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Detection of anti-*Neospora caninum* antibodies in Iranian native cattle

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ABSTRACT

***Neospora caninum* is an Apicomplexan parasite which may cause abortion in cattle. This study investigated occurrences of antibodies against *N. caninum* in Iranian native cattle. From September 2010 to September 2011, blood samples (n=768) of native cows were collected randomly from different rural regions of Hamedan (n=400) and Kurdistan provinces (n=368) located to the western part of Iran. All the samples were evaluated for IgG antibodies against *N. caninum* using Enzyme Linked Immunosorbent Assay. The IgG antibodies to *N. caninum* were found in 14.2% (n=109/768) of serum samples (95% CI: 11.74 - 16.66). There was a significant difference between seropositivity and abortion history (p<0.0001, OR=2.9), unlike to age groups (p=0.105). This is the first report of *N. caninum* infection in Iranian native cattle. In conclusion, *N. caninum* is an important factor in abortion in Iranian native cattle. Further comprehensive studies and designing control strategies for improving management in cattle farms are highly recommended.**

Keywords

Antibody, Iran, Native cattle, *Neospora caninum*, Serology

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INTRODUCTION

Neospora caninum is a heteroxenous Apicomplexan parasite originally reported in dogs with congenital encephalomyelitis and cattle worldwide (Salehi et al., 2010; Gharekhani and Tavoosidana, 2013). *N. caninum* is mainly transmitted by two ways; these are consumption of oocysts contaminated food, and transplacental transmission. However, vertical transmission could be one of the main mechanisms of infection in cattle (Anderson et al., 2000; Gharekhani and Tavoosidana, 2013). Excreting oocysts in feces of final hosts (Canids) is a risk factor for the occurrence of miscarriage and stillbirth associated with neosporosis in cattle (Gharekhani et al., 2013).

Abortion and mortality in congenitally infected calves are the common reproductive problems that lead to major economic losses in cattle husbandry worldwide (Dubey et al., 2007). Several assays are available to detect the antibodies to *N. caninum* in animals, such as enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT) (Nourollahifard et al., 2008).

The seroprevalence of *N. caninum* in cattle varies depending on the region of study (Salehi et al., 2010). In Iran, several serological studies in *N. caninum* have been done in different hosts and regions (Razmi et al., 2006; Sadrebazzaz et al., 2006; Gharekhani and Tavoosidana, 2013; Gharekhani et al., 2013). Also, *N. caninum* DNA was detected in brains of aborted cattle fetuses in Iran (Razmi et al., 2007; Salehi et al., 2012). In an another study, *N. caninum* has been isolated in

semen of bulls using molecular techniques; the semen is considered as an important medium for venereal transmission of bovine neosporosis (Sharifzadeh et al., 2012). The reports mentioned above are indicative that neosporosis is one of the important causes of bovine abortion in Iran (Salehi et al., 2012). However, there is no published data of *N. caninum* infection in Iranian native cattle. Therefore, the present study was performed to determine the prevalence of antibodies to *N. caninum* in Iranian native cattle in western Iran, using ELISA.

MATERIALS AND METHODS

Study area: This study was carried out in Hamedan (34.79° N and 48.51° E) and Kurdistan (35.31° N and 46.99° E) provinces located to the west of Iran. These provinces are of the most mountainous regions in Iran, and have a generally mild and quite pleasant climate throughout the spring and summer. Winters are long and can be very cold with heavy snowfalls. These regions are economically important for crop production and animal husbandry.

Sampling and serology: From September 2010 to September 2011, 768 blood samples were collected randomly from native cows of different rural regions of Hamedan (n=400) and Kurdistan provinces (n=368). The owners were questioned about age of animals (<2, 2-4, and >4 years), and abortion history (yes or no). Sera were collected after centrifugation at 1500×g for 10 min and stored at -20°C until laboratory testing.

Anti-*Neospora* IgG-antibodies of the samples were detected using commercially available ELISA kit (HerdCheck®, IDEXX). The kit was used according to the manufacturer's instructions. The presence or absence of antibody was determined by calculating of sample to positive ratio (S/P>0.5 = positive).

Ethics committee approval: Ethics committee approval was received for this study from the ethics committee of Iranian Veterinary Organization (No: 2010-1446).

Statistical analysis: Data were analyzed by SPSS version 16.0 software. Chi-Square and Fisher's exact test with 95% Confidence Interval (CI) were done for evaluation of differences and association between data. A *p*-value ≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

IgG antibodies to *N. caninum* were found in 14.2% (n=109/768) serum samples (CI=0.142±0.0246). The seroprevalnce of *N. caninum* antibodies was reported as 0.7-97.2% in cattle worldwide (Dubey at al., 2007; Dubey and Schares, 2011). The lowest and highest seroprevalence of *N. caninum* in cattle was reported 10.5% in Northwest and 46% in Northeast of Iran, respectively (Razmi et al., 2007; Nematollahi et al., 2011).

