Journal of Animal and Veterinary Advances 13 (3): 139-143, 2014
ISSN: 1680-5593
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Seroprevalence of *Toxoplasma gondii* Antibodies in Horses at Riyadh City

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Abstract: Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii* is one of the most important zoonotic diseases affecting both animals and humans. It is one of the most destructive factors affecting the reproductive potentials of animals being responsible for abortion, stillbirths, resulting in a great economic loss. In this study, blood samples were collected from 152 horses (99 female and 53 male). The age of the study groups ranged from 2 months and 20 years and during the period from October 2010 to April 2011. Collected samples were labeled and stored at -20°C until tested by the three serological tests for detection of anti-*Toxoplasma gondii* antibodies. The seroprevalence in the adult horses (>11 years old) 44.1%: 15 of 34 was significantly higher than those of young horses (1-10 years old) 13.4%: 13 of 97. Ended, seroprevalence in horses increased with age. Although, the female horses had a significantly higher seroprevalence (18.2%: 18 from 99) than the male (3.8%: 2 from 53). This study describes that high seroprevalence rates and highlights the risk of toxoplasmosis in horses due to environmental contamination with *T. gondii* oocysts.

Key words: Toxoplasma, horse, antibodies, seroprevalence, blood

INTRODUCTION

The intracellular apicomplexan protozoan *Toxoplasma gondii* is found worldwide is capable of infecting almost any cell type within an exceptionally broad host range-across humans, livestock, companion animals and wildlife, making it one of the most successful protozoan parasites on earth (Dubey, 2010). It has been just over 100 years since the discovery of *Toxoplasma gondii* parasite in 1908 in the little hamster-like rodent *Ctenodactylus gundi* by Nicolle and Manceaux. All hosts including humans can be infected by any one of the three forms of the *Toxoplasma gondii* parasite that correspond to three morphological stages: Tachyzoite, bradyzoite and sporozoite form.

Toxoplasma gondii parasites are obligate intracellular apicomplexans that can infect virtually all warm-blooded animals; felids are definitive hosts. The most common sources of human infection are ingestion of tissue cysts in undercooked meat or of food or water contaminated with oocysts shed by felids and transplacental transmission (Cenci-Goga *et al*., 2011).

Toxoplasmosis is a globally distributed zoonosis with a clinical impact in the unborn fetus and in the immunosuppressed individual. Consumption of undercooked meat has been well established as a major risk factor for human *Toxoplasma gondii* infection worldwide. All warm blood animals are receptive to the parasite but with different rates between the species (Dubey, 2010). Toxoplasmosis is a disease where the interests of the diverse medical and veterinary specialties converge (Cenci-Goga *et al*., 2011). *T. gondii* infection does not cause serious illness but in pregnant women and female animals it is a common cause of abortion, blindness, megallocephally and mental retardation to children. Whereas animal fetuses were mummified, macerated and stillborn or may be borne weak and die within weeks after birth and the parasite also emerged as a major cause of death in patients with acquired immunodeficiency syndrome (Innes, 2010).

The objective of the present study was to determine the seroprevalence of toxoplasmosis among horses in Riyadh city using three serological tests named IHAT, IFAT and Indirect ELISA-IgG & IgM.

MATERIALS AND METHODS

Animal data: Blood samples were obtained from 152 horses from different farms in Riyadh, Saudi Arabia (Arabian Peninsula) from October 2010 to April 2011. Horses were fed in-house with no free grazing. The different ages of animals were pooled into three groups: the first group with an age below 1 year old (n = 21), the second group with horses older than 1-10 years old (n = 97) and the third group with an age between 11-20 years (n = 34). The 99 females and 53 males were screened.

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Blood sampling: Blood samples were obtained via a jugular vein as approved by the National Program for Sciences and Technology, centrifuged at 4000 rpm for 10 min and sera were stored at -20°C until use.

