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Seroepidemiology of *Neospora Caninum* in Dairy Cattle Farms with a History of Abortion in Isfahan Province, Iran

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Abstract

Background: *Neospora caninum* is a worldwide-distributed pathogen which causes abortions in dairy cattle, leading to economic losses in the cattle industry. The main objectives of the present work was to determine the seroprevalence of *N. caninum* antibodies in dairy cattle farms in Isfahan province, central Iran and to investigate the factors associated with the infection.

Methods: 611 sera from Holstein dairy cattle were collected from 25 farms with an abortion history. The sera were divided according to age, pregnancy, lactation state, farm type, feed and contact with caniide. The antibodies to *N. caninum* were detected using a commercially available ELISA kit.

Results: *N. caninum* antibodies were detected in 32.1% of the sera and 96% of the farms. The percentage of infection of animals in different farms ranged from 0.0% to 60% which is statistically significant difference ($p<0.05$). *N. caninum* seroprevalence was significantly higher in pregnant cattle as compared to that in non-pregnant cattle ($p<0.05$). Also cattle contact to watch- dog and wild-caniide showed a higher seroprevalence than cattle contact alone to watch-dog ($p<0.05$). No statistically significant difference in *N. caninum* seroprevalence was noted between age groups, lactation states, farm types, and cattle feed groups ($p>0.05$).

Conclusion: The results demonstrate that the seroprevalence of *N. caninum* is relatively high in cattle from the Isfahan province, central Iran. Further investigation is needed for the confirmation of infection and to confirm the effects of co-infection on *N. caninum* infection in cattle.

Keywords: *Neospora caninum*; Sero-epidemiology; Dairy cattle; Isfahan; Iran

Introduction

Neospora caninum (Apicomplexa: Toxoplasmatinae) is a worldwide-distributed pathogen which causes abortions in beef and dairy cattle in many countries around the world, leading to economic losses in the cattle industry [1,2]. *N. caninum* has a two-host life cycle, including intermediate hosts (mainly cattle and other warm-blooded animals) and definitive hosts (dogs and coyotes) [3,4]. In cattle, transplacental transmission from infected cows to their offspring appears to be the major natural route of infection, and congenitally infected calves remain persistently infected and can pass the infection to their offspring [5]. Dogs and coyotes are the only species recognized as acting as the definitive hosts, in which the sexual phase of development of *N. caninum* occurs, resulting from the shedding of oocysts in the feces [4,6,7]. However, the exposure to *N. caninum*, through oocyst-contaminated food or drinking water, is regarded as the most probable cause of infection in some herds [8,9]. Cows infected with *N. caninum*, both acutely and chronically, have up to 5.7–18.9% higher risk of abortion than non-infected cows [10,11]. Diagnosis of *N. caninum* infection in these cases has been based largely on the serological testing by immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) of serum from all or parts of the affected dairy herds.

In Iran, the presence of *N. caninum* antibodies has been described in cattle but a few serological surveys carried out for the detection of *N. caninum* infection in different parts of our country [12]. The main objectives of the present work was to determine the seroprevalence of *N. caninum* antibodies in dairy cattle farms in Isfahan province (in center of Iran), and to investigate the factors associated with the infection.

Materials and Methods

Study area

The study was carried out on Holstein dairy cattle farms around Isfahan (latitude 30°43′–34°27′ N, longitude 49°36′–55°31′ E). Isfahan is located in center of Iran, in the lush plain of the Zayandeh River, at the foothills of the Zagros mountain range. The city situated at 1590 meters above sea level, enjoys a temperate climate and regular seasons and is very hot during the summer with maxima typically around 36 °C (97 °F). However, it has low humidity and moderate temperatures at night. During the winter, days are mild but nights can be very cold. Its annual precipitation is 113 millimetres.

