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ORIGINAL ARTICLE

Serological study of *Toxoplasma gondii* infection in Turkoman horses in the North Khorasan Province, Iran

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Abstract Toxoplasmosis is an important zoonotic disease with worldwide distribution. The infection is observed in an unusually wide range of warm-blooded animals, including most of the livestock and humans. Many studies have shown high prevalence of toxoplasmosis in man and animals in Iran. The present study was conducted to investigate the seroprevalence of toxoplasmosis in Turkoman horses in the North Khorasan Province. During 2011–2012, 100 blood samples from horses were collected and tested for antibodies against toxoplasmosis using indirect fluorescent antibody test. The seroprevalence of toxoplasmosis was detected in 14 % (14) horses. The antibodies titres were detected in the range of 1:20–1:160 dilution. The lowest and highest frequencies of toxoplasmosis were observed in the age groups of <1 year and 1–10 years, respectively ($P < 0.05$). No significant difference was observed between toxoplasmosis frequencies and gender and usage of horses. With regard to the high frequency of toxoplasmosis in the sampled horses, attention must be paid to the animal health for the control and prophylaxis of the disease.

Keywords *Toxoplasma* · Serology · Horse · The North Khorasan Province

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Introduction

Toxoplasma gondii infection is widely prevalent in man and animal worldwide and has been recognised as an important zoonotic disease (Dubey and Porterfield 1986).Both domestic and wild cats are definitive host of this organism and mammals, birds and humans are intermediate hosts (Tenter et al. 2000; Lindsay and Dubey 2007). The most common sources of infection in intermediate hosts are tissue cysts in meat and oocysts in water and vegetables. Horses are usually infected by the ingestion of oocysts in contaminated feed and water (Dubey and Porterfield 1986; Tassi 2007), with clinical toxoplasmosis being a rare condition (Rodostits et al. 2007). Horses are resistant to experimental infection with 1×10^4 or 1×10^5 oocysts. *T. gondii* can persist in edible tissues of horses for up to 476 days (Lindsay and Dubey 2007).The cyst of *T. gondii* has been isolated from the eyes, placenta and brain of aborted foetus in horses (Dubey and Porterfield 1986; Shappan and Ghazy 2007; Turner and Savva 1990, 1991). In France, three cases of acquired toxoplasmosis in humans have been reported, which were caused by the consumption of raw horse meat (Pomares et al. 2011).

Toxoplasma gondii infection stimulates both the cell mediated immunity and humoral immune response as antibody production. Cellular immunity is therefore the key component of the host's immune reaction in the event of attack by *Toxoplasma*. The macrophages, T lymphocytes T (TL) and "natural killer" (NK) cells, on the one hand, and the cytokines, on the other, are the major elements involved in immune response (Filisetti and Candolfie 2004).

Antibodies play a minor role but remain the essential means for diagnosing toxoplasmosis in humans (Filisetti and Candolfie 2004). Parasite-specific IgM, IgA, IgE and IgG2 antibodies have been isolated from human patients,

and detection of parasite specific antibodies is an effective diagnostic tool to distinguish newly infected individuals from those in the chronic stage of infection (Dupont et al. 2012). The prevalence of toxoplasmosis in the horses were detected by different serological tests including dye test (DT) in Saudi arabia, Turkey (Alanazi and Alyousif 2011; Karatepe et al. 2010), by modified agglutination test (MAT) in Argentina, Mexico, Iran, Tunisia, Portugal, Eygypt (Dubey et al. 1999; Alvarado-Esquivel et al. 2012; Raeghi et al. 2011; Hajialilo et al. 2010; Boughattas et al. 2011; Lopes et al. 2013; Shappan et al. 2012) by enzym linked immunosorbent assay (ELISA) in Eygypt (Shappan et al. 2012), by indirect hemagglutination test (IHA) in china (MiaoQ et al. 2013), by indirect fluorescent antibody test (IFAT) in Brazil and The South Korea (Locatelli-Dittrich et al. 2006; Evers et al. 2013; Gupta et al. 2002) by latex agglutination test (LAT) in Czech and Eygypt (Bártová et al. 2010; Shappan et al. 2012).The seroprevalence of toxoplasmosis in horses has been estimated to be a wide range from 0 to 90 % in different areas of the world (Tassi 2007) and from 11.5 to 72 % in Iran (Raeghi et al. 2011; Hajialilo et al. 2010). The Turkoman horse is an oriental breed, which is bred and found in the east of Caspian Sea, Iran. The present study was conducted to detect the seroprevalence of toxoplasmosis in Turkoman horses in the North Khorasan Province, Iran.

