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Short Communication

Molecular Detection of *Theileria equi* and *Babesia caballi* Infections in Horses by PCR Method in Iran

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Abstract

The aim of the current study was to determine the prevalence of *Theileria equi* and *Babesia caballi* infections in horses in the Central and Southwest, Iran. Blood samples were collected from 53 and 37 horses settled in Isfahan and Shahrekord, respectively and a PCR method was used to detect the parasites in blood samples. The results showed that *Theileria equi* detected in 6 horses in Isfahan and in 4 horses in Shahrekord. Based on the findings, the prevalence of equine theileriosis was much higher than babesiosis and it occurred in both Isfahan and Shahrekord regions of the country. To the authors' knowledge, this is the first report of the molecular survey of *Theileria equi* and *Babesia caballi* infections in horses in Iran. This survey could provide further information on different parasitic infections in horses and its epidemiology.

Keywords: *Horses, Babesia, PCR, Theileria equi, Iran*

İran'daki Atlarda *Theileria equi* ve *Babesia caballi* Enfeksiyonlarının PCR Yöntemi İle Moleküler Tayini

Özet

Bu çalışma, İran'ın Orta ve Güneybatısındaki atlarda *Theileria equi* ve *Babesia caballi* enfeksiyonlarının sıklığını belirlemek amacıyla yapıldı. İsfahan ve Shahrekord'ta yaşayan atlardan sırasıyla 53 ve 37 adet kan numunesi toplandı ve kan örneklerinde parazitleri tespit etmek için bir PCR yöntemi kullanıldı. Sonuçlar, İsfahan'ki 6 at ile Shahrekord'taki 4 atta *Theileria equi* tespit edildiğini gösterdi. Bulgulara göre, at Theileriosis'in prevalansı babeziyoz'a göre çok daha yüksekti ve ülkenin hem İsfahan hem de Shahrekord bölgelerinde oluştu. Yazarların bildiği kadarıyla, bu çalışma *Theileria equi* ve *Babesia caballi* enfeksiyonların İran'daki atlarda moleküler olarak araştırıldığı ilk bildirimdir. Bu çalışma, atlardaki farklı parazit enfeksiyonları ve epidemiyolojisi hakkında ayrıntılı bilgi sağlayabilir.

Anahtar sözcükler: *Atlar, Babesia, PCR, Theileria equi, İran*

INTRODUCTION

The *Theileria* species infect a wide range of both domestic and wild animals and are transmitted by ixodid ticks of the genera *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus*. Most of these ticks are renowned for the large economic losses they cause to the agricultural industry due to disease outbreaks, mortalities, damage to hide and poor production in domestic animals [1]. The genus *Theileria* is distinguished by infection of leukocytes by sporozoites, maturation of schizonts into merozoites and subsequent infection of red blood cells to form piroplasms [2].

Theileria equi is a protozoan of the phylum Apicomplexa that is biologically transmitted by ixodid ticks. It causes disease in equids characterized by fever, anemia, icterus, hepatosplenomegaly, intravascular hemolysis, hemoglobinuria and in some cases death can occur [3]. The

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disease has a worldwide distribution and is endemic in most tropical and subtropical areas as well as in some temperate zones of the world [3-6]. It has caused important economic losses in the horse industry, being a serious threat to the horse raising industry and international movement of horses [7].

Theileria equi and *Babesia caballi* is tick-borne haemoparasites that may cause babesiosis of Equidae. Equine piroplasmosis is an economically important tick-borne protozoan disease of horses that has been reported worldwide [3]. In southern Europe *T. equi* is enzootic and infections may occur asymptomatically and more frequently than those due to *B. caballi* [8,9]. *Babesia* parasites destroy host erythrocytes and induce fever, anemia, and icterus in infected horses [10].

Theileria equi is a tick-borne heamoprotozoan parasite, which causes equine babesiosis [11]. The disease is endemic in most tropical and subtropical regions of the world, including Europe, Asia, Africa, America and Australia continents [12,13]. The diseased animal is characterized by anemia, fever, hemoglobinuria, icterus and in some cases death can occur [3].

