

Serological study of *Neospora caninum* infection in dogs and cattle from west of Iran

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Abstract The aim of this study was to determine the seroprevalence of *Neospora caninum* infection in dogs and cattle from Hamedan province (West of Iran). Blood samples were collected from 1,046 cattle and 270 dogs in this area. Cattle and dog samples were tested and analyzed using ELISA and IFAT, respectively. IgG-antibodies to *N. caninum* were found in 27 and 17.4 % of dogs and cattle samples, respectively. In cattle study, The association between infection and type of cattle was statistically significant ($P=0.004$). Also, significant statistical differences were observed regarding to stray canids presence in farm ($P<0.0001$), and abortion history ($P<0.001$), unlike to age ($P=0.195$) and breed ($P=0.077$). In dog study, there was statistical differences among age groups ($P<0.001$) and type of dogs ($P<0.001$) opposite to gender ($P=0.112$). This study is the first report of *N. caninum* infection in dogs and cattle from west of Iran. There is both horizontal and vertical transmission of *N. caninum* in this area, and the presence of stray dogs may be a risk factor for *N. caninum* infection in cattle. *N. caninum* is an important factor in the economic losses of the cattle breeding in Hamedan province.

Therefore, further investigations and designing control strategies for improving management in cattle farms is highly recommended.

Keywords *Neospora caninum* · Cattle · Dog · Hamedan · Iran

Introduction

Neospora caninum is a heteroxenous cyst-forming Apicomplexan intracellular protozoan from the Sarcocystidae family with a wide host range (Dubey et al. 2007; Khanmohammadi et al. 2011). Domestic dogs (*Canis familiaris*), coyotes (*Canis latrans*), and dingoes (*Canis domesticus*) are definitive and intermediate hosts for *N. caninum*. Excreting oocysts in feces and in the environment of definitive hosts is a risk factor for the occurrence of miscarriages and stillbirths associated with *N. caninum* in cattle and other intermediate hosts (Sharifdini et al. 2011; Dubey et al. 2011). After recognizing the dog and wild canids as the definitive host of the parasite, epidemiological studies were established the association between the presence of dogs and the disease in cattle (Wouda et al. 1999; Barling et al. 2000). Additionally, the association of canids with cattle on their premises has been postulated as a risk factor for the disease (Mc-Alister et al. 2000).

Neosporosis in cattle has been associated with endemic, epidemic, and sporadic abortions, neonatal mortality, and decrease in the volume of milk production that cause economic loss worldwide (Salehi et al. 2010). In addition, neosporosis is an important cause of neuromuscular disease in dogs (Dubey et al. 2007, 2011). Although the vertical transmission is the major mode of infection in cattle, the role of the definitive hosts in horizontal transmission is also important (Haddadzadeh et al. 2007).

Study of *N. caninum* infection rate in dogs and cattle is necessary for complete evaluation of neosporosis in each

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region. The seroprevalence of *N. caninum* infection in the hosts varies largely, depending on the country and region under study (Salehi et al. 2010). Some serological studies in cattle and dogs have done in some part of Iran (Sadrebazzaz et al. 2004; Razmi et al. 2006; Haddadzadeh et al. 2007; Malmasi et al. 2007; Nourollahifard et al. 2008; Yousefi et al. 2009; Salehi et al. 2010; Yakhchali et al. 2010; Hosseinejad et al. 2010, 2011; Khanmohammadi et al. 2011; Sharifedini et al. 2011; Nematollahi et al. 2011; Gharekhani et al. 2012). However, there is no published epidemiological information for *N. caninum* prevalence of the dogs and cattle (both rural and industrial) in West of Iran.

This study was performed to determine the prevalence of *N. caninum* infection in cattle and domestic dogs in Hamedan province, West of Iran. Due to similar climate condition of western provinces, neosporosis evaluation in this region can be an adequate model for west part of Iran.

