Iraqi Journal of Veterinary Sciences, Vol. 26, Supplement II, 2012 (39-41)
Proceedings of the 6th Scientific Conference, College of Veterinary Medicine, University of Mosul

Detection of *Toxoplasma gondii* antibodies in horses in Mosul, Iraq

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Abstract

This study was aimed to verify the presence of *Toxoplasma gondii* antibodies in equine sera in Mosul city, Iraq. Seventy nine samples of sera were examined (70 female and 9 male) by latex agglutination test (LAT) and 2–Mercaptoethanol test (2-ME). Results showed that anti-bodies to *T. gondii* using LAT were detected in 72.2% (71.4% female and 77.8% male) whereas 57% (57.1% female and 55.6% male) of infected horses were detected by 2-ME.

Keywords: *Toxoplasma gondii*; Antibodies; Latex agglutination; Equine.
Available online at http://www.vetmedmosul.org/ijvs

الكشف عن أضداد المقوسات الكوندية في الخيول في الموصل، العراق

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فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

هدفت هذه الدراسة إلى الكشف عن تواجد أضداد المقوسات الكوندية في مصول الخيول في مدينة الموصل، العراق. تم فحص ٧٩ عينة مصل (٧٠ فرس و٩ خيول) باستخدام اختباري تلازن اللاتكس والمركابتوايثانول. كانت النسبة المئوية لنتائج اختبار تلازن اللاتكس الموجبة ٧٢,٢% (٧١,٤% إناث و٧٧,٨% ذكور)، أما باختبار المركابتوايثانول فقد كانت النسبة ٥٧% (٥٧,١% إناث و٥٥,٦% ذكور).

Introduction

Toxoplasma gondii is an intracellular protozoan parasite capable of infecting most tissues in mammals and various tissues in avian species (1). Therefore, it is considered as a significant agent with regard to aspects of animal production as it can provoke abortion among different animal species of economic importance, as well as to public health due to its high prevalence in human infection (2). Both domestic and wild felids are the only known definitive hosts of *T. gondii* in which the sexual cycle can take place (3). Hence cats play a central role in the epidemiology of *T. gondii*, constituting the only known source of environmental contamination with the infective oocyst stage (4).

Horses are most commonly infected by ingestion of sporulated oocysts found in feces of infected cats (5). *T. gondii* was isolated in Australia from the diaphragm of one out of four horses (6). In USA, it was isolated from different tissues and organs (heart, diaphragm, spinal cord, brain, tongue, skeletal muscles, liver and kidney) in 4 out of 9 ponies experimentally fed infective *T. gondii* oocyst by mouse inoculation and feeding of cats (7).

T. gondii infection stimulates both the humoral immune response as antibody production, which includes IgM and IgG in addition to cell mediated immunity (CMI). CMI response is essential for host control on intracellular infection like *T. gondii* (8).

Although toxoplasmosis generally causes subclinical infections in horses, it may also lead to symptoms including progressive neurological findings such as ataxia, paralysis, blindness, fever, retinal degeneration and severe encephalomyelitis (1,9-11). A case of an infection in eye of aborted foals in UK has been reported (12). Horses generally display low susceptibility to the disease. However young animals and animals with immunosuppression (sick, pregnant animals) are more susceptible to toxoplasmosis (13). Since *T. gondii* infections generally display sub-

clinical course in horses, serological techniques for the detection of specific antibodies produced in the body against the parasite have great diagnostic value (14,15). Many recent studies in Mosul city in Iraq, deals with toxoplasmosis in rabbits (16), cattle (17), turkeys (18), sheep (19), buffaloes (20) and donkeys (21). Up to our knowledge, there are no known investigations about the prevalence of infection in horses, therefore our study was aimed to verify the presence of *T. gondii* antibodies in equine sera in Mosul city.

Materials and methods

Samples

Blood samples were collected between 2010-2011 from clinically healthy 79 horses of race breed (70 female and 9 male) aged 2-10 years, with a history of abortion.