Animals

The population targeted was 611 dairy cows (Holstein–Friesian) from 25 industrial and non-industrial farms randomly selected in the North, South, East and West of Isfahan. Farms were selected by stratified random sampling method. Based on the previous studies the prevalence of *Neospora* antibody was 30% and a 5% tolerable error was assumed when determining the desired sample size of cattle to be sampled. The cattle sample size (322) was determined according to the random sample size formula where 1.96 was the z value for the desired confidence level (95%). By sampling animals dependent on farm herd size, and reducing the clustering effect a final sample size of 611 animals were sampled and examined in this study.

Range of sampled cows on the farms was from 5 to 56 animals. According to the farm records, abortion had been observed on the all farms. Cattle were grouped according to age (under 12 months: 109; 12-24 months: 105; and over 24 months: 397), Pregnancy (Pregnant: 355; and Non-pregnant:256), Lactation state (Lactated cows: 460; and Non-lactate cows: 151), Farm type (industrial: 489 and non-industrial: 122), Feed (Dried alfalfa+ silage +concentrate: 489; and Fresh alfalfa+ dried alfalfa+ silage +concentrate: 122) and the Contact with caniide (Watchdog: 493; and Watchdog and wild caniide:118).

Sampling

Samples of blood serum from female cattle were examined. Blood samples were taken using disposable needles (venoject) (Ava Co., Tehran, Iran). All samples were immediately transported to the diagnostic laboratory. Serum was removed after centrifugation at 1000 x g for 10 min. All sera were divided equally into two micro tubes and stored at −20 °C until tested.

ELISA test

The antibodies to *N. caninum* were detected using a commercially available ELISA kit (enzyme-linked immunosorbent assay; IDEXX Laboratories Inc., Westbrook, Maine, USA), following the manufacturer's instruction. Briefly each serum sample (diluted 1:100) was added to the *Neospora* antigen-coated microplates, which incubated at room temperature for 30 min. The reaction was revealed adding the supplied chromogen, and after 30 min, the reaction was stopped. Plates were read at 630 nm. Results are expressed as sample to positive (S/P) ratios, as recommended by the manufacturers. Serum samples with S/P ratio is greater than or equal to 0.5 are classified as positive for *N. caninum* antibodies.

Variables studied

A questionnaire addressing epidemiological aspects of the farms relating to *N. caninum* including record of abortions and management system was used. The variables of breed, age, serological, lactation, and pregnancy status were recorded for each animal. The questionnaires were filled out in farms by field observations and recorded data in the cattle farms.

Statistical analysis

The estimated seropositivity was presented by the ratio between the number of positive blood tests and the total number of tests performed, with a confidence interval of 95%. Data were described using descriptive statistics and analysis by chi-square test or ANOWA analysis. The statistical program SPSS package Ver 15 was used to perform the statistical analysis. Statistical significant level of difference was considered at $p<0.05$.

Results

N. caninum antibodies were detected in 196 sera (32.1%) of 611 cows. Frequency of *N. caninum* in 25 cattle farms in Isfahan is shown in Table 1. The percentage of infection of animals in different farms ranged from 0.0% to 60% which is statistically significant difference ($P<0.05$).

As it shown in Table 2, the frequency of *N. caninum* in different age groups is ranging from 29.3% to 33.0%. There were no statistically significant differences among the age groups (Table 2). Of the 611 cows, 355 were pregnant, and 256 non-pregnant. *N. caninum* antibodies were detected in 140 (39.4%) of pregnant and 66 (25.78%) of non-pregnant cows. The differences between frequencies of *N. caninum* in pregnant and non-pregnant were statistically significant (P<0.05) (Table 2). Of the 611 cows, 460 were lactated, and 151 were non-lactated. *N. caninum* antibodies were detected in 159 (34.6%) of lactated and 37 (24.5%) of non- lactated cows.