Materials and methods

Study area Hamedan province by mountainous and mild climate is located in west part of Iran (34.77° N and 48.58° E). It covers an area of 19,546 km² and average annual temperature is 11.3 °C. This province is economically important for crops and animal husbandry, including sheep and cattle breeding. According to Iranian Veterinary Organization information, cattle population in this area is approximately 420,000.

Sample collection and examination A cross-sectional study was performed in the first half of year during 2010 up 2012. Blood cluster sampling were collected randomly from 1,406 cattle (28.4 % rural cattle, 35 % industrial dairy cattle, and 36.6 % industrial beef cattle), and 270 mix breed dogs (26 % stray, and 74 % owner shepherd dogs) (Table 1, 2). Dog samples were selected in around of village and cattle farms. Also, industrial and rural cattle samples were selected in total beef and dairy cattle farms and some different village of Hamedan province, respectively. Information about age and gender in dogs, and age, gender, breed, dog and stray canids presence in farm and abortion history in cattle were taken from owners and physical examination.

All samples were immediately transported to the diagnostic laboratory of Hamedan veterinary office. All sera were removed after centrifugation at 800×g for 15 min and stored at −20 °C until laboratory testing (Haddadzadeh et al. 2007; Nematollahi et al. 2011).

Anti-*Neospora* IgG-antibodies of cattle samples were detected using a commercially available *N. caninum* ELISA (enzyme-linked immunosorbent assay) kit (Herdcheck, IDEXX, USA). 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* ratio according to the formula mentioned inside the manual). The value more and less than 0.5 was considered positive and negative, respectively.

A commercial IFAT (indirect fluorescent antibody test) kit (MegaScreen® FLUONEOSPORA, Horbranz Austria) was used to evaluate of IgG-antibodies of dog samples. The titer 1:50 (cut-off value) was regarded as positive threshold titer (Haddadzadeh et al. 2007; Malmasi et al. 2007).

Statistical analysis Statistical analysis was performed by using the software package SPSS version 16.0 for windows. The differences among variables were evaluated by Chi-square and Fisher's exact tests. A *P* value of less than 0.05 was considered statistically significant.

Results

IgG-antibodies to *N. caninum* were found in 27 % (95 % CI, 21.7–32.3 %) and 17.4 % (95 % CI, 15.4–19.4 %) of dogs and cattle serum, respectively (Tables 1 and 2).

In the study of dogs, there was statistical differences among age groups ($\chi^2=18.026$, $P<0.001$) but the difference between gender was not statistically significant ($\chi^2=2.529$, $P=0.112$). The association between infection and type of dogs was statistically significant ($\chi^2=31.936$, $P<0.001$; Table 1).

In cattle, the association between infection and type of cattle was statistically significant ($\chi^2=11.233$, $P=0.004$). There was not statistical differences between breeds ($\chi^2=5.117$, $P=0.077$), gender ($\chi^2=3.294$, $P=0.069$) and age groups ($\chi^2=4.696$, $P=0.195$) but the difference between

Table 1 Comparison of *N. caninum* seroprevalence in different variables in dogs

	Age groups			Gender		Total	
	<1	1–2	>2	M	F	Stray dog	Shepherd dog
NS	17	139	114	158	112	270	
						70	200
NP%	11.8	17.9	40.3	23.4	32.1	27	
						52.8	18

NS number of sample, NP number of positive, M male, F female

Table 2 Comparison of *N. caninum* seroprevalence in different variables in cattle

Age groups	Gender			Dog presence in farm	Dog contact with herd	Stray canids presence in farm	Breeding			Abortion history		Total		
	<2	2–3	3–4				>4	Na	Hy	Ho	Ru	Id	Ru	Id
NS	415	443	238	310	514	892	1214	709	1058	139	522	745	85	1406
NP%	17.3	16	15.1	21.3	19.8	16	17.2	19	20.9	18.7	20.1	15.3	61.2	17.4
													64	20
													57.1	12.8
													19.8	19.8

