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Serology based comprehensive study of *Neospora* infection in domestic animals in Hamedan province, Iran

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ABSTRACT

This study was conducted to determine seroprevalence of *Neospora* infection in cattle, sheep, horses, donkeys, and dogs in Hamedan province, Iran. Blood samples (n=2254) from the animals were collected randomly during 2009 to 2012. Sera were prepared from the collected blood samples, which were then examined for the presence of antibodies against *Neospora* using enzyme-linked immunosorbent assay (ELISA), *Neospora* modified direct agglutination test (N-MAT), and indirect fluorescent antibody test (IFAT). The seroprevalence rates of *Neospora* were found as 17.4% (n=245/1406) in cattle, 2.2% (n=8/358) in sheep, 40.8% (n=49/120) in horses, 52% (n=52/100) in donkeys, and 27% (n=73/270) in dogs. In this study, higher levels of *Neospora* infection were detected in cattle, horses, donkeys, and dogs. This is the first comprehensive study of *Neospora* infection in domestic animals in Iran. Further researches on molecular and bioassay studies and designing appropriate control strategies against neosporosis in Iran are necessary and strongly recommended.

Keywords

Cattle, Comprehensive study, Dog, Horse, Donkey, *Neospora*, Seroprevalence, Sheep

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INTRODUCTION

Neospora (*N.*) *caninum* is a coccidian parasite that was first recognized in dogs from Norway in 1984 (Dubey et al., 2007). Domestic and wild canids such as dog and coyote are definitive hosts, and a wide-range of animals such as cattle, sheep, horses and donkeys may play the role as intermediate hosts for this parasite (Dubey and Schares, 2011). Excretion of *N. caninum* oocysts could act as a risk factor when these are come out through feces, and finally mixed with environment of definitive hosts; these could cause stillbirths and miscarriages to cattle and other intermediate hosts (Sharifdini et al., 2011).

Neosporosis in cattle has been associated with endemic, epidemic and sporadic abortions causing huge economic loss worldwide (Salehi et al., 2010; Gharekhani, 2014). In sheep, neosporosis can cause abortion, neonatal mortality and clinical signs (Ueno et al., 2009). *N. hughesi* is considered as equine parasite (Dubey and Schares, 2011). In horses, neosporosis can cause abortion, protozoal myeloencephalitis and neuromuscular disorder (Carrie et al., 2007). Neosporosis is an important cause of neuromuscular disease in dogs (Dubey et al., 2007).

Study of *Neospora* infection rate in animals such as dogs and cattle is necessary for comprehensive evaluation of neosporosis (Dubey et al., 2007). Several serological tests including enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), and *Neospora* modified direct agglutination test (N-MAT) have been using for the diagnosis of this infection (Dubey et al., 2007; Dubey and Schares, 2011). Several serological studies on *Neospora* infection have been reported in different hosts of Iran (Sadrebazzaz et al., 2006; Haddadzadeh et al., 2007; Hajikolaei et al., 2007;

Salehi et al., 2010; Hosseini et al., 2011; Asadpour et al., 2012). However, there is no published comprehensive information about neosporosis in the animals in Hamedan province, Iran. Therefore, this study was conducted for a comprehensive study on *Neospora* infection in domestic animals (cattle, sheep, horses, donkeys and dogs) in Hamedan province, Iran.

MATERIALS AND METHODS

Study area: Hamedan province (a mountainous and mild climate region) is located to 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. In Iran, Hamedan province is economically important for crops and animal husbandry.

Ethical approval: The ethical permission was accorded by the Institutional Animal Ethics Committee (Iranian Veterinary Organization) to collect the sample from live animals.

Sampling: A cross-sectional study was performed in Hamedan province from 2009 to 2012. Blood samples (n=2254) were collected randomly from 1406 cattle (400 rural cattle, 492 industrial dairy cattle, and 514 industrial beef cattle), 358 sheep, 120 horses (45 from horse riding clubs, and 75 from rural horses), 100 donkeys, and 270 dogs (70 stray dogs, and 200 owner shepherd dogs).

Serology: Sera were removed from the blood samples after centrifugation at 1500×g for 10 min, and stored at -20°C until use (Haddadzadeh et al., 2007; Nematollahi et al., 2011). The sera of the ruminants (cattle and sheep), equine (horses and donkeys) and dogs were examined for the presence of antibodies against *Neospora* using ELISA, N-MAT and IFAT, respectively.

Enzyme-Linked Immunosorbent Assay (ELISA): Anti-*Neospora* IgG-antibodies in the ruminant samples were detected using a commercially available ELISA kit (HerdCheck® Anti-*Neospora*; IDEXX Laboratories; Switzerland). The presence of antibody was determined by calculating the value% according to the instructions of the manufacturer. A value of ≥40% was considered as positive.

