

Seroprevalence of *Toxoplasma gondii* and *Neospora* spp. Infections in Arab Horses, Southwest of Iran

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Background: Because of the economic importance of the Arab race horses and also the role of *Toxoplasma gondii* and *Neospora* spp. in abortion and reproductive failure of these animals, we decided to perform this study.

Objectives: We designed this study to investigate the seroprevalence of anti-*Toxoplasma gondii* and anti-*Neospora* spp. antibodies in Arab horses from 12 cities of Khuzestan province in southwest of Iran.

Materials and Methods: From October 2009 to March 2011, a total of 235 blood samples were collected from jugular veins of Arab horses of different ages and genders from 12 cities of Khuzestan province. All the sera were tested for anti-*Toxoplasma* antibodies using the modified agglutination test (MAT) and the existence of anti-*Neospora* antibodies were tested using N-MAT for *Neospora* spp.

Results: According to the MAT results, antibodies to *T. gondii* were found in 114 (48.5%) of 235 sera with titers of 1:20 in 84, 1:40 in 19, 1:80 in four, 1:160 in four, and 1:320 in three horses. According to the N-MAT results, antibodies to *Neospora* spp. were found in 47 (20%) of 235 sera with titers of 1:40 in 39, 1:80 in five, and 1:160 in three horses. We did not observe any statistically significant differences regarding age groups and genders between seropositive and seronegative horses for *Neospora* spp. using chi-square (χ^2) test, but it seemed that anti-*Toxoplasma* antibodies were more prevalent in older horses (≥ 10 years old).

Conclusions: The results indicated that Arab horses are exposed to these parasites in southwest of Iran. Further research is required to determine the genomic structures of these parasites in Arab horses in southwest of Iran.

Keywords: *Toxoplasma gondii*; *Neospora*; Agglutination Tests; horses; Iran

1. Background

Toxoplasma gondii and *Neospora* spp. are obligate, intracellular, coccidian protozoan parasites belonging to the Apicomplexa phylum and are closely related tissue-dwelling protozoan parasites with similarity in morphological and biological characteristics, infecting almost all warm-blooded animals (1, 2). Antibodies of these parasites have been found in the sera of many species of domestic and wild animals worldwide (3-6). Both parasites have indirect life cycles. Definitive hosts of *T. gondii* are cats and other felids (7). Mainly dogs and perhaps other canids are the definitive hosts of *N. caninum*, but the definitive host of *N. hughesi* is still unknown (8, 9). These parasites have a wide range of intermediate hosts including sheep, goat, cattle, horse, bison, camel, pig, and deer (10-13). However, horse can serve as the potential intermediate host of these parasites and can be infected following ingestion of sporulated oocysts via contaminated feed or water, as well as via vertical transmission from mother to fetus through the placenta, which is an alternative route (14,

15). Both parasites are related to *Coccidia*, which is reported to cause encephalitis in horses (7). Toxoplasmosis is one of the most common parasitic zoonoses worldwide (16). Antibodies against *T. gondii* were detected in horses in the Czech Republic (17), Turkey (18, 19), Egypt (20), Sweden (21), and Switzerland (22). Toxoplasmosis is a common infection that rarely evolves to clinical disease (23), but may cause fever, ataxia, retinal degeneration, abortion, and severe encephalomyelitis (24).

N. caninum and *N. hughesi* were first described in dogs in Norway in 1984 in the brain and spinal cord of an adult horse in California, USA, respectively (25, 26). In 1990, the first serological evidence of *N. caninum* infection in a horse was described (27). Antibodies against *Neospora* sp. in equine populations have been reported in many parts of the world such as the United States (28), South Korea (29) Iran (30), and Brazil (31). In Europe, antibodies against *N. caninum* have been reported in horses in France (32), Italy (33), Sweden (34), Turkey (35) and the

Czech Republic (36). Horses can be infected by *N. caninum* and *N. hughesi*. Although exposure to *Neospora* spp. in horses seems to be common, clinical diseases are rare. The clinical disease is characterized by abortion, neonatal diseases, and neurological findings of severe encephalomyelitis (14). *N. caninum* and *N. hughesi* are related to abortions and neurological diseases in horses (31).