NS number of sample, NP number of positive, M male, F female, Na native, Hy hybrid, Ho Holesition, Ru rural cattle, Id industrial dairy cattle, Ib industrial beef cattle

the presence of abortion history was statistically significant ($\chi^2=142.247, P<0.001, OR=12.39$). The presence of dogs in farms was not any relation with neospora infections ($\chi^2=0.271, P=0.602, OR=0.9$). The difference between the cattle that contact with dog versus the other one was statistically significant ($\chi^2=3.984, P=0.046, OR=1.37$). The presence of stray canids in farms was statistically significant in cattle ($\chi^2=35.629, P<0.0001, OR=3.56$; Table 2).

Discussion

Several serologic tests including ELISA, IFAT, and Direct Agglutination Test (DAT) are used to detect *N. caninum* (Gharekhani et al. 2012). Infection rate of *N. caninum* were reported 0.7–97.2 % and zero to 67.6 % in cattle and dogs, respectively worldwide (Dubey et al. 2007, 2011).

This study is the first report of *N. caninum* infection in dogs and cattle from west of Iran. In the current study, the prevalence of *N. caninum* infection in dogs was determined 27 %. The results indicated that the infection rate in stray dogs (52.8 %) was higher than shepherd dogs (18 %; $P<0.001$).

The seroprevalence rate of infection in dogs was reported 10.6 to 33 % from Iran (Khanmohammadi et al. 2011; Malmasi et al. 2007). In a similar study in Korea (3.6 %), stray dogs infection was higher than household dogs ($P<0.0001$; Nguyen et al. 2010). Haddadzadeh et al. (2007), reported, the infection rate in farm dogs (28 %) was higher than in urban dogs (11.3 %), and the difference was statistically significant ($P<0.05$). In Malmasi et al. (2007) study, IgG-antibodies to *N. caninum* were seen in 20 and 46 % of household and farm dogs, respectively ($P=0.005$).

The higher seroprevalences in farm dogs could be due to the risk factors that are present in farms. These factors are the possibility to consumption of cattle fetuses or placentas infected with tachyzoites or cysts of *N. caninum*, materials of aborted fetuses, or uterine discharge by dogs living in farm areas (Haddadzadeh et al. 2007; Malmasi et al. 2007). Stray dogs may move between farms and rural area and spread the infection. The high infection of stray dogs in this area is due to contact with wild animals (coyotes and foxes), and other domestic species (horse, sheep, goat, and poultry).

In our study, significant difference was detected among the age groups ($P<0.001$). Similar to other studies in Iran, the seropositivity rates increased with age, suggesting postnatal exposure to *N. caninum* by means of horizontal transmission (Haddadzadeh et al. 2007; Malmasi et al. 2007; Yakhchali et al. 2010). High infection rate has been attributed to a greater probability for exposure to neospora over time, increasing the susceptibility in older dogs (Hosseinejad et al. 2011).

There was no observed significant difference between *N. caninum* infection and gender ($P=0.112$). This finding agrees with research conducted by other authors (Coskun

et al. 2000; Haddadzadeh et al. 2007; Malmasi et al. 2007; Yakhchali et al. 2010; Nguyen et al. 2010; Hosseinijad et al. 2010, 2011; Sharifdini et al. 2011). Gender-related effects were not present. In a study, infection in male dogs was higher than in female dogs (Khanmohammadi et al. 2011). Most of the farmers tend to have male dogs in their farms. Therefore, the male dogs might have been more infectious than the female dogs (Malmasi et al. 2007; Khanmohammadi et al. 2011).

In our study, the highest rate of seropositivity was determined in rural cattle (20 %) followed by beef (19.8 %) and dairy cattle (12.8 %) ($P=0.004$). The lowest and highest previous serological surveys in cattle were reported in 10.5 % (Tabriz, Northwest of Iran) and 46 % (Mashhad, Northeast of Iran) from Iran (Razmi et al. 2006; Nematollahi et al. 2011).

