STUDIES ON TOXOPLASMOSIS IN HORSES IN KHARTOUM STATE-SUDAN

Manal Y Ishag[*] , AM. Majid and MM. Abuobeida

المستخلص

تهدف هذه الدراسة للكشف عن وجود اجسام مناعية مضادة للتوكسوبلازما بين الخيول الموجودة بولاية الخرطوم و كذلك اظهار الدور الذى يلعبه طفيل التوكسوبلازما فى احداث اجهاض الخيول .
عزلت مقوسات و بيض طفيل التوكسوبلازما من احباط مهر . تم اختبار 381 مصلا اخذت من خيول صحية سريريا فى مواقع مختلفة من ثلاثة مدن (الخرطوم , امدرمان و الخرطوم بحرى) و ذلك باستخدام اختبارELISA للكشف عن الاجسام المناعية المضادة للتوكسوبلازما . تم الكشف عن وجود اجسام مناعية IgG فى 24 عينة من جملة عدد 381 عينة (6.3%) , اما الاجسام المناعية IgM فقد وجدت فى 2 عينة من 381 عينة (0.5%) . تم العثور على اجسام مضادة titre عند التخفيف 1:20 فى 10 خيول , 7 خيول عند التخفيف 1:40 , 5 خيول عند التخفيف 1:80 اما عند التخفيف 1:160 فقد وجد فى 2 من الخيول . لا توجد فروقات معنوية بين الاناث و الذكور . سجلت اعلى نسبة لوجود الاجسام المناعية المضادة للتوكسوبلازما فى مدينة امدرمان (10%) تليها مدينة الخرطوم (5.9%) ثم مدينة الخرطوم بحرى و التى سجلت فيها اقل نسبة (2.8%) .

Abstract

The aim of this study was to determine the seroprevalence of *Toxoplasma. gondii* antibodies among horses in Khartoum state and to report the role of *T. gondii* in abortion in equines *T gondii* tachyzoites and oocysts were isolated from an aborted foal. Sera from 381 clinically healthy horses at different locations in 3 cities (Khartoum, Omdurman and Khartoum North) were tested for the presence of *T. gondii* antibodies using ELISA test. IgG antibodies of *T. gondii* were detected in 24 out of 381 (6.3%) , while IgM antibodies of *T. gondii* were detected in 2 horses out of 381 (0.5%). Antibodies titers were found in 10 horses at 1: 20 dilution , in 7 horses at 1:40 dilution , in 5 horses at 1:80 dilution ,and in 2 horses at 1:160 dilution. There are no significant differences ($P<0.5$) within sex . Horses in the three cities were positive with higher prevalence in Omdurman city: 12 (10%) out of 120 horses , followed

[*] Veterinary Researches Institute, Animal Resources Research Corporation, Khartoum , Sudan.

by Khartoum city : 9(5.9%) out of 153 horses. Khartoum North City , showed the lowest prevalence with 3 positive samples (2.8%) out of 108 horses..

Keywords: *Toxoplasma gondii.*, Horses, Isolation, prevalence, Sudan.

Introduction

Toxoplasmosis is a globally distributed zoonotic disease with a clinical impact in the unborn fetus and in the immune-suppressed individual . All warm blooded animals are receptive to the parasite but with different rates between the species (Webster, 2010). Horses are considered one of the less sensitive species to the pathogenic effect of *T. gondii (* Tassi ,2007). *T. gondii* infections in horses progress sub-clinically and, therefore, diagnosis relies largely on serological techniques to detect the parasite-specific antibodies. The sero-prevalence of toxoplasmosis in horses varies among countries , ranging from 1% to 74% (Zardi *et al*., 1968; Eugster and Joyce, 1976; Tizard *et al*.,1978; Beyer and Shevhunova, 1986; Uggla *et al*., 1990; Hejlicek and Literak, 1994). There are reports of *T. gondii* infection in the eyes of aborted foals in UK (Turner and Savva, 1990,1992) and in CNS of horses in Brazil (Macruz et.al.,1975). No data are available on the prevalence of toxoplasmosis in horses in the Sudan. A first case of equine toxoplasmosis has been reported when a mare that aborted 4-months foal was brought for diagnosis at the Diagnostic unit - Central Veterinary Researches Institute, Khartoum State-Sudan, the case led us to make further studies on equine toxoplasmosis through isolation of *T. gondii* cysts and oocysts from the aborted foal and determination of the sero-servallence of *T. gondii* antibodies in clinically normal horses in Khartoum state.

