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Original Article

Neospora caninum Suspects as One of the Most Important Causes of Abortion in Large Dairy Farms in Isfahan, Iran

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Abstract

Background: This study aimed to reveal the serological prevalence of *Neospora caninum* in large dairy farms in Isfahan Province, central Iran.

Methods: Serum samples were collected from 1500 cattle living in four large dairy farms in Isfahan Province, Iran during 2014-2015 and examined for anti *N. caninum* IgG antibodies. Overall, 113 serum samples were also collected from the dogs living in these areas; suspecting to be risk factors for this infection. All the serum samples were investigated to find IgG antibodies by using ELISA. Dogs' sera were also analyzed by indirect fluorescent antibody test.

Results: Totally, 395 out of 1500 bovine samples (26.33%) were positive for *N. caninum*: 34%, 21.61%, 23.03% and 29.01% in four investigated clusters (farms). Infection rate was significantly more in cows with the history of abortion. The infection rate in dogs was 17.69%: (20 out of 113).

Conclusion: The results show a high seroprevalence of the infection and possibly the role of the dogs in horizontal transmission of the infection.

Introduction

Neospora caninum is an apicomplexan protozoan parasite recognized as the most important cause of abortion in cattle in many countries. *N. caninum* causes abortion in both dairy and beef cattle. *N. caninum* associated abortions may occur in any age; from 3 months of gestation to the

end; mostly at 5–6 months of fetus age. Fetuses may die in utero, be resorbed, mummified, autolyzed, stillborn, born alive with clinical signs, or born clinically normal but persistently infected. Neosporosis-induced abortions occur year-round (1).

Dairy and beef cattle with antibodies to *N. caninum* (seropositive) are more likely to abort than seronegative cows (2-4) and up to 95% of calves from seropositive mothers will be congenitally infected without any detectable clinical signs (5).

Dogs (*Canis lupus familiaris*), coyotes (*C. latrans*) and dingos (*C. lupus dingo*) are definitive and intermediate hosts and shed oocysts following ingesting *N. caninum* infected tissues of intermediate hosts (6-8). The presence either of dogs, currently or even within past 10 yr is a risk factor for detecting seropositive cattle (9).

This study was aimed to reveal the serological prevalence of *N. caninum* in large dairy farms in Isfahan Province, Iran, to realize if the parasite is an important agent to induce abortion in these herds and finally if infected dogs are important in seroepidemiology of the infection.

Materials and Methods

Animals

A total of 1500 dairy cows were enrolled in this study. All of them were at least 6 months old. The samples were taken from 4 large dairy farms located in Isfahan Province, Iran. Blood samples were taken and centrifuged immediately.

Serum samples were also collected from 113 dogs in the region in which dairy farms were located. Collected sera were kept in -20 °C until used.

ELISA

An *N. caninum* 38 kDa surface antigen (P38) was affinity purified as described earlier (10) and lyophilized until used for coating the ELISA plates.

To analyses the serum samples taken from dairy cows, the affinity-purified antigen was diluted in coating buffer (0.1 M sodium bicarbonate, pH 8.3) and used to coat ELISA plates (Nunc-Immuno (Polysorb)) at 37 °C for 1 hour. Wells were then washed three times

with PBS-T (PBS, pH 7.2, 0.05% Tween-20) and incubated with blocking solution (PBS-T, 20% horse serum) at 37 °C for 0.5 hours. Wells were emptied and the serum samples (diluted 1:200 in PBST, 20% horse serum) were added. Positive and negative controls were kindly provided by Friedrich Loeffler Institute, Wusterhausen Germany. Positive control was taken from an experimentally infected heifer (heifer 44) and negative control was taken from cow 24, before infection (11). Sera were emptied and ELISA plates were washed. Anti-bovine IgG conjugates were diluted in PBST-2% horse serum and incubated (37 °C, 30 min). After each step, the wells were washed three times with PBS-T.

After the final step, the plates were washed three times with PBS-T and twice with distilled water. Bound antibodies were detected by incubation with a substrate containing 100 mg/ml 3,3',5,5'-tetramethylbenzidine and 0.004% hydrogen peroxide in 0.2 M sodium acetate and 0.2 M citric acid at 37 °C. The reaction was stopped after 15 min by adding sulfuric acid to a final concentration of 2 N, and optical density (OD) values were measured at 492 nm on an ELISA reader.

Sample index values were calculated by the formula $SIn = (S_n - N) / (P - N)$ where SIn is the individual ELISA index value, S_n is the OD value obtained for a single sample, N is the OD value obtained for the negative serum, and P represents the OD values obtained for the positive serum. Sin values of more than 0.153 were regarded positive (12). Evaluation of the serum samples taken from dogs was performed as described earlier (10).

IFAT

Indirect fluorescent antibody test was performed as described previously for serum samples collected from the dogs. IFAT results were regarding positive when the reaction result was positive at least in dilution of 1:16 (10).

Results

Serological evaluation of sera taken from four dairy farms revealed that all the farms had seropositive cows against *N. caninum* with the mean infection rate of 26.33% (395 out of 1500 samples). The infection rates of four dairy farms were 34% (84 out of 247), 21.61% (67 out of 310), 23.03% (114 out of 495) and 29.01% (130 out of 448).

Serological rate of the infection was compared in cows with the history of abortion and cows with no detected abortion. The rate of *N. caninum* infection was significantly higher in cows with the history of abortion ($P < 0.05$). Evaluation of dog sera showed that 20 of 113 (17.69%) dogs had detectable anti-*N. caninum* antibodies. The IFAT titers of dog's sera were compared to the ELISA indices (Fig. 1).