Babesia caballi, like *T. equi*, is a tick-borne protozoan parasite, which causes fever, anemia, jaundice, and edema in the infected horses and sometimes results in death [3,14-16]. These equine babesioses are known to induce significant economic losses in the horse industry [6,16]. These parasites are usually detected in blood smears during the acute stage of infection but not easily in those of the recovered animals that remain carriers of the parasite [17]. Clinical signs are not specific diagnostic measures for babesiosis, especially in asymptomatic or mixed infection in endemic areas [9].

Therefore, detection of *T. equi* and *B. caballi* may be useful for developing effective strategies to treat and control different infections in horses. The diagnosis is routinely done by conventional parasitological techniques like Giemsa stained thin blood smear. Giemsa stained blood smear examination is not a sensitive method to demonstrate parasites in the blood mainly because of the periodically cryptic nature of the parasite. Recently, Polymerase chain reaction (PCR) is considered to be superior to parasite detection and antigen detection tests due to its sensitivity in detecting the prepatent and chronic phase of an infection [13,18]. The lack of molecular data severely hampers our efforts in studying molecular epidemiology of *T. equi* and *B. caballi*. Hence, the objective of this study was to determine the prevalence of *T. equi* and *B. caballi* infections in horses from Isfahan Central and Shahrekord Southwest, Iran using PCR molecular method.

MATERIAL and METHODS

Samples: This cross-sectional study was conducted between February and December, 2014. A total of 53 and 37 horses settled in Isfahan Central and Shahrekord Southwest, Iran, respectively, were randomly selected. The breed, sex and age of each of the horses was noted and appropriately recorded. Blood sample was collected by vein puncture from the external jugular vein from each of the horse using anticoagulant [ethylenediamine tetraceticacid (EDTA)]-containing vacutainer. The samples were transported aseptically in ice packs to the Biotechnology Research Center, and stored at -20°C until needed.

DNA Extraction: Genomic DNA in the blood samples were extracted using DNA extraction kit (Cinnagen, Tehran, Iran) following the manufacturer's instruction. Concentration of extracted DNA from each blood sample was measured spectro-photometrically at 260 nm optical density following the method described by Sambrook and Russell [19]. Extracted DNA samples were kept frozen at -70°C until needed.

Polymerase Chain Reaction: Polymerase chain reaction was performed on the genomic DNA of *T. equi* and *B. caballi*. The primers used for amplification are as shown in *Table 1*. The amplification of *Theileria equi* and *Babesia caballi* DNA was done using thermocycler (Eppendorf, Hamburg, Germany). Polymerase chain reaction products were run using 1.5% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with ethidium bromide and the images were visualized in UVIdoc gel documentation systems (Uvitec, UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany).

RESULTS

Overall Prevalence

The PCR products for *T. equi* (241 bp) and *B. caballi* (180

Table 1. *Primer sequence used for detection of Theileria equi and Babesia caballi genes in horse's blood*
Tablo 1. *At kanındaki Theileria equi ve Babesia caballi genlerinin tespiti için kullanılan primer dizisi*

Organism	Primers Sequences	GenBank Accession Numbers	Annealing Temperature	Size (base pair)
Theileria equi	F: 5´ - GAGGAGCACATCGTCTACACTG - 3´	KC347577	60°C	241 bp
	R: 5´ - ACAAGACCTCCTGGTAGAACTCG-3´			
Babesia caballi	F: 5´ - CGGCTGCTATGGTTATTCAG - 3´	AB017700	60°C	180 bp
	R: 5´ - AGAGTGCAACCGAGCAATGC-3´			

F: *Forward;* ***R:*** *Reverse*

bp) were identified by 100 bp DNA size marker (Fermentas, Germany). A total of 53 samples from Isfahan and 37 from Shahrekord were collected, of these the results of the PCR assays showed that *T. equi* detected in 6 horses in Isfahan and in 4 horses in Shahrekord. In addition, 5 horses were infected by *B. caballi* in each states, Isfahan and Shahrekord. Differences in infection rates were statistically non significant between male and female horses and among different age groups *(Table 2, Table 3)*.