Materials and methods

Field study area

This study was conducted in the North Khorasan Province, located in the northeastern part of Iran at 36°37′–38°17′ N latitudes and 55°53′–58°20′ E longitudes, with an area of more than 28,400 km^2. This province is situated next to the north-eastern border of Iran, near the southern Caspian Sea and south of Turkmenistan, has mountainous areas and receives about 250 mm of rainfall annually. The Turkmen tribes had settled in parts of this province and Turkoman horse rearing is common in the area. A total of 14 villages situated in the border between Iran and Turkmenistan were selected for sampling.

Sampling

Blood samples were collected from the horses from June to August 2011, this was due mainly to easier access to turkeman horses during June–August. Before sample collection, data, including age, gender and usage of horses, were recorded for each horse. The blood samples were collected in clean tubes from the jugular vein. The tubes with the samples were transported to the laboratory under cool condition. The clotted blood was centrifuged at 800×*g* for 10 min at room temperature and the sera were stored at −20 °C until the time of serological examination.

Indirect fluorescent antibody test (IFAT)

The serum samples were analysed for antibodies against *T. gondii* using IFAT as previously described by Razmi and Rahbari (2001). Briefly, the tachyzoites of the RH strain of *T. gondii* were fixed onto clean slides and stored at −20 °C in freezer (Pasteur Institute, Tehran, Iran). For each reaction, sera were diluted in PBS (1:20) and 5 µl of diluted sera were added over the slide holes. The slides were incubated for 30 min at room temperature in humid chamber and were washed three times for 5 min in PBS. The rabbit-anti-horse IgG conjugate (Fuller laboratories, Fullerton, California) was diluted in PBS (1:20) with 0.2 % filtered Evan's blue and 5 µl of the solution were placed over the slide holes. Subsequently, incubation and washing steps were repeated once as outlined earlier. For fluorescence microscopy, the slides were mounted with buffered glycerine, covered with cover slips and examined at 400× magnification. For each slide, positive and negative controls were also used. If the sample was fluorescent positive, a subsequent serial dilution (1:20–1:640) was prepared and re-examined by applying IFAT. A Titre of ≥1:64 was accepted as positivity according to the previous studies in horses (Ghazy et al. 2007; Evers et al. 2013). We used a Zeiss Fluorescence microscope (Carl zeiss, Germany) for microscopy and a digital camera (Olympus, model c-7070 wide zoom, Japan) for photography.

Statistical analysis

Host-related factors such as type of infection, age, gender and activity were analysed by Chi square test to detect any significant relationship.

Results

The antibody titres against *T.gondii* infection were detected in 1:20 dilution in 15 horses, 1:40 dilution in 13 horses, 1:80 dilution in 12 horses and 1:160 dilution in 2 horses. The results of immunoflurescence staining of tachyzoites with sera of infected and uninfected horses were shown in IFAT (Fig. 1a, b). Seropositivity was found in 14 (14 %) of the 100 horses by cut-off titre ≥1:64. The high and low seroprevalence was observed in the age groups of 1–10 years ($P < 0.05$). No significant relationship was observed between the seroprevalence of toxoplasmosis and gender and usage of the horses (Fig. 1; Table 1).

