

Seroprevalence of Toxoplasmosis in horses and animal handlers in Baghdad city

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Abstract

This study was conducted to detect the prevalence of *Toxoplasma gondii* infection in horses and their owners at Baghdad city (Al-Zawraa Zoo, Iraqi Equestrian Federation, Equestrian and Al- Dowanem), during the period 1/November/ 2014 to 31/April/ 2015. One hundred seventy eight sera (92 horses, and 86 animal handlers), were examined by Latex Agglutination Test (LAT) and Enzyme Linked Immunosorbent Assay (ELISA). The results were showed that the infection rate in horses by LAT was 67.39% (58% females and 78.57% males) and 32.6% by ELISA (28% females and 38% males). The higher infection rate 79.16% of horses was recorded in the age group 3->6 years, while a lower infection rate 43.75% was found in the age group <1->3years by the LAT, while in ELISA the higher infection rate 50% was recorded in the age group 9->12 years and a lower infection rate 18.75% was found in the age group <1->3 years. In animal handlers the infection rates were 65.62% and 58.139% by LAT and ELISA respectively and the age group 20-30 years was showed a higher infection rates 76.92% and 65.38%, and the lower infection rate 54.16% was recorded at the age group 30-40 years in Latex and ELISA respectively. Al-Zawraa Zoo, Iraqi Equestrian Federation, Equestrian and Al- Dowanem areas were showed the infection rates 70.37%, 64%, 68% and 66.66% by LAT respectively and the infection rates were 48.14%, 20%, 32% and 26.6% by ELISA in horses, while in animal handlers the infection rates in Al-Zawraa Zoo, Iraqi Equestrian Federation Equestrian and Al- Dowanem areas were 68.18%, 83.33%, 53.33%, 77.27% by LAT and 59%, 58.33%, 63.33%, 50% by ELISA respectively.

Keywords: *Toxoplasma gondii*; Antibodies; Latex; ELISA; Equine.

الانتشار المصلي لداء القطط في الخيول ومربيها في مدينة بغداد

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الخلاصة

هدفت هذه الدراسة الكشف عن نسبة انتشار الإصابة بداء القطط في الخيول ومربيها في مدينة بغداد (حديقة حيوان الزوراء، الاتحاد العراقي للفروسية، إسطبلات نادي الفروسية، ومنطقة الدوانم) خلال المدة من 1/ تشرين الثاني ولغاية 31/نيسان 2015. تم جمع 178 عينة مصل (92 خيول و86 مربي الخيول) وفحصها باختبار تلازن الاتكس ومقايصة الممتز المناعي المرتبط بالإنزيم. أظهرت النتائج ان نسبة الإصابة الكلية في الخيول بلغت 67.39% باختبار اللاتكس (58% إناث و78.57% ذكور) و32.6% بمقايصة الممتز المناعي المرتبط بالإنزيم (28% إناث و38% ذكور). وسجلت اعلى نسبة إصابة 79.16% في الخيول ضمن الفئة العمرية 3->6 سنوات، في حين كانت أوطأ نسبة إصابة 43.75% في الفئة العمرية <1->3 سنة باختبار تلازن اللاتكس، أما مقايصة الممتز المناعي المرتبط بالإنزيم سجلت اعلى نسبة إصابة 50% في الفئة العمرية 9->12 سنة، وبلغت أوطى نسبة 18.75% في الفئة العمرية <1->3 سنة. أما في مربي الخيول فكانت نسبة الإصابة الكلية 62.65%

باختبار تلازن اللاتكس و 58.139% بمقايضة الممتز المناعي المرتبط بالإنزيم، وأظهرت الفئة العمرية 20->30 سنة أعلى نسبي إصابة (67.92% و 65.38%)، في حين سجلت أوطئ نسبة إصابة 54.16% في الفئة العمرية 30->40 سنة بكلا الاختبارين. وأظهرت حديقة حيوان الزوراء، الاتحاد العراقي للفروسية، إسطبلات نادي الفروسية، ومنطقة الدوانم نسب إصابة في الخيول بلغت 70.37%، 64%، 68% و 66.66% باختبار تلازن اللاتكس و 48.14%، 20%، 32% و 26.6% بمقايضة الممتز المناعي المرتبط بالإنزيم على التوالي، بينما اظهر مربي الخيول نسب إصابة في حديقة حيوان الزوراء، الاتحاد العراقي للفروسية، إسطبلات نادي الفروسية، ومنطقة الدوانم بلغت 68.18%، 83.33%، 53.33% و 77.27% باختبار تلازن اللاتكس وكانت نسب الإصابة 59%، 58.33%، 63.33% و 50% بمقايضة الممتز المناعي المرتبط بالإنزيم على التوالي.