Prevalence According to Breed: The distribution of *T. equi* and *B. caballi* in different horse breeds in Isfahan and Shahrekord is indicated in *Table 4*. The result showed that of the 6 *T. equi* detected in Isfahan 2 were from Standardbred, 3 from Thoroughbred and 1 from Arab on the other hand in Shahrekord out of 4 identified *T. equi,* 1 were from Standardbred, 2 from Thoroughbred and 1 from Arab. In addition, of the 5 identified *B. caballi* in each states Isfahan and Shahrekord, 1 were from Standardbred, 2 from Thoroughbred, 1 from Arab and 1 also from Turkoman.

DISCUSSION

In the present study, the occurrence of *T. equi* infection in horses from Isfahan and Shahrekord was investigated by PCR. The results demonstrate that *T. equiis* widespread in the region studied, suggesting high levels of transmission, as there was found a high rate of positive horses by molecular methods. This is probably related to the prevalence and intensity of tick infestation in this region. Arslan et al.[20] and Friedhoff [21] reported that *Dermacentor marginatus* known to transmit *B. equi*. In addition, another study conducted in Hungary showed that the prevalence of *T. equi* infection among 101 horses was 49% with PCR [22]. These differences may be related to management practices and due to a difference in the prevalence of tick vector for *B. equi* between countries, where climatic factors such as temperature, humidity and rainfall influence the habitat for ticks.

Theileria equi is more common and pathogenic than *B.*

Table 2. *Distribution of Theileria equi and Babesia caballi among equine population in Isfahan and Shahrekord*
Tablo 2. *İsfahan ve Shahrekord'taki at nüfusu içinde Theileria equi ve Babesia caballi Dağılımı*

Sex	Number of Samples Collected		Number (%) of Horses Infected			
	Isfahan	Shahrekord	*Theileria equi*		*Babesia caballi*	
			Isfahan	Shahrekord	Isfahan	Shahrekord
Stallion	22	23	1 (4.5)	3 (13.0)	1 (4.5)	2 (8.7)
Mare	31	14	5 (16.1)	1 (7.1)	4 (12.9)	3 (21.4)
Total	53	37	6 (20.6)	4 (20.1)	5 (17.4)	5 (30.1)

Table 3. *Distribution of Theileria equi, Theileria evansi, Babesia caballi, and Habronema among different age groups of horses in Isfahan and Shahrekord*
Tablo 3. *İsfahan ve Shahrekord'taki farklı yaş gruplarındaki atlar arasında Theileria equi, Theileria evansi, Babesia caballi ve Habronema Dağılımı*

Age	Number of Samples Collected		Number (%) of Horses Infected			
	Isfahan	Shahrekord	*Theileria equi*		*Babesia caballi*	
			Isfahan	Shahrekord	Isfahan	Shahrekord
<1	7	3	1 (14.3)	0 (0)	0 (0)	1 (33.3)
1-2	11	4	1 (9.1)	0 (0)	0 (0)	1 (25.0)
2-3	11	6	1 (9.1)	1 (16.7)	1 (9.1)	1 (16.7)
>3	24	24	3 (12.5)	3 (12.5)	4 (16.7)	2 (8.3)
Total	53	37	6 (11.3)	4 (10.8)	5 (9.4)	5 (13.5)

Table 4. *Distribution of Theileria equi, Theileria evansi, Babesia caballi, and Habronema in different horse breeds in Isfahan and Shahrekord*
Tablo 4. *İsfahan ve Shahrekord'taki farklı at ırklarında Theileria equi, Theileria evansi, Babesia caballi ve Habronema Dağılımı*

Breed	Number of Samples Collected		Number (%) of Horses Infected			
	Isfahan	Shahrekord	*Theileria equi*		*Babesia caballi*	
			Isfahan	Shahrekord	Isfahan	Shahrekord
Standardbred	18	13	2 (11.1)	1 (7.7)	1 (5.6)	1 (7.7)
Thoroughbred	21	15	3 (14.3)	2 (13.3)	2 (9.5)	2 (13.3)
Arab	7	7	1 (0)	1 (0)	1 (0)	1 (0)
Turkoman	7	2	0 (0)	0 (0)	1 (0)	1 (0)
Total	53	37	6 (11.3)	4 (10.8)	5 (9.4)	5 (13.5)

caballi in endemic countries [23-25]. The results of the present study demonstrated that *T. equi* was more prevalent than *B. caballi*. Our findings were in agreement with the previous study in Iran [26]. A possible reason for the low prevalence of *B. caballi* could be associated with the earlier removal of the parasite after a short term of infection [27].