The differences between frequencies of *N. caninum* in lactated and non-lactated cows were not statistically significant (Table 2). Of the 611 cows, 489 cows were industrial, and 122 were semi-industrial. *N. caninum* antibodies were detected in 151 (30.9%) of industrial cows and 45 (36.9%) of semi-industrial cows. There were no statistically significant differences between the frequencies of *N. caninum* in industrial and semi-industrial cows (Table 2). Of the 611 cows, 489 cows were feed with dried alfalfa+ silage +concentrate, and 122 were feed with fresh alfalfa+ dried alfalfa+ silage +concentrate (Table 2). *N. caninum* antibodies were detected in 151 (30.9%) of cows that feed with dried alfalfa+ silage +concentrate, and 45 (36.9%) of cows that feed with fresh alfalfa+ silage +concentrate. There were no statistically significant differences among the frequencies of *N. caninum* in two groups. Of the 611 cows, 493 cows had contact with watch dogs, and 118 had contact with watch dogs and wild caniide (Table 2). *N. caninum* antibodies were detected in 108 (21.9%) and 88 (74.6%) of cows that had contact with watch dogs and with both watch dogs and wild caniide. There were statistically significant differences between the frequencies of *N. caninum* in two groups.

Farm No.	Total No. of cows in farm	Number of sampled cows	Sero-positive	Prevalence (%) (95% confidence interval)
1	300	39	11	28.2 (16.5-43.7)
2	546	56	15	26.8 (16.9-39.6)
3	1100	54	18	33.3 (22.2-46.6)
4	200	48	13	27.1 (16.5-40.9)
5	200	43	21	48.8 (34.6-63.2)
6	260	32	10	31.2 (17.9-48.6)
7	1800	36	8	22.2 (11.7-38)
8	598	30	8	26.7 (14.1-44.4)
9	300	28	10	35.7 (20.7-54.2)
10	500	47	17	36.2 (23.9-50.4)
11	300	47	11	23.4 (13.6-37.2)
12	100	29	9	31 (17.3-49.2)
13	50	8	3	37.5 (13.6-69.4)
14	30	11	2	18.2 (5.1-4.8)
15	30	8	2	25 (7.1-59.1)
16	30	12	7	58.3 (31.9-80.6)
17	31	8	4	50.0 (21.5-78.4)
18	45	12	5	41.7 (19.3-68)
19	100	7	3	42.9 (15.8-74.9)
20	30	11	0	0.0 (0-25.9)
21	25	12	4	33.3 (13.8-60.9)
22	27	16	6	37.5 (18.5-61.3)
23	15	7	4	57.1 (25-84.1)
24	15	5	3	60 (23.1-88.2)
25	15	5	2	40 (11.8-76.9)
Total	7277	611	196	32.1 (28.5-35.9)

Table 1: Frequency of *N.caninum* in 25 cattle herds in Isfahan with a history of abortion

Category	Level	Number tested	Sero-positive	Prevalence (%) (95% confidence interval)
Cattle	**All**	611	196	32.1 (28.5-35.9)
Age(month)	**<12**	109	32	29.3 (21.6-38.4)
	12-24	105	33	31.4 (23.3-40.8)
	>24	397	131	33.0 (28.5-37.7)
Pregnancy	**Pregnant**	355	140	39.4 (34.5-44.6)
	Non-pregnant	256	66	25.8 (20.8-31.4)
Lactation state	**Lactated cows**	460	159	34.6 (30.3-39)
	Non-lactate cows	151	37	24.5 (18.3-31.9)
Farm type	**Industrial**	489	151	30.9 (26.9-35.1)
	Semi-industrial	122	45	36.9 (28.8-45.7)

Category	Level	Number tested	Sero-positive	Prevalence (%) (95% confidence interval)
Feed	Dried alfalfa+ silage +concentrate	489	151	30.9 (26.9-35.1)
	Fresh alfalfa+ dried alfalfa+ silage +concentrate	122	45	36.9 (28.8-45.7)
Contact with caniide	Watch dog	493	108	21.9 (18.5-25.7)
	Watch dog and wild caniide	118	88	74.6 (66-81.5)

Table 2: Frequency of *N.caninum* in relation to age, pregnancy, lactation state, farm type, feed condition and contact with caniide of dairy cow in Isfahan

Discussion

Studies of *N. caninum* antibody seroprevalence have been conducted in several parts of the world. In Iran, the presence of *N. caninum* antibodies has been described in cattle however, the role of dogs in the transmition is not well known but according to Malmasi, *et al.* (2007) study *N. caninum* antibodies were seen in 10 (20%) of 50 household dogs and in 23 (46%) of 50 farm dogs in Tehran [12,13]. A few serological surveys carried out for the detection of *N. caninum* infection have shown frequencies from 12.6 % to 32% in cattle, 37% in buffaloes and 3.22% in camel [14-17].