الكلمات المفتاحية: المكورات الكونديه، الأجسام المضادة، اللاتكس، الاليزا، الخيول.

Introduction

Toxoplasmosis is one of the most important zoonotic parasitic diseases worldwide (1, 2). The disease has serious economical and social consequences, owing to spontaneous abortions, new-borne with congenital malformations and owing to psychological disorders in humans (3). It is caused by *Toxoplasma gondii* as an enteric coccidian of the domestic cat (*Felis catus*) and other members of the family Felidae (4), which acts as a definitive host and other, all animal species act as intermediate hosts (5, 6). The most common sources of infection are ingestion of tissue cysts in raw or undercooked meat or food or water contaminated with oocysts shed by felids (7, 8, 9); Also, raw milk and milk products (2). Paratenic hosts become infected with *T.gondii* by ingesting sporulated oocysts from cat feces or bradyzoites in the tissue cyst of other paratenic hosts (4). The distribution of this parasite depends on the regions and weather condition, where the oocysts survive in environment (10, 11). 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 (8). Transplacental transmission of tachyzoite from dam to fetus in utero also occur but varies in importance depending on the species of host involved (4). Generally, worldwide seroprevalence of *T. gondii* infection in horses has been summarized prior, ranging from 0.4% to 48.1% (12). A similar study in Qazvin, Iran, showed a quite higher rate of seropositivity 71.2% in horses (13). The seroprevalence results of *T. gondii* infection in equids were higher than other food animals, including pig, cattle, sheep, chicken, duck, and goose confirm that infection in food animals is wide spread and should be of public health concern (14). Due to the importance of toxoplasmosis and lack information about this disease in horses and horsemen this research was conduct.

Materials and Methods

- **Samples Collection:** One hundred and seventy eight (92 horse and 86 horsemen) of venous blood samples of both sexes were collected at Baghdad city (Al-Zawraa Zoo, Iraqi Equestrian Federation, Equestrian and Al- Dowanem) during the period 1/November/ 2014 to 31/April /2015. They were transported in a cold box to the Zoonotic Unite, Veterinary Medicine College, Baghdad University, and kept at room temperature for one hour in order to complete their clot, then centrifuged at 2000 rpm for 10 minutes to separate the sera, which they were kept in Eppendorf tubes and stored at -20°C till used (15) for *Toxoplasma gondii* Latex Agglutination Test (Toxo-Latex slid agglutination-SPINREACT, Spain) and ELISA (Toxos-MS ver1013GB-France, and Toxo-IgG INF5120901 GB-Germany) which they were used according to the kits procedures.
- **Statistical analysis:** The data analyzed by used SPSS for different groups at levels 0.05 and 0.01 (16).

Results

- The infection rate according to the age of the animals: The higher infection rate 79.16% was recorded in the age group 3-6 years, while a lower infection rate 43.75% was recorded in the age group <1-3 years by using Latex agglutination test, while ELISA showed a higher infection rate 50% was recorded in the age group 9-12 years and a lower infection rate 18.75% was found in the age group <1-3 years without a significant difference ($P>0.05$) in both tests (Table 1).

Table (1) Total infection rate of *Toxoplasma gondii* in horses according to the age of the animals by using Latex and ELISA

Age (years)	No. of samples examined	No. of positive Latex	Percentage (%)	No. of positive ELISA	Percentage (%)
<1->3	16	7	43.75	3	18.75
3->6	24	19	79.16	8	33.33
6->9	20	11	55	6	30
9->12	12	9	75	6	50
12-15<	20	15	75	7	35
Total	92	62	67.39	30	32.6

* $P>0.05$

- The infection rate according to the sex of the animals: There was a different infection rates of *T. gondii* in the horses between males and females. A higher infection rate 78.57% was found in the males and a lower infection rate in the females 58% by Latex test with a significant difference ($P<0.01$); Also, A higher infection rate 38 % was found the in males and a lower infection rate in the females 28% by using ELISA with a significant difference ($P<0.05$) (Table 2)