Serological tests: An Indirect Haemagglutination test (IHA) was performed using a reagent commercially available (Wampole Laboratories, New Jersey, USA). The serum samples were diluted two fold from 1:64-1:2, 048 following the steps described by the manufacturer. All positive serum samples in the IHA test were retested after treatment with 2-Mercaptoethanol (2-ME) in order to verify the presence of IgM antibodies (Camargo *et al*., 1978). Other IHA (M-Toxo) developed by Yamamoto *et al*. (1991) for the serodiagnosis of acute toxoplasmosis was carried out with a standardized suspension of red blood cells coated with a heat-stable alkaline-solubilized extract of *T. gondii* which reacts predominantly with IgM antibodies. To 50 μL of doubling diluted serum as above mentioned, 25 μL of sensitized cell suspensions were added and the agglutination pattern read after incubation of 1 h and 30 min at room temperature. Positive and negative reference serum samples (for IgG & IgM specific to *T. gondii*) were included in all assays.

The indirect immunofluorescence test for detection of IgG antibodies to *T. gondii* (IgG-IFI; Hemagen Diagnostics, Inc., Columbia, Maryland, USA) used in this investigation was similar to that used for diagnosis of human infections (Camargo, 1964). Antigen slides of *T. gondii* were incubated with serum samples screened at 1:64 dilution and positive samples were then diluted two fold until 1:4,096. An isothiocyanate fluorescein labeled rabbit IgG anti-dog IgG (Abcam, Cambridge Science Park, Cambridge, UK) was used as secondary antibody and the optimum titer (1:150) was determined by block titration with positive and negative serum controls. Positive control sera were obtained of the horses with consistently positive serological results by IHA-Hematoxo in interassay and intraassay variation tests. Negative control sera were obtained of dogs healthy with consistently negative serological results by IHA-Hematoxo as mentioned above. The slides were examined by epifluorescent microscope (Olympus, Mod. BH2, Tokyo, Japan).

An Immunoenzymatic test (ELISA) was carried out for detection of IgG and IgM antibodies anti *T. gondii* as described (Mineo *et al*., 1980) with some modifications. Optimum antigen solution and positive, negative and conjugated sera were determined through end block titration. The 100 μL of antigen (at concentration of 10 μ g mL^{-1} for IgG-ELISA and 5 μ g mL^{-1} for IgM-ELISA) diluted in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6 were added to each microplate well and the plate was incubated at 4°C for 18 h in moist-chamber. The plate used for IgG-ELISA and IgM-ELISA were purchased from Genway Biotech, Inc., SanDiego, USA.

Microtiter plates (Genway Biotech, Inc., SanDiego, USA) were coated overnight at 4°C with a soluble antigen preparation (0.25 μg of protein/well), consisting of a sonicated extract of purified *Toxoplasma tachyzoites* diluted in 0.06 M sodium carbonate buffer (pH 9.6). The plates were washed three times with Phosphate-Buffered Saline containing 0.05% Tween 20 (PBST) and incubated with the samples. Doubling dilutions of the horse serum samples, diluted from 1:64-1:16,384 in PBST with 20% Equine Serum (PBST/ES) were added in duplicates to the wells and the plates incubated at 37°C for 45 min. After repeated washing, the secondary antibody, consisting of a peroxidase (horseradish peroxidase, type VI, Sigma Co., St. Louis, USA) labeled to rabbit IgG anti-horse IgG or goat ant-horse IgM, prepared as described (Wilson and Nakane, 1978) and diluted 1:20000 in PBST/ES was added and incubation performed for 45 min at 37°C. After a final wash, the plates were incubated with enzyme substrate hydrogen peroxide and o-phenylenediamine (Merck, Germany) in 0.1 M citrate Na_2HPO_4 buffer (pH 5.5) for 15 min at room temperature. The reaction was stopped by adding 2N H_2SO_4. The reading was taken in an ELISA reader (BioTek) equipped with a 450 and 405 nm filters.

