



Original Article

Serological study of equine toxoplasmosis in southeast of Iran

Shiva Amanollahi ¹, Ehsanollah Sakhaee ^{*2}, Mehdi Golchin ³

¹ School of Veterinary Medicine graduate, Shahid Bahonar University of Kerman, Kerman, Iran

² Department of Clinical Sciences, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

³ Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

ARTICLE INFO

Received: 20 August 2017

Accepted: 12 October 2017

DOI: xxxxxxxxxxxxxx

KEYWORDS :

Toxoplasma gondii

Latex agglutination test

Horse

Iran

ABSTRACT

Toxoplasmosis, is an important worldwide zoonotic disease caused by *Toxoplasma gondii*. This disease is transmitted mainly through the ingestion of tissue cysts in contaminated raw or undercooked meat, or through sporulated oocysts in food, soil and water. The objective of the present study was determination of anti-*Toxoplasma gondii* seroprevalence in the horses in Kerman and Yazd provinces by latex agglutination test. Therefore, 163 serum samples were collected from apparently healthy horses at race clubs in Kerman and Yazd provinces and evaluated by latex agglutination test. According to the results, antibodies against *Toxoplasma gondii* were detected in 71 sera (43.56 %) among 163 samples. So there are some sero-positive cases in horse population of Kerman and Yazd provinces.

مطالعه سروزیک توکسوپلاسموز در اسبان جنوب شرق ایران

شیوا امان الهی^۱، احسان اله سخائی^{*۲}، مهدی گلچین^۳

^۱ دانش آموخته دانشکده دامپزشکی دانشگاه شهید باهنر کرمان، کرمان، ایران

^۲ گروه علوم درمانگاهی دانشکده دامپزشکی دانشگاه شهید باهنر کرمان، کرمان، ایران

^۳ گروه پاتوبیولوژی دانشکده دامپزشکی دانشگاه شهید باهنر کرمان، کرمان، ایران

چکیده

توکسوپلاسموز یک بیماری مشترک مهم محسوب شده که توسط توکسوپلازما گونه ایجاد می شود. عامل بیماری از طریق بلع کیست های بافتی موجود در گوشت خام یا از طریق اووسیست های اسپروله شده در موادغذایی، خاک و آب انتقال می یابد. هدف مطالعه ی حاضر ردیابی پادتن ضد توکسوپلازما گونه ای به روش لاتکس آگلوتیناسیون در اسبان استان های کرمان و یزد می باشد. به این منظور تعداد ۱۶۳ نمونه سرم اسب (از نظر ظاهری سالم) از باشگاه های سوارکاری استان های مذکور اخذ و به روش لاتکس آگلوتیناسیون مورد ارزیابی قرار گرفتند. بر اساس نتایج حاصله تعداد ۷۱ سرم از مجموع ۱۶۳ نمونه مورد آزمایش (۴۳/۵۶ درصد) حاوی پادتن ضد توکسوپلازما گونه ای بود. بنابراین به نظر می رسد که پادتن ضد عامل مذکور در اسبان استان های مورد نظر حضور داشته باشد.

واژه های کلیدی: توکسوپلازما گونه ای، آزمایش لاتکس آگلوتیناسیون، اسب، ایران

* Corresponding author: Ehsan_Sakhaee@uk.ac.ir

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa, order Coccidia [11, 21]. Infection with this parasite, namely, toxoplasmosis that is the most widespread zoonotic disease worldwide which can infect warm blood vertebrates, including mammals and birds species [25]. The life cycle of the parasite involves a definitive (Felids, especially the cat) and an intermediate (all warm-blooded animals and human) host. The two most common forms of transmission are by ingestion of oocysts from feces of infected cats and by the ingestion of cysts in raw or undercooked meats. Other forms of transmission are trans-placental, blood transfusions, and organ transplants [6, 7]. Horses are most commonly infected by ingestion of sporulated oocysts found in feces of infected cats [1]. Toxoplasmosis in horses is generally asymptomatic because they are the most resistant to infection with *Toxoplasma gondii* but, fever, ataxia, retinal degeneration, encephalitis, abortion or still birth and neonatal death in pregnant Equids may occasionally occur [15]. With considering the importance of toxoplasmosis in the context of animal health and of public health, this study was aimed to determine the frequency of equine anti-*Toxoplasma gondii* antibody in Kerman and Yazd provinces. The Latex Agglutination Test (LAT) kit (Zist Faravard Pars Company, Rasht, Iran) used in our study has been evaluated as a serologic screening test for toxoplasmosis in animals and has been widely used in serological surveys with various animal species.