Table (2) The total infection rates of *Toxoplasma gondii* in horse according to the sex of the animals by using Latex and ELISA

Sex	No. of samples examined	No. of positive ELISA	Percentage (%)	No. of positive LATEX	Percentage (%)
Males	42	16	38*	33	78.57**
Females	50	14	28	29	58
Total	92	30	32.6	62	67.39

* $P<0.05$ ** $P<0.01$

- The infection rate according to the areas of the study: There were a significant difference ($P<0.05$) in the *Toxoplasma gondii* infection rates among different areas of the study (Al-Zawraa Zoo, Iraqi Equestrian Federation- (IEF), Equestrian and AL-Dowanem). A higher infection rate 70.37% was recorded by Latex test in Al-Zawraa Zoo, while a lower infection rate 64% was found in IEF.; Also a higher infection rate 48.14% was recorded in Al-Zawraa Zoo, but a lower infection rate (20%) was found in IEF by ELISA. (Table 3).

Table (3) Total infection rates of *Toxoplasma gondii* in horses according to the areas of the study by using Latex and ELISA

Areas	No. of samples examined	No. of positive ELISA	Percentage (%)	No. of positive Latex	Percentage (%)
Al-Zawraa Zoo	27	13	48.14	19	70.37
Iraqi Equestrian Federation	25	5	20	16	64
Equestrian	25	8	32	17	68
AL-Dowanem	15	4	26.6	10	66.66
Total	92	30	32.6	62	67.39

*($P<0.05$)

- Infection rate of the animal handlers according to the age of the study: Table (4) was showed no significant effect ($P>0.05$) of age in the *Toxoplasma gondii* infection rate. The age group 20-30 years was showed a higher infection rates 76.92% and 65.38%.; while the same lower infection rates 54.16% was recorded at the age group 30-40 years in Latex and ELISA respectively.

Table (4) The total infection rates of *Toxoplasma gondii* according to the ages of the animal handlers by using Latex and ELISA

Age (years)	No. of samples examined	No. of positive ELISA	Percentage (%)	No. of positive Latex	Percentage (%)
<20	18	10	55.55	13	72.22
20->30	26	17	65.38	20	76.92
30->40	24	13	54.16	13	54.16
40-50<	18	10	55.55	12	66.66
Total	86	50	58.13	58	67.44

*($P>0.05$)

- The infection rates according to the sex of the animal handlers: The higher *Toxoplasma gondii* infection rate 72.27% was recorded in the females in Latex and 68.18% by ELISA, while in males the lower infection rate 65.62% and 53.125% were recorded by Latex and ELISA respectively with a significant difference ($P<0.05$) in latex test only. (Table5)

Table (5) Total infection rates of *Toxoplasma gondii* according to the sex of the animal handlers by using Latex and ELISA

Sex	No. of samples examined	No. of positive ELISA	Percentage (%)	No. of positive Latex	Percentage (%)
Males	64	35	54.68	42	65.62
Females	22	15	68.18	16	72.72
Total	86	50	58.13	58	67.44

*($P<0.05$)

- The infection rates in animal handlers according to the areas of the study: The higher *Toxoplasma gondii* infection rate was recorded in Iraqi Equestrian Federation 83.33%, while the lower infection rate 53.33% was recorded in Equestrian by using Latex test, but in case of using ELISA a higher infection rate 59% was found in Al-Zawraa Zoo areas, while Al-Dowanem area was showed a lower infection rate 50% without a significant difference ($P\geq 0.05$) in both tests (Table 6).

Table (6) Total infection rates of *Toxoplasma gondii* in animal handlers according to the areas of the study by using Latex and ELISA

Area	No. of samples examined	No. of positive ELISA	Percentage (%)	No. of positive Latex	Percentage (%)
Al-Zawraa Zoo	22	13	59	15	68.18
Iraqi Equestrian Federation	12	7	58.33	10	83.33
Equestrian	30	19	63.33	16	53.33
AL-Dowanem	22	11	50	17	77.27
Total	86	50	58.139	58	67.44

*($P\geq 0.05$)