Positivity criteria for serological assays: The determination of the cut off titers for IHAT, IFAT and ELISA were made based on reports about seroprevalence of *T. gondii* in horses where at least one or two of these tests were used. Thus, serum samples that showed reactivity at dilution ≥1:64 were considered positive samples (O'Donoghue *et al*., 1987; Nieto and Melendez, 1998; Hashemi-Fesharki, 1996).

The titers obtained on the serological tests were defined as low titers when the reactivity was equal or lower than 128. On the other hand, titers higher than 128 were defined as high titers.

Statistical analysis: Differences in the seroprevalence of *T. gondii* infected horses, male and female and different age groups were analyzed using a χ^2-test calculated with Excel 2007 (Microsoft®). The $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The IHA test revealed *T. gondii* antibodies in 28 samples (18.4%) of the 152 horses with titers of 1:64-1:128

Table 1: Summary of the seroprevalence results of indirect haemagglutination test

Factors	Initial samples No.	Positive samples No.	IHAT-low (1:64-1:128)	IHAT-medium (1:256)	IHAT-high (1:512-1:1024)
Sex					
Male	53	5	4	0	1
Female	99	23	20	3	0
Total	152	28	24	3	1
Age					
2-11 months	21	0	0	0	0
1-10 years	97	13	11	1	1
11-20 years	34	15	13	2	0
Total	152	28	24	3	1

Table 2: Summary of the seroprevalence results of indirect immunofluorescence test

Factors	Initial samples No.	Positive samples No.	IFAT-low (1:64-1:128)	IFAT-medium (1:256)	IFAT-high (1:512-1:1024)
Sex					
Male	53	2	1	0	1
Female	99	20	17	3	0
Total	152	22	18	3	1
Age					
2-11 months	21	0	0	0	0
1-10 years	97	9	6	2	1
11-20 years	34	13	12	1	0
Total	152	22	18	3	1

in 24 horses, 1:256 in 3 horses and 1:512-1:1024 in one horse (Table 1). The statistical analysis showed that other factors (gender and age) reported in the present study affected prevalence of infection. The seroprevalence in the adult horses (>11 years old) 44.1%: 15 of 34 was significantly higher than those of young horses (1-10 years old) 13.4%: 13 of 97. Ended, seroprevalence in horses increased with age. In addition to this difference between the age groups, a difference in the subgroups of female and male horses was also significant. Indeed, the female horses had a significantly higher seroprevalence (23.2%: 23 from 99) than the male (9.4%: 5 from 53).

Based on IFA test, *T. gondii* antibodies were found in 22 samples of the 152 horses with titers of 1:64-1:128 in 18 horses, 1:256 in 3 horses and 1:512-1:1024 in one horse (Table 2). Also, seroprevalence in horses increased with age. Where the seroprevalence in the adult horses, (>11 years old) 38.2%: 13 of 34 was significantly higher than those of young horses (1-10 years old) 9.3%: 9 of 97 and zero in young horses. Although, the female horses had a significantly higher seroprevalence (20.2%: 20 from 99) than the male (3.8%: 2 from 53).

In order to further investigate between acute and chronic infection, ELISA test of IgM and IgG antibodies of anti *T. gondii* were performed and the results showed that 20 samples of the 152 horses with IgG positive and zero sample with IgM positive. The seroprevalence rate in the adult horses (Table 3), (>11 years old) 38.2%: 13 of 34, was significantly ($p<0.05$) higher than those of young horses (1-10 years old) 7.2%: 7 of 97 horses. Although, the female horses had a significantly higher seroprevalence (18.2%: 18 from 99) than the male, (3.8%: 2 from 53).

In the recent years, researchers have been observed that the mortality rate in birth and abortion in horses began to appear and some studies have shown that

Table 3: Summary of the seroprevalence results of ELISA-IgG and IgM

Factors	Initial samples number	No. of positive IgG	No. of positive IgM
Sex			
Male	53	2	0
Female	99	18	0
Total	152	20	0
Age			
2-11 months	21	0	0
1-10 years	97	7	0
11-20 years	34	13	0
Total	152	20	0

infection with parasites are one of the primary causes of abortion and neonatal mortality in horses by 1% (Szeredi *et al*., 2008). Boughattas *et al*. (2011) reported that high prevalence level of toxoplasmosis in Arab horses seem to be the most sensitive breed for acquisition of the infection, associated with ecological/climatic conditions, type of farming, the presence of cats and the quality of water this may be attributed to susceptibility to *T. gondii* (Dubey *et al*., 2009).