2. Objectives

Few data has been reported about the prevalence of *T. gondii* and *N. caninum* in Arab horses in Iran. The current research was conducted to investigate the seroprevalence of *T. gondii* and *Neospora* spp. infections among Arab horses, the most popular horse species in Khuzestan province, southwest of Iran, using modified agglutination test (MAT).

3. Materials and Methods

3.1. Study Area

The study area, Khuzestan province, covers approximately 65000 km² and is located in southwest of Iran (31° 3' N, 48° 7' E) (Figure 1). The climate of this area is generally hot and occasionally humid. Summertime temperatures routinely exceed 50°C. Khuzestan province is known to master the hottest temperatures on record for a populated city anywhere in the world.

3.2. Collection of Sera

From October 2009 to March 2011, blood samples (5 to 10 mL) were collected from jugular veins of 235 Arab horses

of different ages and sexes. The samples were from 12 cities (Table 1) of Khuzestan province, southwest of Iran (Figure 1). According to the information taken from owners and the physical examinations performed by veterinarians on age and gender of horses, they were categorized into three age groups (≤ 2 , 2-10, and ≥ 10 years old). The blood samples were centrifuged 10 minutes at 3000 rpm and serum was removed and stored at -20°C until used.

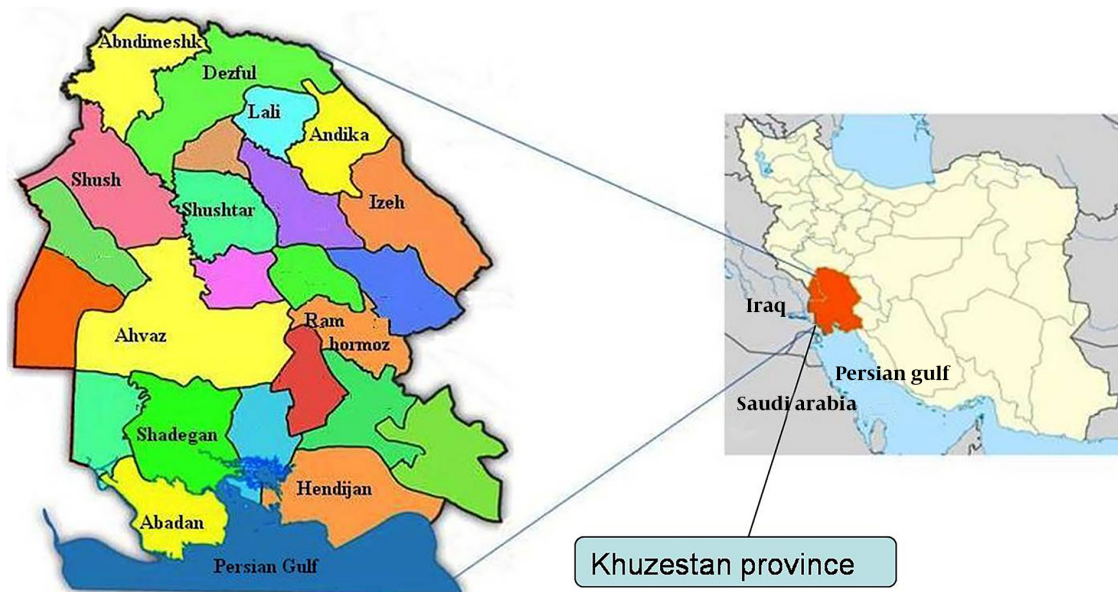
3.3. Parasites

The *T. gondii* and *N. caninum* antigens used in MAT and N-MAT were prepared from the RH strain of *T. gondii* and the NC-1 strain of *N. caninum* tachyzoites, harvested from cells grown in mice in the Pasteur Institute of Tehran and the Razi Institute of Shiraz, Iran, respectively.

3.4. *Toxoplasma gondii* Serology

T. gondii antibodies were investigated using modified agglutination test, as described by Desmonts and Remington (37) and Dubey and Desmonts (38). The sera were diluted two folds (1:20 to 1:320) with phosphate buffered saline containing 0.2 M 2-mercaptoethanol and 50 μ L of each dilution was put in a well of 96 U-bottom ELISA plates. Thereafter, 50 μ L of the whole formalin-preserved *T. gondii* tachyzoites were added to each serum dilution. The wells were then mixed thoroughly by pipetting up and down several times, covered, and then incubated at 37°C overnight. The test was considered positive when a layer of agglutinated parasites was formed in wells at dilutions of 1:20 or higher. Positive and negative controls were included in each test.

