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Seroprevalence of *Neospora caninum* in Holstein dairy cattle in northeast of Iran

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Abstract

Neospora caninum, a cyst forming coccidian parasite, is an important cause of abortion in cattle worldwide. The present work aims to update knowledge on the presence of *Neospora caninum* in cattle from northeast of Iran**.** The study was performed in Mashhad suburb in Khorasan Razavi Province, in northeast of Iran. Serum samples were collected from 116 Holstein dairy cattle that were between three and five months of pregnancy from seven dairy herds. Chi-square tests were used for statistical analyses. The prevalence of *N. caninum* in cattle herds was 24.3 %. There was no significant difference in the proportion of *N. caninum* -seropositive animals within different parity groups but there was significant difference between different parity groups. The results of this study confirmed the presence of *N.caninum* in cows in Mashhad area. Culling seropositive cows and their offspring has been recommended for the control of *N. caninum* in herds with a low prevalence of infection.

Keywords: Abortion, Holstein dairy cattle, Northeast of Iran. *Neospora caninum*

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Introduction

N.caninum is an apicomplexan protozoan which causes abortion in cattle worldwide (Dubey and Lindsay, 1996). Cattle may be infected transplacentally or as result of ingesting oocyst produced by dogs, which represent the definitive host. Cattle at any age may abort from 3 months of gestation to term, and abortion is the main clinical sign observed. Fetuses may die in utero, be resorbed, mummified, autolyzed, stillborn, born alive but diseased or born clinically normal but chronically infected (Dubey and Lindsay, 1996; Dubey et al., 2005). In Iran, antibodies to *N. caninum* were reported in sera of dairy cattle (Razmi et al., 2006; Sadrebazzaz et al., 2004). In a preliminary study in Iran, *N. caninum* DNA had been detected by polymerase chain reaction (PCR) in brains from four out of six aborted fetuses (Habibi et al., 2005).The parasite was first detected in 1984 in dogs with myositis, lameness and encephalitis and named as *N. caninum* (Bjerkas et al., 1984; Dubey et al., 1999). serological tests, including enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody technique (IFAT), direct agglutination test (DAT), and immune blots (IB) can be used to detect anti *N. caninum* antibodies (Bjorkman and Uggla, 1999). *Neospora caninum* is transmitted vertically from an infected cow to her foetus during pregnancy (Anderson et al., 1997). However, despite the fact that Mashhad is the largest dairy-producing region in Iran, there are only two reports of prevalence of *N. caninum* (Sadrebazzaz et al., 2004; Razmi et al., 2006). Our objective was to determine the prevalence of *N. caninum* in seven dairy herds in Mashhad, Northeast of Iran.

Materials and Methods

This study was performed in Mashhad suburb in Khorasan Razavi Province, Northeast of Iran. Serum samples were collected from a total of 116 Holstein

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dairy cattle that were between three and five months of pregnancy from seven dairy herds during years 2010 and 2011. Blood samples were collected from the coccygeal vein of each animal, using disposable needles. All the samples were immediately transported to the laboratory. Serum was removed after centrifugation at 1000×g for 10 min. All sera were transferred to micro tubes having anticoagulant (EDTA) and stored at -20 centigrade degrees until laboratory testing. Serum samples were analyzed for having antibodies to *N. caninum* using ELISA kit (Svanova Biotech AB, Sweden). Sera were started at 1:40 serum dilution for *N. caninum*. Logistic regression and chi-square tests were used for statistical analyses. Variables with P value ≤0.5 were offered to logistic regression analysis. Logistic regression model for this analysis was used as following format (Vittinghoff et al., 2004):

$$E(Y) = Log\left[\frac{P}{1-P}\right] = \{1+\exp(-X\beta)\}^{-1} + e$$

Where, Y is the vector of binary response variable with Bernoulli distribution. In the above model, X is the incidence matrix of the fixed effects and, β is the vector of fixed effects of statistical model (Vittinghoff et al. 2004). The residual parts of the model have zero mean and variance of p (1-p).Each model was applied at the individual cow. Statistical analyses were performed using the statistical software SAS version 9 (SAS, 2002).

Results

Twenty eight serum samples, out of the 116 examined sera, were seropositive for *Neospora caninum* infection. The percentage of seroprevalence of *N. caninum* in herds was 24.3. Positive results were found in all lactations (1-5), (Table 1).From these positive samples, three, one, eleven, five and eight samples were associated to the first, second, third, fourth and fifth month of pregnancy , respectively . There was no significant difference (P>0.05) in the proportion of seropositive animals within parity groups, but there was significant difference (P<0.05) between different parity groups although the proportion tended to increase as animals were over their second parity. Estimated odds ratio for *N. caninum* was 0.98 (Table 2) and represented the increasing trend of *N. caninum* occurrence. P value was 0.0032 for all the tests of the associations. Logistic regression showed that seropositivity of cows to *N. caninum* is associated to prevalence of *N. caninum*.