Because various diagnostic techniques and variety of cut-off points have been employed by different investigators comparison of different results is difficult. In the present study, *N. caninum* antibody sero-prevalence was 32.07%, twice that obtained by Moore, *et al.* and Gondim, *et al.*, who observed values of 16.6% and 15.6%, respectively by indirect fluorescent antibody test (IFAT) [18,19].

After primary *N. caninum* infection the avidity of specific antibodies increases over time. Usually high avidity IgG responses are observed in cattle naturally infected for more than 6 months [3].

Among positive cattle, the frequencies of *N. caninum* in different age groups were ranging from 29.35% to 33.0% which it was not significant difference. According to study of Davison, *et al.* in Britain and Dyer, *et al.* in the United States the infection was less in those whose ages ranged 12–24 months when compared to those over 24 months [20,21]. This fact suggests the existence of sporulated *N. caninum* oocysts in the environment, which characterizes horizontal transmission, as observed by Dijkstra, *et al.* [9]. However, this feature of transmission has not proved frequent in some regions of the world, such as in California, where the major percentage of cattle is infected by the transplacental route, and most calves are born seropositive [22].

In this study we found that seroprevalence of *N. caninum* in pregnant cattle was significantly higher than non-pregnant cattle. Cattle with antibodies to *N. caninum* are more to abort than seronegative cows. Foetus may die in utero, resorbed, mummified, autolyzed or stillborn or born alive with or without clinical signs [3]. Detection of *N. caninum* antibody in this animal category is an alarm for losses during gestation and developing measures for the control of neosporosis in this animal category is very important.

N. caninum antibodies were detected in 21.90% and 74.57% of cows that had contact with only watch dogs and watch dogs+wild caniide, respectively. The differences between the frequencies of *N. caninum* in two groups were statistically significant means that wild caniide like foxes and jackals may play critical role in transmission of *Neospora* infection in dairy cattle farms of the region. Our results is on the contrary with Bartels, *et al.* study, who showed the presence of dogs on the farms as a significant associated factor, Barling, *et al.* observed that the presence of a dog among the cattle was a protective factor against *N. caninum* infection, explaining that the domestic dogs of the farm probably drove off stray and wild dogs that might be more important sources of infection than tame dogs [23,24].

As to the general characteristics of the farms in relation to cattle, among variables analyzed, the feed sources silage and farm-produced concentrate behaved as protective factors against *N. caninum* infection. Cattle fed in this way would thus present a lower prevalence of infection by *N. caninum*. This finding, especially in relation to silage, may be related to preparation and storage, which hinder access by dogs; thus, avoiding contamination of the feed by *N. caninum* oocysts shed by the dogs. However, the study by Bartels, *et al.* showed that damp silage given during the summer is a risk factor [23]. Many mycotoxins suppress immune function by decreasing the proliferation of activated lymphocytes, impairing phagocytic function of macrophages, and suppressing cytokine production, but some induce hypersensitive responses in different dose regimes. The authors give as a probable cause the reactivation of latent *N. caninum* infection owing to the ingestion of mycotoxins in the silage, leading to intoxication with resulting immunosuppression.

Conclusion

These finding explain, at least in this region of Iran, there is a high percentage of *N. caninum* seropositive cattle and further investigation is needed for the confirmation of infection. In this study several important factors were investigated about the *N. caninum* seropositivity. We confirmed that present of watch dogs+wild caniide on farm related to increasing of seropositivity for *N. caninum*. The difference between *N. caninum* seropositivity on farms was due to differences in management conditions between

farms. Further research is needed to confirm the effects of other pathogens such as bovine leukemia virus (BLV), bovine viral diarrhea virus (BVDV), *Mycobacterium avium* subspecies paratuberculosis (MAP) and mycotoxins on *N. caninum* infection in cattle.

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