Figure 1. Map of Iran and Khuzestan Province



Khuzestan is highlighted with green. Cities of this province are distinguished by colures. The map of Khuzestan province by Uwe Dering highlighted by Dr. Blofeld.

3.5. *Neospora* spp. Serology

The N-MAT was used for detection of antibodies to *Neospora* spp. in horses, described by Romand et al. (39), which is very similar to MAT which is used for detection of toxoplasmosis in sera, except that *Neospora* tachyzoites were used as antigens. Briefly, the sera were diluted with phosphate buffer saline (pH = 7.2) containing 2-Mercaptoethanol (2-ME) and screened with 1:40 and 1:80 dilutions. Positive sera at 1:80 dilutions were subsequently submitted to serial dilutions (1:160 to 1:640) (29).

3.6. Statistical Analysis

Differences in antibody prevalence of horses infected with *T. gondii* and *Neospora* spp. with regard to gender, age and geographical regions were analyzed using chi-square test and were calculated with SPSS (version 16.0). P value < 0.05 was considered statistically significant.

4. Results

All the equines remained clinically normal and there was no evidence of abortion. The serological results are summarized in Tables 1, 2 and 3. The horses' sera were collected from different cities of Khuzestan province (Table

1). Statistical analysis showed that some factors like age affected the prevalence of *T. gondii* infection. In this study, we divided all the horses into three groups based on age, to 53 (≤ 2 years old), 121 (2-10 years old), and 61 (≥ 10 years old) horses. The *T. gondii* seroprevalence in adult horses (≥ 10 years old) was significantly higher than other age groups (63%: 38/61). The results showed that *T. gondii* seroprevalence in horses increased with age. Toxoplasma antibody with MAT in horses in the age group of 2-10 was 49% (59/114) and was 33% in the age group of ≤ 2 (17/53). However, we could not find any correlation regarding antibodies and age between the three groups. We found 31 (13/2%) samples that showed antibodies for both of these parasites in at least the 1:20 titer.

4.1. *Toxoplasma gondii*

Antibodies to *T. gondii* were detected in 114 (48.5%) Arab horses. The majority of the positive sera had titers ≤ 1:20 (Table 2). The seroprevalence in horses varied from 1.8% to 28% between regions. No differences in seroprevalence were found between male and female horses. Antibodies to *T. gondii* were found in 84 (35.8%) of 235 horses with titers of 1:20, 19 (8.1%) with titers 1:40, 4 (1.7%) with titers of 1:80, 4 (1.7%) with titers of 1:160, and 3 (1.3%) with titers of 1:320. MAT was repeated twice in this step.

Table 1. The Number of Samples From Different Sites in Khuzestan Province and Their Serological Status Against *Toxoplasma gondii* and *Neospora caninum*

City	Samples, No.	Males, No.	Females, No.	<i>T. gondii</i> (+)		<i>Neospora</i> spp. (+)	
				Male	Female	Male	Female
Abadan	31	16	15	4	7	2	2
Ahvaz	58	27	31	21	12	7	11
Ramhormoz	15	9	6	3	5	1	1
Shush	18	9	9	4	2	1	4
Shushtar	15	10	5	4	5	2	2
Dezfol	24	13	11	6	4	1	2
Lali	10	6	4	2	1	-	1
Andika	10	3	7	3	1	1	1
Andimeshk	15	7	8	3	3	-	-
Hendijan	10	6	4	3	4	1	-
Izeh	13	8	5	5	3	2	-
Schadegan	16	8	8	5	4	2	3
Total	235	122	113	63	51	20	27

Table 2. Modified Agglutination Test Titers for *T. gondii* in Sera From 235 Arab Horses of Khuzestan Province

	Titers				
	1:20	1:40	1:80	1:160	1:320
Male	51/122 (42)	7/122 (5.8)	2/122 (1.7)	1/122 (0.8)	2/122 (1.7)
Female	33/113 (30)	12/113 (10.7)	2/113 (1.8)	3/113 (2.7)	1/113 (0.9)
Total	84/235 (35.8)	19/235 (8.1)	4/235 (1.7)	4/235 (1.7)	3/235 (1.3)

^a Data are presented as No. (%)

Table 3. Direct Agglutination Test to *Neospora* spp. in Sera From 235 Arab Horses in Khuzestan Province^a

	Titers		
	1:40	1:80	1:160
Male	17/122 (14)	2/122 (1.7)	1/122 (0.8)
Female	22/113 (20)	3/113 (2.7)	2/113 (1.8)
Total	39/235 (16.6)	5/235 (2.2)	3/235 (1.3)

^a Data are presented as No. (%)

4.2. *Neospora* spp.