Materials and Methods

Serum sample was taken from a mare suffering from abortion-which was brought at the Diagnostic unite – Central Veterinary Researches Institute, Khartoum state-Sudan. ELISA test was carried out for detection of anti-*T.gondii* antibodies using IgM kit (DRG Instruments GmbH,Germany), the test was carried out as the manufacture description. Also Rose Bengal test was carried out to detect anti-*Brucella* antibodies . Portions of the aborted foal tissues (brain, heart, lymph nodes & thigh muscles) were embedded in formalin 10% for histopathology .Three albino mice were fed on the remainder portions. After serial passage through mice, three naïve kittens (2-weeks old) were fed on the infected mice brains, one kitten had been kept as a control .

Animal data: Blood samples were obtained from 381 horses (129 females and 252 males) from different locations in 3 cities (Khartoum, Omdurman and Khartoum North). The different ages of animals were pooled into two groups: one group with an age below 10 years old (n = 210) and the second group with horses older than 10 years old (n = 171).

Blood sampling and serological examination: Blood samples were obtained via a jugular vein, centrifuged at 2500 rpm for 10 min and sera were stored at -20°C until use. *T. gondii* antibodies were determined using IgM and IgG

ELISA kits (DRG Instruments GmbH, Germany), the tests were carried out as the manufacture description.

Statistical analysis: Differences in the seroprevalence of *T. gondii* between the different regions, sexes and different age groups were analyzed using a Chi square test calculated with Excel 2007 (Microsoft). The P value < 0.05 was considered statistically significant. The correlation between the rates of infection in different horses were analyzed by SPSS for windows v17 software.

Results

Toxoplasma gondii IgM antibodies were detected in the aborted mare serum . On the other side infected kittens began shedding *T.gondii* oocysts in feces, 3-5 days after feeding infected foal tissues (Fig . 1).The kittens were continued shedding *Toxoplasma* oocysts for 5-7 days. *T. gondii* tachyzoites had been detected in the histopathology sections of the aborted foal tissues (Fig.2).

Figure 1: *Toxoplasma gondii* oocysts in a fecal-float of cat._400

Figure 2:*T. gondii* tachyzoites from aborted foal brain -400

The serological survey which was based on ELISA test results , showed that , IgG antibodies of *T. gondii* were detected in 24 out of 381 (6.3%) horses , while IgM antibodies of *T. gondii* were detected in 2 horses(0.5%). Antibodies titers were found in 10 horses at 1: 20 dilution , in 7 horses at 1:40 dilution , in 5 horses at 1:80 dilution and in 2 horses at 1:160 dilution. There is no significant difference ($P<0.5$) within sex . Horses from the three cities were positive, with higher prevalence in Omdurman city : 12 (10%) from 120 horses , followed by 9 (5.9%) from 153 horses in Khartoum city. Khartoum North City , showed the lowest prevalence with 3 positive samples (2.8%) from 108 horses (Table 1). The seroprevalence in the adult horses (>10-year-old) was 7.6% and it was significantly higher than those of young horses (≤ 10-years-old) 5.2% .

Table 1: Summary of the sero-prevalence results

	Omdurman		Khartoum		Khartoum North	
	Ni	N+	Ni	N+	Ni	N+
Sex						
Males	75	7	100	6	81	3
Females	45	5	53	3	27	-
Total	120	12	153	9	108	3
Age						
2-10 years	92	7	113	3	76	1
> 10 years	28	5	40	6	32	2
Total	120	12	153	9	108	3

Ni: initial samples number, N+: positive samples number

Discussion

In this study *T. gondii* oocysts had been isolated from kittens fed tissues of aborted foal , Al Khaldidi and Dubey, (1979) were previously isolated *T. gondii* oocysts from cat fed tissues pooled from several horses. There are reports of *T. gondii* infection in the eyes of aborted foals in UK (Turner and Savva , 1990, 1992) and in CNS of horses in Brazil (Macruz *et.al*.,1975). Also this study evaluated clinically normal horses from Khartoum state for the presence of *T. gondii* antibodies . Horses from the three cities were positive with higher prevalence in Omdurman city (10%), this may be due to cats which freely present and also due to the uncontrolled water that supplied to the horses . Toxoplasmosis seems to be slightly less in others cities. The difference could be, thus, associated with type of farming, the presence of cats and the quality of water. The seroprevalence in the adult horses (>10-year-old) was 7.6% and it was significantly higher than those of young horses (≤ 10-years-old) 5.2% . Seroprevalence in horses increased with age ,the 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. In addition to this difference between the age groups, a difference in the subgroups of females and males horses was not significant. Indeed, the females had a seroprevalence, (6.2%) while the males, (6.3%). The overall seroprevalence

was 6.3%, which was far less than those reports from other countries in Africa. The serological survey of this parasite has previously been conducted only in three countries on the African continent: Nigeria , Egypt and Tunisia. In the first country, the overall rate of anti-*Toxoplasma* antibodies, exceeds 30% in horses (Aganga,1983). In Egypt a first study reported an upper rate of 40% (Ghazy et al.,2007) but the sera samples were collected from horses with neurological clinical manifestations, and a prevalence rate of 25% had also been reported in Egypt among draught horses (Haridy et. al.,2009). In Tunisia antibodies of *T. gondii* were found in 17.7% (Sonia et.al.,2011). Interpretation of the horse survey results using criteria from previous camel studies (Manal *et.al*.,2005) suggest that , the prevalence of *T. gondii* antibodies in horses in Sudan is low, also antibodies titers were generally low, these results suggest that horses in the present study might have been exposed to only a few oocysts.

