



Evaluation of *Neospora caninum*-Associated Abortion in a Seroepidemiological Study of Cattles Using IFAT Method

Seyed Razi Bahavarnia^{1*}, Pedram Ahli², Arash Rasouli³

¹Department of Parasitology, Islamic Azad University, Tabriz Branch, Tabriz, Iran

²Department of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

³Department of Obstetrics and Reproductive Diseases, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Abstract

Background: *Neospora caninum* is a cyst forming coccidian parasites and one of the important factors in abortion of cows and neurologic disease in dogs around the world. This study was conducted to investigate the seroprevalence of *N. caninum* in dairy cows of Tabriz city using immunofluorescence method and related risk factors.

Methods: In this study conducted in 2018, 100 samples from industrial cattle farms and 100 samples from traditional cattle farms of Tabriz were randomly collected. By fixing tachyzoites cultured in a culture medium, the kit was prepared in this study and the slides were examined by fluorescence microscopy (Zeiss). The results obtained in relation to the study variables (type of farming system, age, contact with dogs, and abortion history) were analyzed using SPSS software and chi-square test.

Results: *Neospora caninum* antibodies were detected in 33 samples (16.5%). The relationship of positive serums with abortion and contact with dogs was significant statistically ($P < 0.05$).

Conclusions: Due to the significant relationship of infection rate in cows with abortion history and contact with dogs, further studies are needed to determine the exact role of *Neospora* in abortion of cows.

Keywords: *Neospora caninum*, IFAT, Cattles, Abortion rate

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Background

Neospora caninum is an obligate intracellular protozoan that is considered as one of the main causes of abortion and encephalitis in cattle (1). The importance of this parasite is due to direct damage caused by abortion and indirect losses, including costs of diagnosis, insemination of cattle, and reduction in milk production (2). The global importance of Neosporosis is due to the wide dissemination in the world and its great economic losses. This parasitic protozoan can infect a wide range of different species of animals. Dogs and coyotes are definitive hosts of *N. caninum* and cow and other domestic animals are considered as natural intermediate hosts of parasites (3). *N. caninum* in dogs causes paralysis of the posterior motor limbs, difficulty in swallowing, paralysis of the jaw, muscle flaccidity, muscle atrophy, and sometimes dermatitis. Cows may be infected by ingesting *N. caninum* oocytes (the horizontal transmission), but the vertical transmission is the main way of infection in cows. This form of transmission of infection can take place in the next pregnancies of a cow. Therefore, the infection could be transmitted to the next generation and stabilized

in infected cattle. Infection by this parasite is usually chronic and can threaten the cattle until the end of its life (4).

Non-pregnant adult cows infected with *N. caninum* show no symptoms of the disease, but the infection in pregnant cows may cause abortion or the birth of the weak calves with subclinical infection.

In fact, the only symptom of Neosporosis is abortion in infected pregnant cows, which may occur at any age. It seems that the rate of abortion in infected cattle is three to seven times higher compared to non-infected cattle and abortion occurs usually at 5-6 months of pregnancy. The fetus may die in the womb and autolysis and mummification may occur. Although there is no accurate knowledge of the economic loss due to Neosporosis around the world, it has been estimated to be millions of dollars (5).

As the diagnosis of Neosporosis is not possible solely through clinical symptoms, it should be based on observing pathological effects of parasites on aborted fetuses and relying on serological tests, and diagnosis in live cattle is usually done by serologic methods including

*Corresponding Author: Seyed Razi Bahavarnia, Email: Razi.bahavarnia@yahoo.com

IFAT, ELISA, Neospora agglutination test (NAT), and western blot (6). Immunofluorescence method was used for the first time by Dubey for confirming antibodies of *N. caninum* in dogs, and it was the first serological criterion for the diagnosis of *N. caninum* infection in cows.