Antibodies to *Neospora* spp. were detected in 47 (20%) among Arab horses. Sera were examined by the developed *Neospora* agglutination test (NAT) as described by Romand et al. (39) NAT is similar to MAT for *T. gondii* except that tachyzoites of *Neospora* spp. are used instead *T. gondii* tachyzoites. Antibody titers to *Neospora* spp. in examined horses are shown in Table 3. At 1:40 dilution, the prevalence of antibodies to *Neospora* spp. was 14% for males and 20% for females, while at 1:80 and 1:160 dilutions prevalence was 1.7% and 0.8% for males and 2.7% and 1.8% for females respectively.

5. Discussion

This is the first report of *T. gondii* and *Neospora* spp. infections in Arab horses in southwest of Iran. Results of this investigation indicated that 114 (48.6%) of 235 Arab horses had been exposed to *T. gondii* and antibodies to *Neospora* spp. were detected in 47 (20.5%) of them. MAT is considered as one of the highly specific and sensitive tests for detection of *T. gondii* and *Neospora* spp. in animals, especially in horses (23, 40). Although antibodies to *T. gondii* have been reported in horses by MAT, ELISA or IFAT from many countries, there is no clear evidence that this protozoan causes clinical diseases in horses (23). We did not find any significant differences between the seroprevalence of *T. gondii* and *Neospora* spp. and horses gender. Dubey et al. the first time detected tachyzoites of *N. caninum* in a fetus lung (27) and *N. hughesi* tachyzoites were isolated from an adult horse in the US (8). Horizontal transmission of *Neospora* spp. in horses appears to be a major mode of transmission (41). Because of serological cross reactivity between *N. caninum* and *N. hughesi*, we could not confirm which species of *Neospora* infected Arab horses using MAT. The seroprevalence of *T. gondii* in older horses indicated more prevalence rates, which was in agreement with Boughattas's study in Tunisia (42). In a recent Iranian survey in northeast of Iran on *Neospora* in horses, they detected *Neospora* antibodies in 30% of horses using N-MAT (43). The serological results of this study indicated that 47 of 235 (20%) horses were exposed to *Neospora* spp. which was comparable to those reported from the northwest, north, south and west of Iran as 28%, 30%, 32% and 40.8%, respectively (30, 43-45).

Our results were comparable to those reported from the US, France and Italy (27, 33, 46). According to hajjalilo's study in Qazvin, Iran, 71.2% of sport horses were seropositive for *T. gondii* (47).

The prevalence of *Neospora* spp. antibodies in cattle in the southeast of Iran was 12.6% (48) and in water buffaloes in the southwest of Iran it was 37% (49). This results imply that exposure to this parasite is common in south of Iran. Dogs could be one of the main definitive host in this region. According to the study of Hosseininejad et al. the prevalence of *N. caninum* in dogs from west and central parts of Iran was 26.8% (50). Another study in west of Iran (Hamedan province) indicated that the prevalence of *N. caninum* in dogs was 27% (51). Further research on the epidemiological and molecular evidence for identification of *Neospora* strains is required. This study was the first investigation on *T. gondii* and *Neospora* spp. in Arab horses of Khuzestan province, Iran, and indicates that there is exposure to these parasites in this region. Therefore, designing control strategies including restriction of the existent stray cats and dogs in farm hoes as well as follow up and treatment of the owned dogs is recommended and further studies such as molecular and sequencing methods are needed to distinguish *N. caninum* from *N. hughesi*.

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Authors' Contributions

Tavalla Mehdi: study design, study execution, data analysis and interpretation, and preparation of the manuscript. Sabaghan Mohammad: data collection, study execution, and preparation of the manuscript. Abdizadeh Rahman: study execution and preparation of the manuscript. Shahram khademvatan: data analysis and interpretation and preparation of the manuscript. Rafiei Abdollah: preparation of the manuscript.

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