A similar rate of infection was reported in Brazil, Greece, Peru, Australia, Canada, Ireland, Korea, and Spain and was in contrast with other countries (Dubey et al. 2007, 2011). Different serological techniques, study design, and sample size are main causes of varied results.

Considering the investigation results in the same region of world and with Nourollahi et al., in Kerman, there was no significant difference in seroprevalence between the different age groups, similar to our study (Pare et al. 1998; Atkinson et al. 2000; Chanlun et al. 2002; Kyaw et al. 2004; Dubey et al. 2007). In contrast to our result, Razmi et al. (2006) and Gharekhani et al. (2012) reported statistical significance in different age groups. Sadrebazaz et al. (2004) and Wouda et al. (1998) 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 in cattle >2 years of age is due to the decrease of antibody in congenital infection. It seems that the relationship between age and seroprevalence rate is speculative.

In the current study, difference in seroprevalence between different breeds was not statistically significant, similar to study in France (Ould et al. 1999). In Spain, prevalence of *N. caninum* in dairy cattle was reported higher than beef cattle (Quintanilla et al. 1999; Hemphil et al. 2000). This may be related to different production systems for dairy and beef cattle rather than to breed differences. There are indications that *N. caninum* seroprevalence differs according to the cattle breed (Dubey et al. 2011). Considering less studies on breed, planning and conducting extensive research on the impact and role of different breed in the infection prevalence is essential.

In the present study, 61.2 % of samples by abortion history were seropositive that are similar to some previous results (Pare et al. 1998; Anderson et al. 2000; Lopez et al. 2005; Dubey et al. 2011; Gharekhani et al. 2012). Razmi et al. (2006), reported prevalence of abortion seropositive was higher than seronegative cattle in Mashhad ($P<0.05$, OR=1.78). Evaluation of seropositive case in previous studies

showed that the risk of abortion were 4-, 5.3-, and 8-fold higher than seronegative cattle (Jenkins et al. 2002; Vaclavek et al. 2003; Schares et al. 2004; Lopez et al. 2005). Rate of abortion after *N. caninum* infection might also be affected by the breed of cattle (Dubey et al. 2011). Taken together with previous works, these results support the idea that the seropositive rate correlated with abortion.

In the current study, *N. caninum* infection was reported in 17.2 % (209/1214) and 20.9 % (221/1058) of cattle in farms with the presence of dog and stray canids (fox and jackal). Also, 19 % (135/709) of cattle were in contact with dogs that were seropositive. According to our results, 3.56 and 1.37-fold increases in the rate of infection was observed in cattle with the presence of stray canids in farm and dog contact by cattle in farms, respectively.

Studies in Spain and France have also found positive associations between the seropositivity of cattle and the presence or the number of farm dogs (Ould et al. 1999). Barling et al. (2001), observed the presence of dogs in farms as a putative protective factor. That study was conducted in Texas, in the same region where it had been demonstrated that the abundance of wild canids could explain the seroprevalences in cattle. Our result is in contrast with Kyaw et al. (2004) findings.

After the confirmation of the dog as a definitive host, 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 (Dubey et al. 2007).

We found association between the presence of a seropositive dog on a farm and high prevalence of infection in the cattle, indicating a relationship between *N. caninum* infections in both species. Although increasing evidence shows that cattle may be infected by exposure to canine oocysts. It is doubtful that all *N. caninum* abortion storms are the immediate result of a recent exposure to oocyst shedding farm dogs.

This result shows that the presence of stray and farm dogs may be a risk factor for *N. caninum* infection in cattle farms in this area of Iran. However, to confirm this hypothesis, a further molecular characterization of extracted DNA and isolation of parasites by bioassay examination is necessary.

There is both horizontal and vertical transmission of *N. caninum* in this area. In conclusion, *N. caninum* is an important factor in the economic losses of the cattle breeding in Hamedan province. Therefore further investigations and designing control strategies for improving management in cattle farms is highly recommended.

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Conflict of interest We declare no conflict of interest.

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