Toxoplasma gondii is a protozoan parasite of warm-blooded animals, it has a worldwide distribution, cats including all felines are the definitive hosts which excrete environmentally-resistant oocysts in their feces and it is the main source directly and continuously to the infection (Skjerve *et al*., 1998).

Hosts become infected by ingestion of food or drink contaminated with oocysts or by ingesting undercooked meat from infected animals with *T. gondii*. It causes mental retardation and loss of vision in congenitally-infected children and abortion in pregnant women (Dubey *et al*., 2009).

Toxoplasma gondii is most important parasite that affects the production capacity of animals which caused the incidence of abortions and deaths of deformed embryos which leads to significant economic losses (Dubey and Welcome, 1988).

The symptoms of toxoplasmosis disease in horses be lurking asymptomatic and therefore the infection is difficult to diagnose clinically so the diagnosis is still based on the use of different serological tests to detect antibodies to the parasite *T. gondii* (Dubey and Welcome, 1988).

The results of this study showed a significant proportion of the *T. gondii* parasite among horses in the city of Riyadh (18.4, 14.5, 13.2 and 0%) using the three serological tests IHA, IFA, ELISA-IgG and ELISA-IgM, respectively.

In the present research, prevalence of *T. gondii* infection by serological test IHA (18.4%), nearly similar results of incidence of toxoplasmosis in USA (20%) were recorded by Riemann *et al.* (1975), respectively whereas lower incidence rates (11.8 and 6.3%) was obtained by Chhabra *et al.* (1985) in India horses and Filha in Brazil horses.

The IFA test has shown in the present study infection rate (14.5%) while showed the same test lower rate in South Korea 2.6% by Gupta *et al.* (2002) and higher rates in Germany 55% by Erb Filha in Egypt 40.5% by Ghazy *et al.* (2007).

The ELISA-IgG test was demonstrated in the present study infection rate (13.2%) while showed the same test lower rate in Sweden 1% by Uggla *et al.* (1990) as higher rates in Egypt 38.1% by Ghazy *et al.* (2007) while no ELISA-IgM detected in the present research this indicates the absence of acute case of this disease.

The present study showed that older horses were more likely to be seropositive than horses under 10 years old which provided further evidence for the increased risk of *T. gondii* infection with acquisition of age through longer contact with infective oocysts from the environment which is in agreement with results previously obtained by Boughattas *et al.* (2011) and Riemann *et al.* (1975).

In this study indicated that female horses are more sensitive to the infection by the parasite than male horses which is in agreement with results previously obtained that females can cause transmission of the parasite through the placenta (Remington *et al.*, 2006) thus causing of congenital toxoplasma disease in the fetus (Fortier *et al.*, 1991).

CONCLUSION

It is conceivable that high seroprevalence rates for toxoplasmosis in horses are a result of environmental contamination with *T. gondii* oocysts. However, horses in the Kingdom as the rest of the animals herbivores are contracting the parasite which urges to health education programmes for farmers and further studies and research aimed at monitoring the diseases of horses and economic importance these practices should be reviewed and special awareness, advice and recommendations made by physicians to the target population.

ACKNOWLEDGEMENTS

This research project was supported by a grant from the Research Center of the Center for Female Scientific and Medical Colleges, Deanship of Scientific Research, King Saud University. Serological experimentations were performed by AF and AE, statistical analysis and drafted the manuscript. AF sampled blood specimens from animals and was responsible for acquisition of data and their analysis. AF participated in statistical analysis. EM has been involved in revising the manuscript critically for important intellectual content. AE conceived the study and has given financial support. All researchers approved the final version of the manuscript.

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