IFAT is considered as the gold standard method compared to other serological techniques due to trace or negative cross-reactivity with other Apicomplexa. The test has a sensitivity of 99% and specificity of 98% (7). This study was conducted to serologically investigate *N. caninum* in dairy cows of Tabriz city using immunofluorescence method.

Materials and Methods

In this study conducted in 2018, 100 samples from industrial cow farms and 100 samples from traditional cow farms of Tabriz were selected randomly. Then, blood samples were isolated after centrifugation at 2000 rpm for 5 minutes and they were kept at -20°C until experiment time.

By fixing tachyzoites cultured in a culture medium, the kit was prepared in this study. To prepare immunofluorescence slides, approximately 5-10 µL of culture medium containing *N. caninum* tachyzoites was poured on microscopic lam. After tachyzoites being dried, acetone was used to fix them on the lam surface, and after acetone evaporation, the range of each bezel fixed by tachyzoites was determined using lac to prevent the integration of sera added in the next stages and to increase the accuracy of experiment. The recommended cut-off point to determine the seroprevalence of *N. caninum* in adult cows using IFAT technique is 1/200 and it is 1/25 in dogs.

Then, 10.5 µL of serum diluted in PBS were added to each bezel, slides were humidified in an incubator for 30 minutes, and the temperature was set at 27°C. After rinsing slides three times by PBS and drying the surface of slides, 5-10 µL of conjugate was added to each of the bezels, and FITC conjugated affinity purified anti-bovine Ig-M (VmrD Inc., Pullman, Washington, USA) was used for cow samples.

The slides were again placed in humidity chambers at 27°C for 30 minutes, and their surface was rinsed three times by PBS and dried. Then, the slides were stored in a solution containing 50% glycerol and 50% phosphate buffer and were examined by fluorescence microscopy (Zeiss) (8). The results obtained in relation to the study variables (type of farming system, age, contact with dogs, and abortion history) were analyzed using SPSS software and Chi-square test.

Results

The results of serological investigations using IFAT technique indicated the presence of antibody in 16.5% of serum samples (33 out of 200) obtained from cows of Tabriz city. Infection in industrial cows was 5%, while

it was 28% in traditional cows. The results of statistical tests for the comparison of these two groups indicate a significant difference in terms of infection between cows from traditional and industrial cattle farms (Table 1).

This study showed that rate of infection was different in varied age groups and the highest infection rate was found in the age group of over 4 years (25%). Based on the results of statistical analysis, a significant correlation was found between infection and age (Table 2).

Considering the role of canines in increasing seropositivity, statistical tests indicate the significance of this difference in two studied groups (Table 3).

In addition, the investigation conducted to determine the relationship between *N. caninum* infection and the frequency of abortion in studied cows showed that there is significant correlation in this regard. In other words, Neosporosis can be an effective factor in the reoccurrence of abortion in infected cows (Table 4).

Table 1. Prevalence of *Neospora caninum* Antibodies in Industrial and Traditional Cattle Farms

	Total Samples	Positive Serum Samples	Infection Percentage
Industrial livestock farming	100	5	5
Traditional livestock farming	100	28	28
Total	200	33	16.5

Table 2. Prevalence of Anti-*Neospora caninum* Antibodies in Different Age Groups

	Total Samples	Positive Serum Samples	Infection Percentage
Under 2 years	66	7	10.60
2-4 years	66	9	13.63
Over 4 years	68	17	25
Total	200	33	16.5

Table 3. The Prevalence of *Neospora caninum* Antibodies in Cows with and without Contact with Dogs

	Total Samples	Positive Serum Samples	Infection Percentage
Contact with dogs	78	22	28.2
No contact with dogs	122	11	9.00
Total	200	33	16.5

Table 4. Prevalence of *Neospora caninum* Antibodies in Cows With and Without a History of Abortion

	Total Samples	Positive Serum Samples	Infection Percentage
With abortion history	20	17	85.0
Without abortion history	180	16	8.88
Total	200	33	16.5

Discussion

Neospora caninum is one of the main causes of abortion and encephalitis in cows. Given the importance of virulence in Neosporosis and considering that the cost spent on diagnosis and prevention of abortion is lower than the loss imposed to the producer as a result of abortion in a herd of dairy cows, and that no appropriate drug or vaccine has been recognized (9), studies are very important to reduce economic losses and side effects caused by Neosporosis. Determination of infection rate of *Neospora* in cows depends on factors such as geographic location, type of test, and cut-off point (10).