The results of molecular and microscopic examinations confirmed the simultaneous infection of horses in the study region with both equine Babesia species, which was consistent with findings of Seifi et al.[26] and Abedi et al.[28]. They reported mixed infection of *T. equi* and *B. caballi* in horses of Turkmen region in Iran. Results indicated that even subclinical and latent carrier infections diagnosed by molecular means are responsible for inducing pathogenicity. In our study no differences were observed between the *T. equi* and *B. caballi* prevalence in all age and sex groups of the horse examined. It may be due to high number of ticks in this area and continuous exposure of young and old horses to infected ticks [29].

The present study suggests the existence of *T. equi* and *B. caballi* in different horse breeds in Isfahan and Shahrekord. Thus, our results indicate that *T. equi* occurs more frequently than *B. caballi* in the investigated geographical region. However, no diversity was observed among the isolates within the studied regions. The detection of the pathogenic species of *T. equi* and *B. caballi* in asymptomatic horses indicates that the relationship between parasite species/subspecies and clinical signs of infection in horses deserves further investigation. We further recommend investigating the prevalence of *T. equi* and *B. caballi* in other domestic animals, like sheep and goats, living in the same environment.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

All authors contributed equally to this work. All authors read and approved the final manuscript.

REFERENCES

1. Bishop R, Musoke A, Morzaria S, Gardner M, Nene V: *Theileria*: Intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitol* 129, S271-S283, 2004. DOI: 10.1017/S0031182003004748

2. Uilenberg G: Babesia - A historical perspective. *Vet Parasitol*, 138, 3-10, 2006. DOI: 10.1016/j.vetpar.2006.01.035

3. Schein E: Equine babesiosis. **In,** Ristic M (Ed): Babesiosis of Domestic Animals and Man. 197-208, CRS Press, Boca Raton, Florida, 1988.

4. De Waal DT: Equine piroplasmosis: A review. *Br Vet J*, 148, 6-14, 1992. DOI: 10.1016/0007-1935(92)90061-5

5. Brüning A: Equine piroplasmosis an update on diagnosis, treatment and prevention. *Br Vet J*, 152, 139-151, 1996. DOI: 10.1016/S0007-1935(96)80070-4

6. Kizilarslan F, Yildirim A, Duzlu O, Inci A, Onder Z, Ciloglu A: molecular detection and characterization of *Theileria equi* and *Babesia caballi* in horses *(Equus ferus caballus)* in Turkey. *J Equine Vet Sci*, 35, 830-835, 2015. DOI: 10.1016/j.jevs.2015.08.002

7. Friedhoff KT, Tenter AM, Muller I: Haemoparasites of equines: Impact on internacional trade of horses. *Rev Sci Tech*, 9, 1187-1194, 1990.

8. Beck R, Vojta L, Mrljak V, Marinculić A, Beck A, Zivicnjak T, Cacciò SM: Diversity of *Babesia* and *Theileria* species in symptomatic and asymptomatic dogs in Croatia. *Int J Parasitol*, 39, 843-848, 2009. DOI: 10.1016/j.ijpara.2008.12.005

9. Bashiruddin JD, Camma C, Revelo E: Molecular detection of *Babesia equi* and *Babesia caballi* in horse blood by PCR amplification of part of the 16S rRNA gene. *Vet Parasitol*, 84, 75-83, 1999. DOI: 10.1016/S0304-4017(99)00049-7

10. Knowles DP: Control of *Babesia equi* parasitemia. *Parasitol Today*, 12, 195-198, 1996. DOI: 10.1016/0169-4758(96)10007-7

11. Holbrook AA, Johnson AJ, Madden PA: Equine piroplasmosis: Intra-erythrocyte development of *Babesia caballi* (Nuttal) and *Babesia equi* (Laveran). *Am J Vet Res*, 29, 297-303, 1968.