Five ml of blood were individually harvested by jugular vein punctured into non-heparinized tubes to obtain sera which were separated by centrifugation at 3000 rpm for 15 minutes. The collected sera were stored at -20°C until analysis (22).

Serological Examination

Latex Agglutination test (LAT): The titer of *Toxoplasma* antibodies values in serum were estimated using commercial kits (Biokit, Spain SA, toxo-cell-latex), according to the procedure outlined by the manufacturer, which detecting total *Toxoplasma* antibodies of IgG and IgM.

2-ME test: This test was performed for seropositive samples in paragraph (a) to detect *Toxoplasma* antibodies of IgM to confirm active toxoplasmosis (23).

Results

Among 79 horses tested for the presence of *T. gondii* antibodies, 57 were found to be seropositive using latex test, (50 female and 7 male), the LAT detect the total immunoglobulin IgG and IgM (Table 1).

Table (1): Percentage of *T. gondii* reactors using LAT.

No. of examined sera	Positive reactors No.	%	Negative reactors No.	%
Total 79	57	72.2	22	27.8
Female 70	50	71.4	20	28.6
Male 9	7	77.8	2	22.2

Results were also showed that 45 horses were positive by using 2-ME (40 female and 5 male), (Table 2).

Table (2): Percentage of *T. gondii* antibodies reactors using 2-ME.

No. of examined sera	Positive reactors No.	%	Negative reactors No.	%
Total 79	45	57	34	43
Female 70	40	57.1	30	42.9
Male 9	5	55.6	4	44.4

The distribution of different titers in seropositive animals by using LAT was variable, the titers were ranging between 1:20 to 1:640 (Table 3).

Table (3): Distribution of antibody titers by LAT according to genders.

Titer	No. of total infected animals 45	Male 7 of total 9 No.	%	Female 50 of total 70 No.	%
1:20	3	1	14.3	2	4
1:40	3	-	-	3	6
1:80	13	2	28.6	11	22
1:160	25	4	57.1	21	42
1:320	12	-	-	12	24
1:640	1	-	-	1	2

While the positive results in horses by using 2-ME have different titers ranging between 1:20 to 1:160 (Table 4).

Table (4): Distribution of antibody titers by 2-ME according to genders.

Titer	No. of total infected animals 45	Male 5 of total 9 No.	%	Female 40 of total 70 No.	%
1:20	7	-	-	7	17.5
1:40	14	2	40	12	30
1:80	16	3	60	13	32.5
1:160	8	-	-	8	20

Discussion

The prevalence of equine *Toxoplasma* in different regions appear to be associated to environmental factors such as humidity, temperature and height (24). In general, prevalence is greater in warm and humid areas than in arid and semi-arid regions. Other epidemiological factors must be considered, such as the feline population infected, age, and type of animal management (25).

The seroprevalence rates variation to *T. gondii.* in horses between our results and those previously reported may be due to the serologic test used; initial serum dilution;

the virulence of *T. gondii* strains, the immune status, age and management of investigated animals in different localities (9).

The prevalence of *T. gondii* infection in horses at the different localities in the world is extremely variable. It was 57% in the present study, which is more than that reported in other parts of the world (215, 25-31). The reasons for these discrepancies may be explained by spatial, temporal and many other factors determining the prevalence of toxoplasmosis in animals as well as possible differences among laboratories and testing procedure and number of animals tested (15). It is conceivable that high seroprevalence rates for toxoplasmosis in horses in this study is a result of environmental contamination with *T. gondii* oocysts. However, horses examined here were bred in farm and villages; this increases the risk of exposure to animal of felidae family and feces of cats.

Protection to *T. gondii* turned out to be complex involving innate and specific immunity. In the 1940s humoral antibodies were found to kill extracellular but not intracellular tachyzoites (32). Protective immunity was found to be mediated largely by immune lymph cells (8). Mapping of *T. gondii* genes was achieved recently and undoubtedly will help in search for better antigens for diagnosis and protection, and mechanism of disease (33). It is concluded that horses in Mosul city are highly infected with toxoplasmosis, and this may affect breeding of race horses.

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