Discussion

The prevalence of *T. gondii* infection in horses at the different localities in the world is extremely variable (17). Worldwide seroprevalence of *T. gondii* infection in horses has been summarized prior, ranging from 0.4% to 48.1%.(18) or 1% and 37% had been reported in a

variety of studies conducted on equines in different countries [19,20]. Generally, the positivity for *Toxoplasma gondii* infection was more than 40%, with variations between 26 and 70% (3); 69.6% in the Fernando de Noronha, Brazil (21) 10.8% in Southern Spain (22), 25.6% in donkeys in Southern Spain (22) by Modified agglutination test (MAT). Also, *T. gondii* seropositivity has been studied in many regions of Turkey and seropositivity ranging between 1.8% and 42.2% (17). In a study conducted in the Kayseri region, Turkey 19.2% equines were found to be seropositive (23), 28% in horses bred for sport purposes in the province of Ankara, Turkey, (24), 8.33% and 2% in Ankara, using Sabin field man dye test -SFDT, 6.4% in Malatya, (25), 7.5% in Sanliurfa (26) 11.5% in Urmia (27) and 20.6% in Kars, (28). In Czech Republic, *T. gondii* antibodies were found to be 7.7% and 4.1% using the Sabin Feldman dye test (SFDT) and the complement fixation (CF) test, respectively (29) and, It had been reported to be 24% by latex agglutination test (LAT) (30). In Brazil (31) was detected seropositivity in 17% of horses, 1% in Sweden using ELISA (20). Whereas seropositivity rate of horses 2.6% was obtained in South Korea using IFAT (32), 31.6% in Riyadh Province, Saudi Arabia by Sabin-Feldman dye test (SFDT) (33), 20% of horses in Argentina (34), while (35) were reported seropositivity in 17.4% of horses in North America by using Sabin Feldman aye test (SFDT). The infection of toxoplasmosis in both sexes were showed a high infection rates were recorded in males than in females. These results were agreement and disagreement with some researches report before, (36) was determined the presence of seropositivity in females and males to be 6.0% and 9.9% respectively, but, the same samples were studied using LAT and 24.1% were in females and 23.6% in males from various regions of Turkey. *Toxoplasma gondii* antibodies in equine sera in Mosul city, Iraq; Seventy nine samples of sera were examined (70 female and 9 male) by LAT and 2-Mercaptoethanol test (2-ME). Anti-bodies were detected in 72.2% (71.4% female and 77.8% male) by LAT, whereas 57% (57.1% female and 55.6% male) were detected by 2-ME (17), that may be due the activity of males that consumption more food and water and exposure to environmental contamination by cat feces than females, which increase the infection rate. The seroprevalence differences can be attributed to epidemiological factors effective on *T. gondii* parasite. It can be noted that sampling techniques, husbandry method used in different regions, frequency of cats on the farms and climatic variations from one region to another, which are essential elements in epidemiological studies and different results (27) and the prevalence of equine *Toxoplasma* in different regions appear to be associated to the environmental factors such as humidity, temperature and height (17). 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 (37). Also, Horses are most commonly infected by ingestion of sporulated oocysts found in feces of infected cats (19). A same results in the horses, the horsemen had a high infection rate of toxoplasmosis (58.13% and 67.44%), which divided into 53.12%, 65.62% in males and 72.72%, 72.72% in females by ELISA and LAT respectively, that agreement with some research before; A total seroprevalence of *T.gondii* IgG and IgM among pregnant (66.6 and 50%, respectively) and non pregnant women (62.5 and 50%, respectively) of the age group 35-45 years. These high risk group women showed also the highest positive PCR results (62.550 for pregnant and 37.5% for non pregnant). The prevalence of IgG antibodies to *T. gondii* in Italy by ELISA being 48.5% with correlation of infection with age, showed a significant increase of positivity until 30-40 approximately years, that maybe due to the prevalence of the infection with *T. gondii* increases with age and there are considerable geographic differences in prevalence rates. Older age group of 35 years had a significantly higher seroprevalence than that of the younger age group of 15-25 years (38). The public health significance of toxoplasmosis is not only due to direct contact with the definitive host (Family Felidae, such as domestic cats) but also due to consumption of infected meat and milk which can facilitate zoonotic transmission (2,8, 39,40). Also, The distribution of this parasite depends on regions and weather condition where the oocysts survive in environment (10,11,41). The ingestion of undercooked infected meat is considered as an important source of the infection for humans (7, 42) that may be explain the difference in the infection rates between areas of the study in spite of the high infection rates in all areas. In conclusion, horses and horsemen had a high seroprevalence of toxoplasmosis.

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