MATERIALS AND METHODS

Sample collection and processing

One hundred and sixty-three serum samples were collected from 93 male and 70 female

clinically healthy horses at 9 race clubs in Kerman and Yazd provinces, Iran, from February to September 2015. The breed composition comprised of Arabian horses and Arabian horse crosses and non-Arabian horses consisted of Darreh Shouri, Turkmen, Thoroughbred and local breeds. The samples were submitted to Immunology laboratory of School of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran. Sera were extracted and stored in 1.5 ml sterile microtube (Eppendorf) at -20°C until analysis.

Animals and Sampling Procedures

A total of 120 donkeys (52 females, 43.33%; 68 males, 56.67%) with an age range of 1 to 12 years old (median 5.0 years) were selected and divided into two age groups (group A: below 5 years of age, 57 heads; group B: above 5 years of age, 63 heads). The eyes were carefully examined and proved to be clinically healthy. The specimens were obtained from the inferior conjunctival sac of both eyes by sterile dry swab without touching the eyelids, eyelashes and vibrissae. The swabs were placed in tubes containing sterile normal saline and submitted immediately to microbiology laboratory in a cool box.

Latex Agglutination Test (LAT)

Latex Agglutination Test Kit (Zist Faravard Pars Co., Rasht, Iran) was used according to the manufacture's protocols. Briefly, 10 μl of serum and 10 μl of antigen coated latex particles were added onto an agglutination card and mixed with a plastic stirrer. The card was rocked from side to side for up to 5 min to provoke the agglutination reaction. Any specimen that showed agglutination in this period was recorded as positive, and otherwise negative. Incomplete agglutination was recorded as "suspected infection". Positive and negative controls were tested each day.

RESULTS

According to the results, antibodies were detected in 71 sera out of 163 (43.56%) (43 cases < 7 years old and 28 cases > 7 years old). Based on the results presented on Table 1, 38 (27 cases less than and 11 cases more than 7 years old) stallions (23.31%) and 33 (16 cases less than and 17 cases more than 7 years old) mares (20.24%) were sero-positive against toxoplasmosis.

DISCUSSION

Our results showed the total seropositivity for all 163 horses examined in this study was 43.56%. *Toxoplasma gondii* causes subclinical infection in horses and has its importance due to economic losses and the risks for public health [16]. Equine toxoplasmosis is potentially an important source of infection for feral and zoo carnivorous animals which are sometimes fed on equids [22]. To the best

knowledge of the authors, many studies in different regions of world, have documented the detection of *Toxoplasma gondii* antibodies in horses using different serological tests, while there is no documented study in southeast of Iran. Commercially available latex agglutination test is simple, rapid and reliable diagnostic assay in all species [17]. In LAT, white polystyrene antigen (*Toxoplasma gondii*) coated particles agglutinate in the presence of antibodies, which is visible to naked eye. LAT is of widespread use in serological survey with various animal species [2, 4, 12, 14, 20]. Tatsunori, et al., (2016) indicated that LAT and (indirect anti-fluorescence antibody) IFAT had similar capabilities for detection of equine anti-*Toxoplasma gondii* antibodies [23]. LAT however, is quicker, cheaper and requires less sophisticated facilities to perform; and hence we chose the test for our purpose.

Results of studies of Hejlícek and Literak (1994) performed by DT and Bartova et al., (2010) by LAT in Czech Republic indicated

Table 1. The number and frequency of positive, negative and suspected samples (based on the presence of antibodies against *Toxoplasma gondii*) among 163 horses in Kerman and Yazd provinces, Iran

Province	Gender	Age								Total										
		≤ 7 years old				> 7 years old				P		N		S		T				
		P	N	S	T	P	N	S	T	No	F	No	F	No	F	No	F			
		No	F	No	F	No	F	No	F	No	F	No	F	No	F	No	F	No		
Yazd	Male	15	31.25	17	35.42	16	33.33	48	28.57	2	28.57	3	42.86	7	30.9	19	34.55	19	34.55	55
	Female	9	25.71	12	34.29	14	40	35	58.34	7	8.33	4	33.33	12	34.04	13	27.66	18	38.3	47
Kerman	Male	12	54.54	6	27.27	4	18.19	22	56.25	9	0	7	43.75	16	55.26	6	15.79	11	28.95	38
	Female	7	63.63	1	9.1	3	27.27	11	83.34	10	8.33	1	8.33	12	73.91	2	8.7	4	17.39	23
Total		43	37.07	36	31.03	37	31.9	116	59.58	28	4	15	31.91	47	43.56	40	24.54	52	31.9	163

No: Number of cases, F: Frequency (percent), P: Positive, N: Negative, S: Suspected, T: Total

that sero-prevalence of equine anti-Toxoplasma gondii antibodies were 7.7 % and 23% respectively [4, 10], while the results of the present study show sero-positivity of 43.56%. It may be due to methods using in the mentioned studies.

