

Seroprevalence of *Neospora* infection in horses and donkeys in Hamedan province, Western Iran

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Abstract

Aim: The aim of the present study was to determine the seroprevalence of *Neospora* infection in horses and donkeys in Hamedan province, Western Iran.

Materials and Methods: In cross-sectional study, Blood samples (n=220) were collected from 120 horses and 100 donkeys in 2012 year. All sera were screened for *Neospora* using *Neospora* modified direct agglutination test (N-MAT).

Results: Antibodies to *Neospora* infection in horses and donkeys were reported in 40.8% and 52%, respectively. There was not significant correlation demonstrated between infection rates in different age groups and genders.

Conclusion: The current study is the first report of *Neospora* infection in donkeys from Iran. Further investigations and designing control strategies is recommended.

Key words: donkey, Hamedan, horse, Iran, *Neospora*, N-MAT,

Introduction

Neospora caninum with global distribution is a coccidian parasite that was first recognized in dogs from Norway in 1984 [1]. Wide range of animals such as cattle, sheep, horses and donkeys may be play the role of intermediate hosts for this parasite [2]. Another species of *Neospora*, *N. hughesi* is considered as equine parasite [2]. Neosporosis can cause abortion, protozoal myeloencephalitis and neuromuscular disorder signs in horse [3].

Several assays are available for detection of antibodies to *N. caninum* in animals [4]. *Neospora* Agglutination Test (NAT) is the most used serological method for *Neospora* detection in horses [5]. Some serological study of *N.caninum* infection in cattle [6], dog [7], water buffalo [8], camel [9], horse [10], and sheep [11], have done in different region of Iran. There is no published information on *Neospora* infection in equine of Hamedan.

The aim of this study was to determine the seroepidemiology of *Neospora* spp. in domestic equine (Horses and Donkeys) from Hamedan province, West of Iran.

Materials and Methods

Sampling: A cross-sectional study was performed during April to December 2012. Blood samples (n=220)

were collected randomly from 120 horses (*Equus caballus*) (37.5% in horse riding clubs, and 62.5% in rural horses), and 100 in donkeys (*Equus africanus asinus*) from different region of Hamedan province. Information about age and gender was taken from owners and physical examination was done. The animals were categorized into three age groups (<3 yr, 3-6 yr and >6 yr).

Neospora modified direct agglutination test (N-MAT): All sera were removed after centrifugation at 1200×g for 10min and stored at -20°C until laboratory examing. Anti-*Neospora* antibodies of samples were detected using the *Neospora* modified direct agglutination test (N-MAT) [12]. In brief, sera were doubling diluted from 1:10 to 1:80 with phosphate-buffered saline containing 0.2 M 2-mercaptoethanol, and 50 µl of each dilution was put in a well of 96 U-bottom microtiter plates. Then, 50 µl of 3.5×10^7 /ml tachyzoites suspension (NC-1 strain of *N. caninum*) resuspended in alkaline buffer (7.02 g of NaCl, 3.09 g of H3BO3, 24 ml of 1N NaOH, 4 g of horse serum albumin [fraction V], 50 mg of eosin Y, dH2O to 1liter, 0.1% sodium azide as a preservative; pH: 8.7) were added to each serum dilution of samples as well as positive and negative controls. The wells were then mixed thoroughly by pipetting up and down several times; covered, and incubated overnight at 37°C with 5% CO₂. A cut-off titer of 1:80 was considered as significant for the presence of antibodies [13]. when the tachyzoites were spread on entire bottom of micro plate wells, and those showing button formation were

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Table-1. Seroprevalence of *Neospora* sp. in different gender and age groups in horses and donkeys from western Iran.

Parameters	Horse			Donkey	Total
	Riding club	Rural	Total		
Sex					
Male	26(57.9%)	17(52.9%)	43(46.5%)	22(36.1%)	65(43%)
Female	19(42.1%)	58(36.2%)	77(37.7%)	78(56.4%)	155(47%)
Age groups (year)					
<3	3(0%)	53(45.3%)	56(42.8%)	77(51.9%)	133(48.1%)
3-6	40(47.5%)	20(30%)	60(41.7%)	23(52.2%)	83(44.6%)
>6	2(0%)	2(0%)	4(0%)	0(0%)	4(0%)
Total	45(42.2%)	75(40%)	120(40.8%)	100(52%)	220(45.9%)

Figures in bracket shows positive no.

considered positive and negative, respectively.

Study area: Hamedan province by mountainous and mild climate is located in west part of Iran (34.77°N and 48.58°E). This region is economically impressed by an agricultural and animal husbandry. According to veterinary office report of 2010, the equine population in this area was approximately 22,000.

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* test. *P*-value of less than 0.05 was considered statistically significant.

Results

The frequency of prevalence for *Neospora* antibodies was shown as much as 45.9% (95% CI: 43.9%-48%) (Table-1). Seroprevalence of *Neospora* in horses and donkeys were found in 40.8% (95% CI: 34.77%-46.83%) and 52% (95% CI: 42.01%-61.99%), respectively ($\chi^2=2.738$, $P=0.098$). In horses, seroprevalence rate of riding club samples (42.2%) was higher than rural samples (40%) ($\chi^2=0.057$, $P=0.811$). The most and least frequency rates were shown in equines <3 yr (48.1%), and >6 yr (0%), respectively. Furthermore, there was not found statistical differences among age groups ($\chi^2=0.570$, $P=0.752$) and genders ($\chi^2=0.298$, $P=0.585$).

Discussion

As per our knowledge, this study is the first report of *Neospora* seroprevalence in donkeys from Hamedan province, West of Iran.

The seroprevalence rate of *Neospora* was reported from zero to 77.7% in horses worldwide [1]. The previous serological studies in *Neospora* sp. in horses were reported 28% in Northwest, 30% in Northeast, and 32% in South of Iran [10,12,13]. In the present study, *Neospora* seroprevalence in horses (40.8%) was obtained similar to investigation from Southern Brazil [14]. The prevalence rate in riding club samples was higher than rural samples, due to intensive system and their direct contact by dogs inside the club. Neurological signs and abortion history were not showed in any samples, similar to our results. Different serological assays, study design, and geographical conditions are main cause of varied results.

In current study, there was no statistical significant between the seroprevalence samples and

different gender, as similar to other investigations [5,10,12,13,15,16,17].

There are both way (horizontal and vertical) transmission of *Neospora* in animals [1,2]. 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 [1].

Our results and Gharekhani [6] study in cattle from Hamedan indicate that exposure to *Neospora* is common in West of Iran, and the horizontal way is a major mode of transmission in equine from this region. The results of this survey can provide baseline information for the future investigations. However, comprehensive studies in other *Neospora* hosts and a further molecular characterization of extracted DNA, and isolation of parasites by bioassay examination is necessary.

Conclusion

The current study is the first report of *Neospora* seroprevalence in donkeys from Iran. The seroprevalence of *Neospora* in horse is reported in some region of Iran, before our study. Therefore further investigations and designing control strategies is highly recommended, since donkeys may be play significant role in the wild animal (Coyotes and Foxes) infection. In Iran, The donkeys living outdoor, therefore maybe eaten by wild animals and complete the life cycle.

Authors' contributions

JG: project manager and designer, sampling and testing. GRT: technical guidance. GRN: carried out the statistical analysis. All authors drafted an revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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