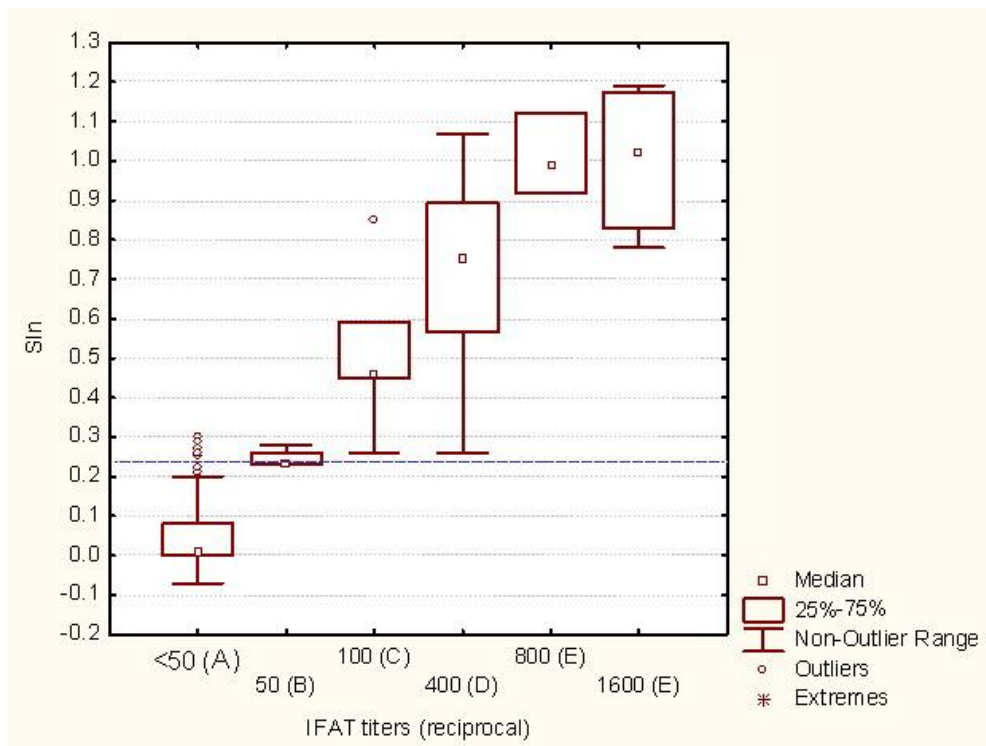


Fig. 1: ELISA indices of dogs' sera in comparison to the IFAT titers

Discussion

Seroepidemiology of *N. caninum* has been investigated worldwide in dogs, dairy cattle, beef cattle, other domestic animals, wildlife and zoo animals and human (9). Although each serological method has its properties and the results, of one's serological investigation cannot be compared with the others but overall the results show that many species of mammals are exposed to this parasite worldwide. Minor cross reactivity may occur be-

tween *N. caninum* and *Toxoplasma gondii* although it can be ignored (9).

The prevalence of 26.33% observed in this study is relatively high. In another study in Iran, *N. caninum* seroprevalence showed a considerable difference. The prevalence of infection was investigated in Khorasan was 18% and 21% from Ahwaz although it was 44% in another study performed in Khorasan, Iran (13-15). Prevalence of infection is more than what has been reported from neighbor countries (16, 17) and from most of European countries although there are huge differences

reported from different regions of one country (9). There are significant differences among different countries, cities, regions, and between beef and dairy cattle. However, differences in serologic techniques, study design, and sample size used should be kept in mind when evaluating these results. Results show that different regions, within a particular region, and among different management systems infection risk differs.

This study showed that the rate of abortion was more in cows with the history of abortion. This is nearly a common finding in other serological studies (2, 3).

There are two patterns of *N. caninum* associated abortions. In the first pattern named *epidemic pattern*, the cause of abortion is postnatal infection of un-infected cattle; most likely due to exposure to the food or water sources contaminated with oocysts (18, 19). This pattern of abortion may lead to abortion storms reported 57% of pregnant cows abort just in few weeks-months (1, 20, 21). If the abortion rate exceeds 10%, 12.5% or 15% of cows at risk within 4, 6 or 8 wk, the abortion will be epidemic (3, 19, 22). Dog feces can represent an important source for parasite-infection. From the time that the dogs were recognized as the definitive hosts for *N. caninum*, the question of its importance in the epidemiology of related abortion was raised (23). The introduction of a new dog in a cattle herd has been reported to increase the risk of epidemic *N. caninum*-related abortion. The newly coming dog was infected with *N. caninum* by infected materials from cattle. It transmitted the infection to other cattle by oocysts shedding (23).

The second pattern of *N. caninum* associated abortion is endemic abortion in which the major route of infection is vertical (19). The re-activation of latent infection during gestation is the reason for an increased abortion risk (24). Chronically infected seropositive cattle have an about two to three fold more risk of abortion compared to seronegative ones (25). Less than 5 percent of cows may have re-

peated abortion due to neosporosis (26). *N. caninum* transmits vertically in cattle for several generations (27). Most abortions are endemic and most likely due to vertical transmission (1).

Conclusion

Relatively high *N. caninum* seroprevalence in dogs living inside or in the region where the farms are located suggests the role of dogs to transmit the parasite horizontally. A wide study is needed to detect the exact role of the dogs in the epidemiology of the infection in these farms.

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Conflict of interest

The authors declare that there is no conflict of interest.

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