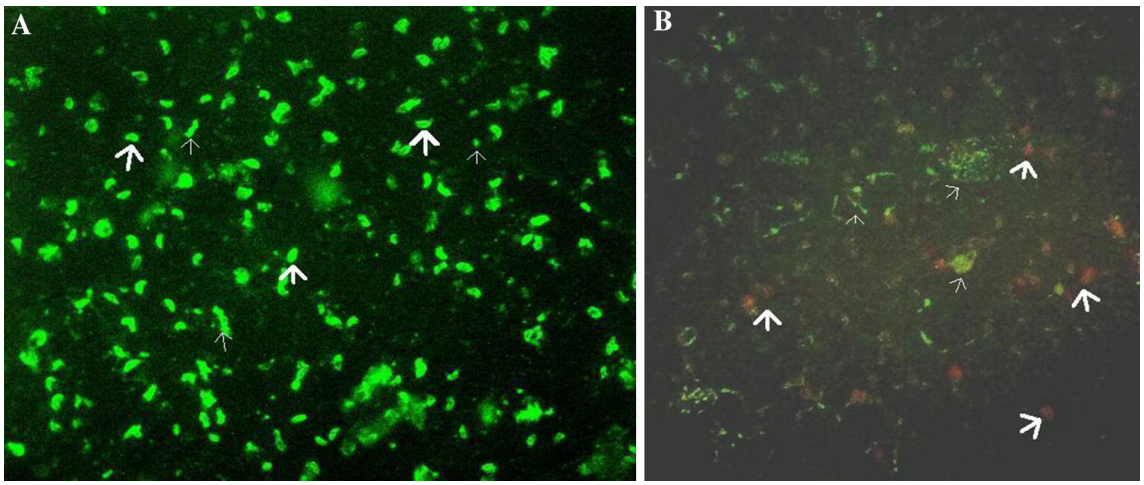

Fig. 1 Immunofluorescenct staining of tachyzites of *T.gondii* with serum of infected horse (**a**) but not staining with serum of uninfected horse (**b**). *Thick arrows* point to tachyzites of *T.gondii* and *thin arrows* to artifacts

Table 1 Seroprevalence of toxoplasmosis in 100 Turkoman horses with respect to age, activity and sex in the North Khorasan province, Iran

Risk factors	Negative	Positive		Total	*P* values
	No	No	%		
Age (year)	–	–	–	–	$P < 0.05$
<1	7	0	0	7	–
1–10	64	14	17.9	78	–
>10	15	0	0	15	–
Activity	–	–	–	–	$P > 0.05$
Racehorse	59	8	11.9	67	–
Stud	27	6	18	33	–
Gender	–	–	–	–	$P > 0.05$
Male	18	3	14.2	21	–
Female	68	11	13.9	79	–
Total	86	14	14	100	–

Discussion

Different serological methods have been used for the detection of antibodies against toxoplasmosis in man and animals. The IFAT has high sensitivity and specificity of serological diagnosis of toxoplasmosis in man when compared with other serological tests (Piergili Fioretti 2004; Wilson et al. 1990), while a comparative serological study about toxoplasmosis in horses showed that ELISA gave better results than IFAT.

IFAT revealed a seroprevalence of toxoplasmosis in 14 % of the horses examined. Recently, in Iran, seropositivity of toxoplasmosis was detected in 71 % of the racing horses in Qazvin area (Hajialilo et al. 2010) and 11.5 % of the horses in Urmea area (Raeghi et al. 2011). Similar studies have been conducted in other countries and the seroprevalence of toxoplasmosis in horses was noted to be 7.2 % (Karatepe et al. 2010)and 28 % (Zeynep et al. 2007) in Turkey, 31 % in Saudi Arabia (Alanazi and Alyousif 2011), 34 % in Czech (Bártová et al. 2010), 24 % in Spain (García-Bocanegra et al. 2012), 17 % in Tunisia (Boughattas et al. 2011) and 30–50 % in Egypt (Shappan et al. 2012). The difference in the results may be owing to the type of serological methods employed, climatic conditions, the age of animals and even to the hygienic condition of the farms and farm management.

In the present study, the highest antibody titre of 1:160 was detected by IFAT. Similar serum dilutions from 1:100 to 1:200 have also been reported in horses in Iran (Raeghi et al. 2011; Hajialilo et al. 2010), Argentina (Dubey et al. 1999) and South Korea (Gupta et al. 2002). The results obtained in the present study indicated the high seroprevalence of toxoplasmosis in older horses. These findings were predictable because the chance of oocyst ingestion in older horses is higher than that in younger horses. The results obtained in the study were in agreement with those

obtained in a previous study conducted in Tunisia (Boughattas et al. 2011), which showed that the seroprevalence of toxoplasmosis in adult horses was significantly higher than that in young horses. However, this finding was in contrast to that reported in studies performed in Turkey (Karatepe et al. 2010) and Brazil (Finger et al. 2013). Furthermore, the difference in the seroprevalence of toxoplasmosis in male and female horses was not statistically significant. This result was in agreement with the previous findings obtained in Turkey (Karatepe et al. 2010) and Brazil (Karatepe et al. 2010), but is contradictory to those observed in Tunisia (Boughattas et al. 2011) and Egypt (Shappan et al. 2012). The Turkoman horses graze in the pasture and the seroprevalence of toxoplasmosis in the mare similar to that in horses with different activities. Previous studies have shown that the usage of horses with different feeding significantly affected the seroprevalence rate of infection (Alvarado-Esquivel et al. 2012; Kouam et al. 2010). In conclusion, in the present study, toxoplasmosis was found to be highly prevalent among Turkoman horses and horse-meat consumption increased the risk of transmission of this organism to the final host (Family: Felidae) and intermediate hosts (humans and other animals).

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