Neospora caninum is the cause of abortion in cows. In Argentina, 18.9% of beef cows and 43.1% of dairy cows, 60% of adult cows and 20% of calves in Uruguay (11), and 18.9% of cows in Northeast China were positive for *N. caninum* infection (12).

In Iran, the prevalence of *N. caninum* antibodies in cow was investigated in 2004 (13) and it was reported to range from 7 to 53.71%. It was reported that the prevalence of *N. caninum* antibody was 32% in 2009 in the northern areas of Iran. Researchers reported that the prevalence of antibodies in dairy cows of Mashhad was 15.18% (14).

Researchers in 2008 reported that the infection rate was 21% in cows of Ahvaz (15). In the study conducted by Ranjbar-Bahadori et al, the infection was observed in 38.5% of dairy cows with abortion (16-22). In a study conducted on cows by Yousefi et al in 2009, a seroprevalence of 7% was reported in Ardebil, 45.2% in Garmasar, and 57.3% in Babol. Additionally, Nourollahi Fard et al in 2008 reported a seroprevalence of 12.6% in Kerman (20,23,24).

In the present study, *N. caninum* antibodies were present in 16.5% of the samples (200) using immunofluorescence technique. Investigations show that factors such as the weather can play a significant role in this parasite epidemic. Moreover, it is obvious that temperature and other environmental factors are very effective. By disposal of parasite oocyte by final host and its presence out of the host body, the crucial role of moisture, temperature, and other environmental factors is displayed to pass the Sporogony stages and create infection stages in intermediate host (17).

The results of this study indicated that infection in industrial cows was 5%, and it was 28% in traditional cows. In 2009, in the north of Iran, infection rate was reported to be 25.8% in industrial cows and 43.9% in local cows (18).

In the current study, the highest infection rate was observed in the age group of 4 years and age had a significant impact on the infection rate. There are different views on the relationship between age and the infection rate in cows. Jesen et al in a study conducted on the rate of *N. caninum* infection in Danish cows found that the infection rate increases with age (13). In the study conducted by Gharekhani et al in 2012, considering that

the highest rate of infection was observed in 4-year-old cows and the lowest infection rate in cows under two years, the horizontal transmission is probably the main way of infection in cows of this region (10).

However, in a study conducted by Ranjbar-Bahadori et al (18), this relationship was not significant. Bartlet et al showed that the impact of the presence of dogs on the infection rate was significant. They observed that the presence of a dog among the cows is a protective factor against *N. caninum* infection and this protection can be due to it since the presence of herd dogs causes stray dogs and other canines, that are the main causes of infection, to be away from them (3). In the study conducted by Ranjbar-Bahadori, a significant relationship was also found between Neosporosis and the number of abortions so that 95% of dairy cows in which *N. caninum* antibody was positive had abortion history (18).

Conclusions

Due to the significant relationship of infection rate in cows with abortion history and contact with dogs of the area, the need for complementary studies to determine the exact role *Neospora* in the abortion of cows is essential. IFAT technique is the first applied serological test in diagnosing Neosporosis and it has been used more than other methods in recent years. This test is more cost-effective than ELISA test in studies in which a great number of samples are used. As there is no proven method to control or treat Neosporosis, control methods should be conducted to decrease the vertical and horizontal transmission.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Issues

In this research, ethical considerations have been fully observed.

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Authors' Contribution

SRB did writing and editing of the manuscript. PA designed and did data collection. AR designed, did data collection and statistical analysis.

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