***Neospora* modified direct agglutination test (N-MAT):** Anti-*Neospora* antibodies in the equine samples were detected using N-MAT (Hosseini et al., 2011). In brief, using phosphate-buffered saline (PBS) containing 0.2M 2-mercaptoethanol, the sera were double-diluted from 1:10 to 1:80. An amount of 50 µL of each dilution was taken in a well of 96-U-bottom microtiter plate. Then, 50 µL of tachyzoites suspension (3.5×10^7/mL; NC-1 strain of *N. caninum*) resuspended in alkaline buffer [7.02g of NaCl, 3.09g of H_3BO_3, 24 mL of 1N NaOH, 4g of horse serum albumin (fraction V), 50mg of eosin Y, dH_2O to 1L, 0.1% sodium azide as preservative; pH: 8.7] was added to each well of serum dilution, and to positive and negative controls. Then, the wells were mixed properly by pipetting. The plate was then incubated overnight at 37°C with 5% CO_2. As per Gharedaghi (2012), a cut-off titer of 1:80 was considered as significant for the presence of antibodies. When the tachyzoites were found as spreading condition on bottom of each well, then it was considered as positive reaction. On the other hand, negative reaction showed button formation at the bottom of each well.

Indirect Fluorescent Antibody Test (IFAT): A commercial IFAT kit (MegaScreen® FLUONEOSPORA, Horbranz Austria) was used to evaluate the IgG-antibodies of dog samples. A titer 1:50 (cut-off value) was regarded as a positive threshold titer (Haddadzadeh et al., 2007).

Statistical analysis: Statistical analysis was performed by using the software package SPSS version 16.0 for windows. Odds Ratios (OR), confidence Interval (CI), χ^2 and *p*-value were calculated separately for each variable. A *p*-value ≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The seroprevalence of *Neospora* infection were reported as 17.4% (n=245/1406) in cattle, 2.2% (n=8/358) in sheep, 40.8% (n=49/120) in horses, 52% (n=52/100) in donkeys, and 27% (n=73/270) in dogs (**Table 1**). Among the cattle, higher rate of seropositive was detected in rural cattle (20%; n=80/400) followed by beef cattle (19.8%; n=102/514) and dairy cattle (12.8%; n=63/492) (p=0.004). Seroprevalence rate in riding club horses (42.2%; n=19/45) was higher than rural samples (40%; n=30/75) (p=0.811). In dogs, the seroprevalence rates were 52.8% (n=37/70) and 18% (n=36/200) in stray and shepherd dogs, respectively (p<0.001). A prevalence of 61.2% and 1.9% were observed in cattle and sheep having history of abortion, respectively (**Table 2**).

As worldwide scenario, infection rates of *Neospora* were reported as 0.7-97.2% in cattle, 0.45-63% in sheep, 0-77.7% in equine, and 0-67.6% in dogs (Dubey et al., 2007; Dubey and Schares, 2011). However, in Iran, these rates were reported as 15.8-46% in cattle (Razmi et al. 2006; Nematollahi et al., 2011; Gharekhani et al., 2012), 1.1-5.7% in sheep (Asadpour et al., 2012;

Table 1: The seroprevalence of *Neospora* infection in different age and gender groups in animals.

Species	Age groups (year)		Gender		Total (%)	CI 95%
	1 - ≤2 (%)	>2 – 3 (%)	Male (%)	Female (%)		
Cattle	415(17.3)	991(17.4)	514(19.8)	892(16)	1406(17.4)	15.4-19.3
Sheep	124(4.8)	234(0.85)	0(0)	358(2.2)	358(2.2)	2.05-2.35
Horse	49(49)	71(35.2)	43(46.5)	77(37.7)	120(40.8)	34.7-46.8
Donkey	63(55.5)	37(45.9)	22(36.4)	78(56.4)	100(52)	42-62
Dog	156(17.3)	114(40.3)	158(23.4)	112(32.1)	270(27)	21.7-32.3

CI = Confidence Intervals

Table 2: The seroprevalence of *N. caninum* in animals having history of abortion.

Species	Abortion history		*p*-value	OR
	No. of sample	No. of positive (%)		
Cattle	n=85	52 (61.2)	<0.001*	12.4
Sheep	n=155	3 (1.9)	0.737	0.78

*A *p*-value ≤0.05 was considered as significant; OR = Odds Ratios

Ezatpour et al., 2012), 28-32% in horses (Hosseini et al., 2011; Moraveji et al., 2011; Gharedaghi, 2012), and 10.6-33% in dogs (Haddadzadeh et al., 2007; Malmasi et al., 2007; Hosseininejad et al., 2010; Yakhchali et al., 2010; Hosseininejad and Hosseini, 2011).

In our study, the seroprevalence rate was determined between 12.8-20% among different types of cattle. In a study conducted in Spain, Quintanilla-Gozalo et al. (1999) found that prevalence of *N. caninum* in dairy cattle was higher than beef cattle. This variation in prevalence might be due to difference in production systems of dairy and beef cattle, rather than differences in breed (Dubey and Schares, 2011).

In our study, no significant correlation was found among different age and gender groups of cattle (p=0.961, p=0.069), horses (p=0.131, p=0.344) and donkeys (p=0.353, p=0.096); unlike to age group in sheep (p=0.015, OR=5.9). In contrast, Razmi et al. (2006) and Gharekhani et al. (2012) reported significant correlations among different age groups. Sadrebazzaz et al. (2004) and Wouda et al. (1999) reported equal levels of seroprevalence in all age groups for most herds. On the other hand, Jensen et al. (1999) suggested that seroprevalence increased with age and depended on sample size.