Seroprevalence of equine Toxoplasma gondii antibody in Urmia, northwest, Iran [19], Qazvin, Iran [9] and Southwest of Iran [24] were 11.5 %, 71.2 % and 48.5% respectively. Comparison between the results of the present and similar above mentioned studies in Iran shows the prevalence of anti-Toxoplasma gondii antibodies varies from region to region. Distribution of anti-Toxoplasma gondii antibodies in the equine population of various countries using LAT is different, too. Alshahery and Mansour (2012) in Iraq, Ahmed et al., (2013) in Sudan, Bartova et al., (2010) in Czech Republic and Saqib et al., (2015) in Pakistan indicated the prevalence of anti-Toxoplasma gondii antibodies was 72.2%, 32.7%, 23%, 23,5% respectively [2, 3, 4, 22]. It may be attributed to some epidemiological factors effect on Toxoplasma gondii parasite. Sampling techniques, husbandry method used in different regions, distribution of cat population and climatic variation are essential elements in epidemiological studies and variation of results [8, 18, 24]. The results of our study show that the prevalence of anti-Toxoplasma gondii antibodies is 47.14% and 40.86% in mares and stallions, respectively. Papini et al., (2015) in Italy has given similar results with the IFA method [18]. However, the results of some other studies did not show any significant differences between mentioned groups [22, 24].

It seems that different results in various regions are due to some factors such as; serological methods [5, 8, 18] , climate variation, amount of animals contact with cats [8], target population [5, 18], geographical

areas [18], health management [8], age [8, 18], gender and breed [22], and any other factors or any combination(s).

CONCLUSION

According to the results and regarding similar prevalence reported in adjoining provinces, the prevalence of equine anti-Toxoplasma gondii antibodies in Iran is noticeable. Therefore, it seems that Iranian equestrian clubs should improve their management and health levels to increase their proficiencies. Feline and other possible shedding hosts should be identified and dealt with if the aim is eradication of the condition in the horses. Extension efforts should be carried out to increase awareness and prevent feeding raw horse meat to domestic and wild animals.

ACKNOWLEDGMENTS

This research was financially supported by the Research Council of Shahid Bahonar University of Kerman, Iran.

REFERENCES

- [1] Aganga AO, Kwanashie GG, Belino ED. Toxoplasma antibodies in polo horses of Nigeria. *International journal of zoonoses*. 1983; 10(2):155-8.
- [2] Ahmed SM, Ibrahim AM, Alshafie NK, Mohammed RH, Ismail AA, Angara TE. The First Report on Sero-Prevalence of Toxoplasma gondii in Working Horses and Donkeys in the Sudan. *Journal of Life Sciences*. 2013; 1:7(12).
- [3] Alshahery MN, Mansour RS. Detection of Toxoplasma gondii antibodies in horses in Mosul, Iraq. *Iraqi Journal of Veterinary Sciences*. 2012; 2:26(Supplement II):39-41.
- [4] Bártová E, Sedlák K, Syrová M, Literák I. Neospora spp. and Toxoplasma gondii antibodies in horses in the Czech Republic. *Parasitology Research*. 2010; 107(4):783-5.
- [5] Coiro CJ, Langoni H, da Silva RC. Epidemiological aspects in the Leptospira spp. and Toxoplasma gondii infection in horses from Botucatu, São Paulo, Brazil. *Journal of Equine Veterinary Science*. 2012; 32(10):620-3.

