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

Mehdi Tavalla 1,2,*; Mohammad Sabaghan 1; Rahman Abdizadeh 3; Shahram Khademvatan 4; Abdollah Rafiei 1,2; Anahita Razavi Piranshahi 1

1Department of Medical Parasitology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
2Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
3Department of Medical Parasitology and Mycology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, IR Iran
4Department of Medical Parasitology, Urmia University of Medical Sciences, Urmia, IR Iran

*Corresponding author: Mehdi Tavalla, Department of Medical Parasitology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran. P. O. Box: 61357-33118, Tel: +98-6113367543, Fax: +98-6113367545, E-mail: am.tavalla@gmail.com

Received: September 19, 2013; Revised: April 19, 2014; Accepted: November 2, 2014

**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 14, 1:160 in 10, and 1:320 in 1 horse. According to the N-MAT results, antibodies to *Neospora* spp. were found in 47 (20%) of 235 sera with titers of 1:40 in 19, 1:80 in 13, 1:160 in 3 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 ($\chi^2$) test, but it seemed that anti-*Toxoplasma* antibodies were more prevalent in older horses ($\geq 10$ 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 serorevalence 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 65,000 km² and is located in southwest of Iran (31º 3’ N, 48º 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 µL of each dilution was put in a well of 96 U-bottom ELISA plates. Thereafter, 50 µL 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%) 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. | T. gondii (+) Male | T. gondii (+) Female | Neospora spp. (+) Male | Neospora spp. (+) 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                   | -                     | -                       |
| Andika    | 10           | 3          | 7            | 3                 | 3                   | -                     | -                       |
| 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 *

| Titer | Male       | Female     | Total     |
|-------|------------|------------|-----------|
| 1:40  | 17/112 (14) | 2/113 (1.8)| 22/225 (10) |
| 1:80  | 2/112 (1.7) | 3/113 (2.7)| 5/235 (2.1) |
| 1:160 | 1/112 (0.8)| 2/113 (1.8)| 3/235 (1.3) |

* 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 of 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. (41) 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 hajialilo’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 houses 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.

Acknowledgements

This study was conducted using personal financial sources. We are very grateful to Dr. Mohammad M. Namavari from RAZI Vaccine and Serum Research Institute, Shiraz, Iran, whose cooperation is deeply appreciated, especially for providing the Neospora spp. tachyzoites.

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. Rafie Abdollah: preparation of the manuscript.

References

1. Dubey JP. Toxoplasmosis of animals and humans. 2nd ed.BocaRaton,Florida, New York: CRC press; 2010.
2. Goodswen SJ, Kennedy PJ, Ellis JT. A review of the infection, genetics, and evolution of Neospora caninum: from the past to the present. Infect Genet Evol. 2013;13:133-50.
3. Sediak K, Bartova E. Seroprevalences of antibodies to Neospora caninum and Toxoplasma gondii in zoo animals. Vet Parasitol. 2006;136(3-4):223-31.
4. De Craeye S, Speybroeck N, Ajzenberg D, Darde ML, Collinet F, Tavernier P, et al. Toxoplasma gondii and Neospora caninum in wildlife: common parasites in Belgian foxes and Cervidae? Vet Parasitol. 2011;178(1-2):64-9.
5. Kamga-Waladjo AR, Gbati OB, Kone P, Lapo RA, Dombou E, Chataignon G, et al. Neospora caninum and Toxoplasma gondii in lion...
Bjerkas I, Mohn SF, Presthus J. Unidentified cyst-forming spora
ycke Parazitol Derg. 2003;1(3):195–9.

Dubey JP. History of the discovery of the life cycle of Toxoplasma
gondii. Int J Parasitol. 2009;39(8):877–82.

McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire
AM. Rapid communication: Dogs are definitive hosts of Neospora
caninum. Int J Parasitol. 1999;29(9):1247–9.

Dubey JP, Scharen G, Ortega-Mora LM. Epidemiology and control
of neosporosis and Neospora caninum. Clin Microbiol Rev. 2007;20(2):523–67.

Dubey JP. Review of Neospora caninum and neosporosis in ani-
mals. Korean J Parasitol. 2003;41(3–6).

Bartova E, Sedlak K. Seroprevalence of Toxoplasma gondii and
Neospora caninum in slaughtered pigs in the Czech Republic.
Parasitology. 2011;138(1):13–6.

Hamidinejad H, Ghorbanpour M, Rasooli A, Nouri M, Hekmati-
moghaddam S, Namavari MM, et al. Occurrence of anti-Toxo-
plasma gondii and Neospora caninum antibodies in camels (Camelus
dromedarius) in the center of Iran. Turkish J Vet Anim Sci. 2013;37(1):277–81.