Our results is similar to studies conducted in Italy (14% using IFAT), Argentina (14.2% using IFAT), USA (14.7%, using ELISA, IDEXX), Brazil (14.9% using IFAT) and Australia (14.9% using IFAT) (Stoessel et al., 2003; Oshiro et al., 2007; Moore et al., 2009; Dubey and Schares, 2011). In a similar study in rural regions of Northern Iran, 43.9% of native cattle were found to be seropositive (Youssefi et al., 2009). The main causes of varied results might be due to differences in using different diagnosis methods, study design, climatic variations and frequency of final hosts in the farms (Gharekhani et al., 2013).

Our results indicated that 26% of cases with history of abortion were seropositive (**Table 1**; X^2=24.304, DF=1, *p*<0.0001 and Odds Ratio=2.9). Youssefi et al. (2010) reported 7%, 45.2% and 57.3% of aborted cattle were seropositive to *N. caninum* in Ardebil (Northwest of Iran, cold climate), Garmsar (Central of Iran, warm and dry climate) and Babol (North of Iran, mild climate), respectively. In a study from Northeast of Iran, the abortion prevalence in seropositive cattle was reported higher than seronegative (*p*<0.05, OR=1.78) (Razmi et al., 2006); this was similar to our findings and reports of

Table 1: Prevalence of IgG antibodies to *Neospora caninum* in native cattle in different age groups, abortion history and study area.

Study area	Number of sample (% seropositive)								CI 95%
	Age groups (year)				Total	History of abortion			
	<2	2-4	>4	*p*-value		Yes	No	*p*-value	
Hamedan	82 (7.3)	185 (16.8)	133 (32.3)	0.000	400 (20)	50 (64)	350 (13.7)	0.000	16.08-23.92
Kurdistan	41 (9.8)	64 (9.4)	263 (7.2)	0.759	368 (7.8)	115 (9.6)	253 (7.1)	0.418	5.07-10.53
Total	123 (8.1)	249 (14.9)	396 (15.7)	0.105	768 (14.2)	165 (26)	603 (10.9)	0.000	11.74-16.66

CI 95% was calculated for Hamedan and Kurdistan regions, and total samples, separately.

others (Pare et al., 1998; Anderson et al., 2000; Dubey et al., 2007; Dubey and Schares, 2011). In contrast to our finding, Dubey and Schares (2011) reported that the native breeds are genetically resistant to neosporosis.

Antibodies level is expected to be peak if *N. caninum* is involved in abortion (Dubey and Schares, 2011). Evaluation of seropositive case in previous studies showed that the risk of abortion was higher than seronegative cattle (Schares and Barwald, 2004; Lopez-Gatius et al., 2005; Sharifzadeh et al., 2012; Gharekhani and Heidari, 2014). As compared to previous studies, our findings are in consistent with the idea that the seropositive rate correlated with abortion.

The seroprevalence of *N. caninum* was detected as 8.1%, 14.9% and 15.7% in <2, 2-4 and >4-yr of age groups, respectively; however, no significant differences was found (**Table 1**, X^2=4.500, DF=2, p=0.105). This finding is similar to result of Nourollahifard et al. (2008) in Southeast of Iran and other researchers (Pare et al., 1998; Atkinson et al., 2000; Kyaw et al., 2004; Dubey et al., 2007).

Razmi et al. (2006) and Gharekhani et al. (2013) reported significant relations among different age groups (p<0.05). Youssefi et al. (2010) found significant increase in seropositivity in 4-5-yr age group. Wouda et al. (1998) and Sadrebazzaz et al. (2004) reported equal levels of seroprevalence in all age groups for most herds. Jensen et al. (1999) suggested seroprevalence increases with age and depends on sample size. Lower seroprevalence, found in our study, in cattle of <2 age was might be due to decrease of antibody in congenitally infection. It seems relationship between age and seroprevalence rate is speculative.

The prevalence of *N. caninum* in dairy cattle was reported higher than beef cattle (Pare et al., 1998; Hemphil and Gottestin, 2000). This might be related to different production systems for dairy and beef cattle rather than to breed differences. As few studies have been conducted on the association of breed, planning and conducting extensive research on the impact and role of different breed in the infection prevalence is recommended.

The most of cattle husbandry farms are traditional in rural regions of Iran, and the animals have a direct contact with dogs. The presence of dogs in farm has been assumed to provide the greatest chance of horizontal transmission through the ingestion of oocysts, shed by infected dogs. In addition, dogs kept in the neighborhood of farms may pose an infection risk (Gharekhani et al., 2013).

CONCLUSIONS

The findings of this study provide baseline information for the future studies. There are both horizontal and vertical transmissions of *N. caninum* in native cattle of western Iran. Therefore, evaluation of *Neospora* infection in other hosts is necessary for designing appropriate control strategies. This is the first report of *N. caninum* infection in Iranian native cattle. Further comprehensive studies and designing control strategies for improving management in cattle farms are highly recommended.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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