Conclusion

In this study the isolation of *Toxoplasma gondii* tachyzoites from aborted foal and *T*. oocysts from kittens fed on the aborted foal tissues reported that *Toxoplasma gondii* plays a role in equine abortions in Sudan. Also the current study evaluated clinically normal horses from Khartoum state (the three cities) for the presence of anti-*T. gondii* antibodies . The overall sero-prevalence of anti-*T. gondii* antibodies in horses in the surveyed animals is lower than those reported from other countries in Africa (6.3%) , also antibodies titers were generally low. Although eating equine meat is not a Sudanese habitat, the existence of a possible risk arouses by the presence of parasitic stages in infected horses, which resulted in the infection of cats and/or mice.

References

Aganga AO, Kwanashie GG, Belino ED. **1983**. *Toxoplasma* antibodies in polo horses of Nigeria. *Int J Zoonoses.* **10**:155–158.

Al-Khalidi N.W., Dubey J.P. **1979.** Prevalence of *Toxoplasma gondii* infection in horses. J. Parasitol., *65*, 331–334.

Beyer T.V., Shevhunova E.A. **1986.** A review of toxoplasmosis of animals in the U.S.S.R. Vet. Parasitol. 19,225–243.

Dubey, J.P., Desmonts, G. **1987**. Serological responses of equids fed *Toxoplasma gondii* oocysts. Eq. Vet. J. 19, 337–339.

Eugster A.K., Joyce J.R. **1976** . Prevalences and diagnostic significances of *Toxoplasma gondii* antibodies. Vet. Med. Small. Anim. Clin., 71, 1469–1473.

Ghazy AA, Shaapan RM, Abdel-Rahman EH. **2007**. Comparative serological diagnosis of toxoplasmosis in horses using locally isolated *Toxoplasma gondii*. *Vet Parasitol*. **145**:31

Haridy FM, Shoukry NM, Hassan AA, Morsy TA. **2009.** ELISA-seroprevalence of *Toxoplasma gondii* in draught horses in Greater Cairo, Egypt. *J Egypt Soc Parasitol*. **39**:821–826.

Hejlicek K., Literak I. **1994**. Prevalence of antibodies to *Toxoplasma gondii* in horses in the Czech Republic. Acta Parasitol. 39, 217–219.

Macruz, R., Lenci, O., Ishizuka, M.M.. **1975**.Toxoplasmose em eqinos PSI: estudo serologico. Rev. Fac. Med. Vet. Zootec. Univ. Sao Paulo 12, 2777–2782. Manal

Y.I, Majid A.M.and Magzoub E. **2005.** Seroprevalence of *Toxoplasma gondii* antibodies in camels (*Camelus dromedaries*) in Sudan. Al Buhuth, The Sudan J .of Sci. Res. , 9 (1):94-102.

Sonia Boughattas, Ramzi Bergaoui, Rym Essid, Karim Aoun, and Aida Bouratbine , **2011**. Seroprevalence of *Toxoplasma gondii* infection among horses in Tunisia. Parasit Vectors. 4:

Tassi P. **2007**. *Toxoplasma gondii* infection in horses. A review. *Parassitologia*.;**49**:7–15. .

Tizard I.R., Harmeson J., Lai C.H. **1978**. The prevalence of serum antibodies to Toxoplasma gondii in Ontario mammals. Can. J. Comp. Med. 42, 177–183.

Turner,C.B.,Savva,D. **1990**. Evidence of *Toxoplasma gondii* in an equine placenta. Vet .Rec. 127,96.

Turner, C.B., Savva, D. **1992.** Transplacental infection of a foal with *Toxoplasma gondii*. Vet. Rec., 131,180.

Uggla A., Ma□son S., Jun□i N. **1990** . Prevalence of antibodies to *Toxoplasma gondii* in cats, dogs and horses in Sweden. Acta Vet. Scand. 31, 219–222 .

Webster Joanne P. **2010**. In: *ParasitVectors.* Second. Dubey JP, editor. Vol. 3. Review of "Toxoplasmosis of Animals and Humans; p.112.

Zardi O., Giorgi G., Vendii G., Drisaldi D. **1968** . Serological studies on *T. gondii* infection in a limited number of animal species. Zooprofilassi. *22*, 223–237.