Discussion

In all, 24.3% cows were infected by *N. caninum*. Sadrebazzaz et al. (2004) found a prevalence of 15.1% for Neospora antibodies from cattle in North East of Iran (Mashhad). In Middle East, the prevalence was reported from Jordan to be 35%, which is higher than the prevalence in the present study (Abdelsalam et al., 2013).The prevalence of antibodies against *N. caninum* in the southeast of Iran in cattle was 12.6% (Nourollahi Fard et al., 2008) and in water buffaloes was 37% (Hajikolaei et al., 2007). The results indicate that exposure to this parasite in this area is common. One of the most important factors associated with the occurrence of *N. caninum* and related abortions is the presence of dogs on the farms (Abdelsalam et al., 2013). After the assured recognition that dogs are a final host (Mcallister et al., 1998; Lindsay et al., 1999), it was obvious that the presence of dogs on farms can have an important role in the horizontal spread of *N. caninum* through oocysts excreted to the environment. Pare et al. (1998) clearly showed that the presence and the number of dogs have positive association with seropositivity and frequent abortions. The same opinion has been supported by other researchers (Moen et al., 1998; Bartels et al., 1999; Wouda et al., 1999; Dijkstra et al., 2002). The studies have not shown an association between serological status and cow age (Waldner et al., 1998; Davison et al., 1999). Sanderson et al. (2000) determined that seropositivity in cows under three-years of age was higher than in cows above six-years of age. The age effect on seropositivity in dairy cattle may vary in different study regions. In

Table 1: Seroprevalence of *Neospora caninum* in relation with parity

parity	No.of animals tested	No. of Positive Cases	Seroprevalence (%)	OR	95%CI $_{OR}$	P value
1	12	3	25.0(%)	1.02	[0.96, 1.08]	0.48
2	18	1	5.50(%)	0.99	[0.94 ,1.03]	0.68
3	53	11	20.7(%)	0.96	[0.95 ,0.98]	0.02
4	19	5	26.3(%)	0.64	[0.17 ,2.40]	0.51
5	14	8	57.1(%)	0.99	[0.96,1.01]	0.39
Total	116	28	24.3(%)	0.98	[0.98,0.99]	0.003

OR = Odds ratio; 95% CI $_{OR}$ = 95% level confidence limit interval for odds ratio

Table 2: Logistic regression analysis of variables associated with seropositivity to *Neospora caninum*

Variable	B	SE	Chi-Square	DF	P value	OR	95%CI $_{OR}$
Neospora	-0.009	0.0040	8.6763	1	0.0032	0.988	[0.98,0.99]
Constant	1.485	0.8790	2.8575	1	0.0909	-	-

OR = Odds ratio; 95% CI $_{OR}$ = 95% level confidence limit interval for odds ratio

Denmark, Jensen et al. (1999) and in the United States Dyer et al. (2000) reported that the risk of being seropositive may increase with age in dairy cattle, while in Sweden, the situation was the opposite (Bartels et al., 2006). Our results showed association between seroprevalence and parity. Cows at their fifth parity had the highest proportion of seropositive animals with 57.1%. Estimated odds ratio for *N. caninum* was 0.98. This means that Seropositive cows were 1 time more seropositive than seronegative cows (odds ratio = 0.98, P value = 0.003). Davidson et al. (1999) in a study, found a strong relationship between seropositivity and abortion, 3.5 times more often than in seronegative cows (odds ratio = 3.49, P value <0.05). The transplacental transmission (vertical transmission) plays a major role in the maintenance and spread of *N. caninum* in cattle, ranging from 72 to 95% (Davison et al., 1999, Abdelsalam et al., 2013). The horizontal transmission (post-natal transmission) through ingestion of food or drinking water contaminated by sporulated oocysts from the environment is the only documented natural mode of *N. caninum* infection in cattle after birth (De Marez et al., 1999; Abdelsalam et al., 2013). Transmission of *N.caninum* oocysts within and between herds by other means such as airborne transmission, by worker's clothing, contaminated equipment, and persons, might be possible. (Abdelsalam et al., 2013). Roaming dogs shedding *N. caninum* oocysts play a major role in *N. caninum* transmission in the farm (Dubey et al., 2007; Abdelsalam et al., 2013). The tested dairy herds had one roaming dog in the farm. Other factors have been reported to be associated with *N. caninum* seroprevalence in dairy cattle. Ould-Amrouche et al. (1999) reported that the presence of poultry, rabbits and ducks on the farm was significantly associated with seropositivity for *N. caninum.*

Conclusions

The results of this study confirm the presence of *N. caninum* in cows in Mashhad area.The present work aims to update knowledge on the presence of *N. caninum* in cattle from northeast of Iran. Vertical transmission is the major route of transmission, but elimination of vertical transmission may not be enough to eliminate the infection from a herd, because horizontal transmission may occur. Culling seropositive cows and their offspring has been recommended for the control of *N. caninum* in herds with a low prevalence of infection. Minimizing farm workers and visiting other farms, and limiting presence of dogs on the farm are important measures to minimize *N. caninum* transmission to a healthy herd.

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