12. Kuttler KL: Chemotherapy of babesiosis. **In,** Ristic M (Ed): Babesiosis of Domestic Animals and Man. 227-243, CRC Press, Boca Raton, Florida, 1988.

13. Khamesipour F, Doosti A, Koohi A, Chehelgerdi M, Mokhtari-Farsani A, Chengula AA: Determination of the presence of *Babesia* species in blood samples of cattle, camel and sheep in Iran by PCR. *Arch Biol Sci*, 67, 83-90, 2015. DOI: 10.2298/ABS140410009K

14. Purnell RE: Babesiosis in various hosts. **In,** Ristic M, Krier JP (Eds): Babesiosis. 25-64, Academic Press, New York, 1981.

15. Friedhoff KT: The piroplasms of equidae. Significance for the international equine trade. *Berl Muench Tieraerztl Wochenschr*, 95, 368-374, 1982.

16. Brüning A, Phipps P, Posnett E, Canning EU: Monoclonal antibodies against *Babesia caballi* and *Babesia equi* and their application in serodiagnosis. *Vet Parasitol*, 58, 11-26, 1997. DOI: 10.1016/S0304-4017(96)01074-6

17. Holbrook AA: Biology of equine piroplasmosis. *Am J Vet Med Assoc*, 155, 453-454, 1969.

18. Brun R, Hecker H, Lum ZR: Trypanosoma evansi and *T. equiperdum*: Distribution, biology, treatment and phylogenetic relationship (a review). *Vet Parasitol,* 79, 95-107, 1998. DOI: 10.1016/S0304-4017(98)00146-0

19. Sambrook J, Russell DW: Molecular cloning: A laboratory manual. 10-120, Cold Spring Harbor Laboratory Press, New York, 2001.

20. Arslan MÖ, Umur Ş, Aydın L: Kars yöresi sığırlarında Ixodidae türlerinin yaygınlığı. *Türkiye Parazitol Derg*, 23, 331-335, 1999.

21. Friedhoff KT: Transmission of Babesia. **In,** Ristic M (Ed): Babesiosis of Domestic Animals and Man. 23-52, CRC Press, Boca Raton, Florida. 1988.

22. Farkas R, Tánczos B, Gyurkovszky M, Földvári G, Solymosi N, Edelhofer R, Hornok S: Serological and molecular detection of *Theileria equi* infection in horses in Hungary. *Vet Parasitol*, 192, 143-148, 2013. DOI: 10.1016/j.vetpar.2012.09.035

23. De Waal DT: Distribution, transmission and serodiagnosis of Babesia *equi* and Babesia *caballi* in South Africa. *PhD Thesis,* University of Pretoria, 1995.

24. De Waal DT: Global importance of piroplasmosis. *J Protozool Res*, 10, 106-127, 2000.

25. Zweygarth E, Lopez-Rebollar LM, Nurton J, Guthrie AJ: Culture, isolation and propagation of *Babesia caballi* from naturally infected horses. *Parasitol Res*, 88, 460-462, 2002. DOI: 10.1007/s00436-002-0609-4

26. Abedi V, Razmi GH, Seifi H, Naghibi A: Seroprevalence of Theileria equi and Babesia caballi infection in Turkoman breed horses in Iran. *IJVST,* 5 (2): 45-52, 2013.

27. Salim BO, Hassan SM, Bakheit MA, Alhassan A, Igarashi I, Karanis P, Abdelrahman MB: Diagnosis of *Babesia caballi* and *Theileria equi* infections in horses in Sudan using ELISA and PCR. *Parasitol Res*, 103, 1145-1150, 2008. DOI: 10.1007/s00436-008-1108-z

28. Seifi HA, Mohri M, Sardari K: A mixed infection of *Babesia equi* and *Babesia caballi* in a racing colt: A report from Iran. *J Equine Vet Sci*, 20, 858-860, 2000. DOI: 10.1016/S0737-0806(00)80117-3

29. Razmi GR, Naghibi A, Aslani MR, Fathivand M, Dastjerdi K: An epidemiological study on ovine babesiosis in the Mashhad suburb area, province of Khorasan, Iran. *Vet Parasitol*, 108, 109-115, 2002. DOI: 10.1016/S0304-4017(02)00203-0