In the present study, 61.2% cattle were found to be seropositive that had abortion history (**Table 2**), which was in support to several previous studies (Paré et al., 1998; Anderson et al., 2000; López-Gatius et al., 2005; Dubey and Schares, 2011; Gharekhani et al., 2012). Similarly, Razmi et al. (2006) reported a higher abortion rate in seropositive cattle as compared to seronegative cattle (p<0.05, OR=1.78). The risk of abortion in seropositive cattle was reported as 4 (Václavek et al., 2003), 5.3 (Schares et al., 2004) and 8 (López-Gatius et al., 2005) folds higher than seronegative cattle. In another study, Youssefi et al. (2010) reported that 7%, 45.2%, and 57.3% of aborted cattle were seropositive for *N. caninum* infection in Ardebil (Northwest of Iran, cold climate), Garmsar (Central of Iran, warm and dry climate) and Babol (North of Iran, mild climate), respectively.

The overall seroprevalence rate in sheep (2.2%; **Table 2**) found in our study was similar to the studies that were conducted in Italy and Australia (Ghaffari et al., 2006; King et al., 2010). In another study in Iran, this rate was reported as 1.13% in aborted sheep, and 1.7% in healthy sheep (Ezatpour et al., 2012). On the other hand, Asadpour et al. (2012) reported that 5.7% of sheep and 8.5% of ovine fetus in Northwest Iran were infected to neosporosis.

In the current study, higher seroprevalence was reported at age 1-≤2-yr (4.8%) in sheep (p=0.015, OR=5.9). In contrast to our findings, no significant difference was recorded relating to age group of sheep in Brazil, Italy, Spain, United Kingdom, Pakistan, and Iran (Lorestan province), (Ghaffari et al., 2006; Ueno et al., 2009; Panadero et al., 2010; Ezatpour et al., 2012; Nasir et al., 2012).

In this study, *Neospora* in horses (40.8%) of >2-3 years age group showed higher seroprevalence as compared to 1-≤2 years of age group, which was similar to that of the findings of Locatelli et al. (2006). The infection rate in riding club horses was higher than rural samples, which might be due to intensive management system and their direct contact with dogs of inside club. No

significant correlation was seen between the infection rate and genders, similar to the findings of some other previous studies (Mc-Dole and Gay, 2002; Pitel et al., 2003; Jakubek et al., 2006; Moraveji et al., 2011; Gharedaghi, 2012).

Current survey is the first report of *Neospora* infection in donkeys in Iran. The donkey carcasses are being used as food for carnivores in zoo, and are fed to stray canids in suburb of villages in Iran; which might be responsible for transmission of infection among animals.

In our finding, *Neospora* infection rate in stray dogs (52.8%) was higher than shepherd dogs (18%) (p<0.001). This result was in support of Nguyen et al. (2010) who conducted researches in South Korea. Haddadzadeh et al. (2007) and Malmasi et al. (2007) reported high infection rate of *Neospora* infection in farm dogs as compared to urban and household dogs. Similar to some other studies, the seroprevalence rates in dogs increased with age, which suggested postnatal exposure to *N. caninum* through horizontal transmission (Malmasi et al., 2007; Haddadzadeh et al., 2007; Yakhchali et al., 2010). High infection was attributed to greater chances for exposure to *Neospora* over time, increasing the susceptibility of older dogs (Hosseininejad and Hosseini, 2011). There was no significant difference in gender, which was in agreement with some other reports (Haddadzadeh et al., 2007; Nguyen et al., 2010; Hosseininejad and Hosseini, 2011; Sharifdini et al., 2011). Farmers in Iran mostly reared male dogs in their farms. Therefore, the male dogs might have been infected with *Neospora* more than females (Hosseininejad et al., 2010; Khanmohammadi and Fallah, 2011). However, different serological tests and cut-off values, study design, climatic variations and frequency of canids on the farms and around of animals were the main causes of varied results (Dubey et al., 2007).

Most of the animal breeding farms are traditional in Iran, and the animals had a direct contact with the dogs and other canids. Oocyst-contaminated pastures, fodder, and drinking water were considered as potential sources of postnatal infection in animals. Thus, type of feeding practices might pose on increased level of infection risk (Dubey et al., 2007).

Both horizontal and vertical routes of *Neospora* transmission in animals are present in Hamedan region. After the confirmation of dog as a final host, the presence of dogs in farm was assumed to provide the higher chance of horizontal transmission through ingestion of oocysts that were shed by the infected dogs (Dubey et al., 2007).

CONCLUSIONS

To the best of our knowledge, this is the first comprehensive study on *Neospora* infection in domestic animals in Iran. In this study, higher levels of infection were detected in cattle, horses, donkeys and dogs; which could be an important reason for economic losses in this region. Molecular and bioassay studies of *Neospora* and design of appropriate control strategies against neosporosis in Iran are suggested.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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