- [6] Domingues PF, Langoni H. Manejo sanitario animal, 1st ed. EPUB: Rio de Janeiro, Brasil; 2001. p. 860.
- [7] Dubey JP, Jones JL. Toxoplasma gondii infection in humans and animals in the United States. *International journal for parasitology*. 2008; 38(11):1257-78.
- [8] Gharekhani J, Gerami-Sadeghian A, Tavoosidana G, Sohrabei A. Seroepidemiology of Toxoplasma gondii infection in dogs and domestic equine from western Iran. *Comparative Clinical Pathology*. 2015; 24(2):255-8.
- [9] Hajjalilo E, Ziaali N, Harandi MF, Saraei M, Hajjalilo M. Prevalence of anti-Toxoplasma gondii antibodies in sport horses from Qazvin, Iran. *Tropical animal health and production*. 2010; 42(7):1321-2.
- [10] Hejlícek K, Literák I. Prevalence of antibodies to Toxoplasma gondii in horses in the Czech Republic. *Acta Parasitologica*. 1994; 39(4).
- [11] Klevar S. Tissue cyst forming coccidia; Toxoplasma gondii and Neospora caninum as a cause of disease in farm animals. *Acta Veterinaria Scandinavica*. 2007; 49(1):S1.
- [12] Machacova T, Bartova E, Di Loria A, Sedlak K, Mariani U, Fusco G, Fulgione D, Veneziano V, Dubey JP. Seroprevalence of Toxoplasma gondii in donkeys (Equus asinus) in Italy. *Journal of Veterinary Medical Science*. 2014; 76(2):265-7.
- [13] Masatani T, Takashima Y, Takasu M, Matsuo A, Amaya T. Prevalence of anti-Toxoplasma gondii antibody in domestic horses in Japan. *Parasitology international*. 2016; 65(2):146-50.
- [14] Matsuo K, Kamai R, Uetsu H, Goto H, Takashima Y, Nagamune K. Seroprevalence of Toxoplasma gondii infection in cattle, horses, pigs and chickens in Japan. *Parasitology international*. 2014; 63(4):638-9.
- [15] Miao Q, Wang X, She LN, Fan YT, Yuan FZ, Yang JF, Zhu XQ, Zou FC. Seroprevalence of Toxoplasma gondii in horses and donkeys in Yunnan Province, Southwestern China. *Parasites & vectors*. 2013; 6(1):168.
- [16] Naves CS, Ferreira FA, Carvalho FD, Costa GH. Soroprevalência da toxoplasmose em equinos da raça Mangalargamarchador no município de Uberlândia, Minas Gerais. *Veterinária Notícias*. 2005; 11(1):45-52.
- [17] OIE A. Manual of diagnostic tests and vaccines for terrestrial animals. By: Office International Des Epizooties, Paris, France. 2008:1092-1106.
- [18] Papini RA, Buzzone G, Nardoni S, Rocchigiani G, Mancianti F. Seroprevalence and genotyping of Toxoplasma gondii in horses slaughtered for human consumption in Italy. *Journal of Equine Veterinary Science*. 2015; 35(8):657-61.
- [19] Raeghi S, Akaberi A, Sedeghi S. Seroprevalence of Toxoplasma gondii in sheep, cattle and horses in Urmia North-West of Iran. *Iranian journal of parasitology*. 2011; 6(4):90.
- [20] Ramzan M, Akhtar M, Muhammad F, Hussain I, Hiszeczyńska-Sawicka E, Haq AU, Mahmood MS, Hafeez MA. Seroprevalence of Toxoplasma gondii in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Tropical animal health and production*. 2009; 41(7):1225.
- [21] Rorman E, Zamir CS, Rilkis I, Ben-David H. Congenital toxoplasmosis-prenatal aspects of Toxoplasma gondii infection. *Reproductive toxicology*. 2006; 21(4):458-72.
- [22] Saqib M, Hussain MH, Sajid MS, Mansoor MK, Asi MN, Fadya A, Zohaib A, Sial AU, Muhammad G, Ullah I. Sero-epidemiology of equine toxoplasmosis using a latex agglutination test in the three metropolises of Punjab, Pakistan. *Tropical Biomedicine*. 2015; 32(2):276-85.
- [23] Tatsunori M, Yasuhiro T, Masaki T, Aya M, Tomohiko A. Prevalence of anti-Toxoplasma gondii antibody in domestic horses in Japan. *Parasitology International*. 2016; 65:2, 146-150.
- [24] Tavalla M, Sabaghan M, Abdizadeh R, Khademvatan S, Rafiei A, Piranshahi AR. Seroprevalence of Toxoplasma gondii and Neospora spp. Infections in Arab Horses, Southwest of Iran. *Jundishapur Journal of Microbiology*. 2015; 8(3).
- [25] Tenter AM, Heckerroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. *International Journal for Parasitology*. 2000; 30(12):1217-58.