Number
Positive	45.6	41
Negative	54.4	49

Table 3 showed that the frequency of repeat breeder was 44.4%, abortion was 38.9%, and healthy was 16.7%.

Table 3 Frequency of repeat breeder, abortion and healthy

	Number	Frequency
Repeat Breeder	40	44.4
Abortion	35	38.9
Healthy	15	16.7

Table 4 shows no significant difference between positive and negative samples regarding the number of calves ($p < 0.05$).

Table 4 Comparison of negative and positive samples in terms of number of births

	-	+	K square value	P-value
Under two calving abdomen	16	14	0.627	0.731
Between two and five calves	18	12		
More than five calves	15	15		
	49	41		

Table 5 shows no significant difference between the repeat breeder, abortion and healthy samples ($p < 0.05$).

Table 5 Comparison of repeat breeder, abortion and healthy samples in terms of number of births

	Repeat Breeder	Abortion	Healthy	K square value	P-value
Under two calving abdomen	14	13	3	2.76	0.598
Between two and five calves	15	9	6		
More than five calves	11	13	6		
	40	35	15		

Table 6 shows that the factors of repeat breeder, abortion and healthy in positive and negative samples show a significant difference between the samples ($p < 0.05$).

Table 6 Comparison of repeat breeder, abortion and healthy samples in terms of positive and negative

	-	+	K square value	P value
Repeat Breeder	17	23	15.34	0.0001
Abortion	17	18		
Healthy	15	0		
	49	41		

Discussion

The causative agent of neosporosis is a compound called *Neospora caninum*, a branch of the epicomplex. This Parasite can infect various species of domestic and wild animals. In nature, it is found in dogs, cattle, sheep and goats [14]. Neosporosis is one of the leading causes of bovine abortion. This disease is important due to its widespread prevalence and economic losses [15]. Infectious cattle is often chronic and remains infected throughout the animal's life [16]. The disease is transmitted vertically in cattle and placenta to the fetus through the placenta and continues in subsequent pregnancies. A vaccine that can stop the infection through endogenous (placenta) is unavailable [17]. The results obtained in the present study showed that 45.6% of the sample was infected with *Neospora caninum*. Also, the frequency of repeat breeder was 44.4%, abortion was 38.9%, and healthy was 16.7%. On the other hand, there was no significant difference between the repeat breeder, abortion and healthy samples ($p < 0.05$). In 2013, Macedo et al. Found that in dairy cows slaughtered by ELISA, the consumption rate was 41.6% in pregnant cows, 43.3% in non-pregnant cows, and 5.5% in fetuses the presence of DNA in PCR analysis. 3.3% of cows and 6.6% reported abortions [18]. The researchers found that the highest infection rate in aborted fetuses was 4-6 months.

In comparison with the present study's findings, it does not show a significant difference in terms of the frequency of positive samples. In a serological survey of *Neospora caninum* infection in dairy cows in Golestan province, it was shown that out of 800 cows tested, 107 cows (36.2%) showed anti-*Neospora* antibody in the blood serum. According to this study, the rate of abortion in dairy cows with *Neospora caninum* protozoa is higher than cows without neosporosis infection. Also, no significant difference was observed in the incidence of *Neospora caninum* in different age groups (under two years, between 2 to 4 years and over four years) [19]. The results obtained in the above study and the results obtained in the present study indicate a significant infection of dairy cows in Golestan province with the Parasite *Neospora caninum*. In a study examining the prevalence of *Neospora caninum* in ruminant milk by molecular method, they stated that out of a total of 440 milk samples, 54 samples (12.27%) were infected with *Neospora caninum*. Cow's milk had the highest (26%), and sheep's milk had the lowest (4%) prevalence of *Neospora caninum*. Milk samples collected in winter had the highest (22.85%), and samples collected in summer had the lowest (8.57%) prevalence of *Neospora caninum*. Milk samples collected from Chaharmahal and Bakhtiari province had the highest (14.94%), and samples collected from Isfahan had the lowest (6.38%) prevalence of *Neospora caninum* [20].

In a study, by detecting the Parasite *Neospora caninum* in aborted embryos in Arak city farms by polymerase chain reaction (PCR) method, they stated that 26.3% of the brain of aborted embryos contained DNA of *Neospora caninum*. In the cerebellum and the spinal cord of aborted fetuses, the presence of DNA *Neospora caninum* was not observed. There is a statistically significant relationship between *Neospora caninum* infection, number of calves, history of abortion, and dogs' presence in the herd. The results of this study showed a significant relationship between neosporosis infection and the number of abortions performed in the studied cows; For this reason, it seems that this protozoan can be considered as one of the important causes of abortion epidemics in dairy herds of cattle in Arak city [13]. In the present study, it was observed that there is a significant difference between the factors of repeat breeder, abortion and healthy in positive and negative samples ($p < 0.05$) and the number of positive models between cows with a history of abortion and repeat breeder are more common than healthy cows. A study comparing the infection of different tissues of aborted cow embryos with *Neospora caninum* parasite by PCR method stated that infection with this Parasite in the brain tissue of 34 aborted fetuses (41.5%) was identified. In 2 (2.4%) and 4 (4.9%) separate aborted embryos, respectively, the parasite DNA was isolated from lung and liver tissues in addition to brain tissue [21]. In Argentina, the prevalence of infection was reported in dairy herding dogs (48%) and broiler dogs (54.2%) higher than in urban dogs (2.22%) [22]. In a study, the seroepidemiology of *Neospora caninum* in Iranian native cows was studied and reported that the total serum frequency of *Neospora caninum* antibody was determined to be 20%. The highest and lowest frequency of infection was observed in cows over four years of age, 32.33%, and under two years of age, 7.32%, respectively [1].

Conclusion

Neosporosis in cattle has been reported in many countries and is almost universally prevalent. In some countries, the Parasite is a significant contributor to abortion epidemics. In California, 20 to 30% and in the Netherlands, 17% of all bovine abortions are reported. According to various statistics from Iran and other countries, 12 to 42%

of aborted fetuses in cows are infected with this Parasite. In the present study, it was found that 45.6% of the samples were contaminated with *Neospora caninum*. Also, the frequency of repeat breeder was 44.4%, abortion was 38.9%, and healthy was 16.7%. On the other hand, there was no significant difference between the repeat breeder, abortion and healthy samples ($p < 0.05$). It was also found that there was a substantial difference between the factors of repeat breeder, abortion and healthy in positive and negative samples ($p < 0.05$), and the number of positive samples between cows with a history of abortion and repeat breeder was more than Cows are healthy.

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