Meng QL, Qiao J, Wang WS, Chen CF, Zhang ZC, Cai KJ, et al. Seropre-
valence of Toxoplasma gondii and Neospora caninum in Tarim
Red Deer (Cervus elaphus yarkandensis) from Xinjiang Province,
Northwest China. J Anim Vet Adv. 2012.

Lindsay DS. Neosporosis: an emerging protozoal disease of hors-
es. Equine Vet J. 2003;35(2):316–8.

Tassi P. Toxoplasma gondii infection in horses. A review. Parasit-
ologia. 2007;49(1–2):7–15.

Abca A, Babur C, Arslan MO, Gicik Y, Kara M, Kilic S. Prevalence
of antibodies to Toxoplasma gondii in horses in the province of
Kars, Turkey. Veterinar Medicina-UZPI (Czech Republic). 2004;49(1).

Hejlicek J, Literak I. Prevalence of antibodies to Toxoplasma
gondii in horses in the Czech Republic. Acta Parazit. 1994;
39(4):227–32.

Babur C, Cakmak A, Biyikoglu G, Piskin FC. The detection of anti-
Toxoplasma gondii antibodies in sport horses from Qazi-
sia. Acta Parasitol Turcica. 2010;5(1):68–70.

Kilbas ZG, Adanir A, Avcioglu H. Seroprevalence of Neos-
pora caninum in racehorses in Ankara, Turkey. Acta Parasitol.
2008;53(3):315–6.

Bartova E, Sedlak K, Syrova I, Literek I. Neospora spp. and Toxo-
plasma gondii antibodies in horses in the Czech Republic. Para-
sitol Res. 2010;107(4):785–83.

Desmonts G, Remington JS. Direct agglutination test for diagno-
sis of Toxoplasma infection: method for increasing sensitivity
and specificity. J Clin Microbiol. 1980;11(5):562–4.

Dubey JP. Desmonts G. Serological responses of equids fed Toxo-
plasma gondii oocysts. Equine Vet J. 1987;19(4):337–9.

Romand S, Thulliez P, Dubey JP. Direct agglutination test for se-
rologic diagnosis of Neospora caninum infection. Parasitol Res.
1996;84(1):50–3.

Dubey JP, Thulliez P, Weigel RM, Andrews CD, Lind P, Powell EC.
Sensitivity and specificity of various serologic tests for detection
of Toxoplasma gondii infection in naturally infected sows. Am J
Vet Res. 1995;56(8):1030–6.

Corbellini LG, Smith DR, Pescador CA, Schmitz M, Correa A, Stef-
fen DJ, et al. Herd-level risk factors for Neospora caninum se-
roprevalence in dairy farms in southern Brazil. Prev Vet Med.
2006;74(2–3):330–41.

Boughattas S, Bergaoui R, Essid R, Anon K, Bouratbine A. Seropre-
valence of Toxoplasma gondii infection among horses in Tuni-
sia. Parasitol Vectors. 2013;4:218.

Tavalla M et al. 2015;58(3):14939

Gupta GD, Lakritz J, Kim JH, Kim DY, Kim JK, Marsh AE. Seropreva-
ience of Neospora, Toxoplasma gondii and Sarcocystis neurona
antibodies in horses from Jeju island, South Korea. Vet Parasitol.
2002;106(3):293–291.

Gharekhani J, Tavosidana GR, Naderteif GF. Seroprevalence of
Neospora infection in horses and donkeys in Hamadan prov-
ince, Western Iran. Vet World. 2013;6(9):620–2.

Villalobos EM, Furman KE, Lara Mdo C, Cunha EM, Finger MA,
Busch AP, et al. Detection of Neospora sp. antibodies in cart hors-
es from urban areas of Curitiba, Southern Brazil. Rev Bras Paras-
itol Vet. 2012;21(1):68–70.

Pitel PH, Romand S, Promost S, Fourcher N, Gargala G, Maillard K,
et al. Investigation of Neospora sp. antibodies in aborted mares
from Normandy, France. Vet Parasitol. 2003;118(1–2):3–6.

Ciaromella P, Corona M, Cortese L, Piamento D, Santoro D, Di
Loria A, et al. Seroprevalence of Neospora spp. in asymptomatic
horses in Italy. Vet Parasitol. 2004;123(1–2):1–5.

Jakubek EB, Lunden A, Uggla A. Seroreivalence of Toxoplasma
gondii and Neospora sp. infections in Swedish horses. Vet Parasiti-
rol. 2006;138(3):494–9.

Kilbas ZG, Adanir A, Avcioglu H. Seroprevalence of Neos-
pora caninum in racehorses in Ankara, Turkey. Acta Parasitol.
2011;56(3):215–7.

5

Jundishapur Microbiol. 2015;58(3):14939