Epidemiology of *Theileria equi* in Persian Arab horses from Iran

S. Bahrami, A.R. Ghadrdan, M. Pourmahdi Borujeni, M. Vafayi Salarpur

Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

ABSTRACT: The Khuzestan province in south-western Iran is the centre of Persian Arab horse breeding and training. The present study was aimed at determining the prevalence of *Theileria equi* in the equids of this province. A total of 165 blood samples from healthy Persian Arab horses from twenty four stables were examined for the presence of *T. equi* infection using molecular methods. For detection of *T. equi*, primers targeting the 18SrRNA gene were selected. The PCR method gave 47 (28.5%) positive results. Age (P = 0.68), sex (P = 0.88), contact with cattle (P = 0.26) and type of activity (P = 0.06) were not determined as risk factors for *T. equi* infection in this study. However, there was a significant geographical variation in the prevalence of *T. equi* infection ranging from 8.3% (2/24) in Shushtar to 55.6% (10/18) in Ramhormoz (CI, 2.46–76.82) (P = 0.003). In conclusion, equine theileriosis has the potential of posing a significant problem for Iran's Persian Arab horse industry and should remain a major concern to the horse community and regulatory agencies.

Keywords: *Theileria equi*; horse; polymerase chain reaction; Iran

Equine piroplasmosis (EP) is an important tick-borne protozoan disease that poses a serious threat to the horse industry and has important implications for the international movement of horses (Friedhoff et al. 1990). The disease is caused by *Babesia caballi* and/or *Theileria equi* and is endemic in many tropical and subtropical areas as well as in some temperate regions (De Waal 1992). The host spectrum of these parasites consists of different equid species (horse, donkey, mule and zebra). Altogether, 21 species of ixodid ticks of the genera *Dermacentor, Hyalomma* and *Rhipicephalus* are listed as vectors for equine piroplasms (APHIS 2008). *Theileria equi* and *B. caballi* infections are generally characterised by fever, anaemia, icterus and haemoglobinuria. In acute cases death may occur from one to four weeks after the onset of clinical signs and these infections are therefore of considerable veterinary economic importance, particularly to the horse breeding industry (Kuttler 1988; Schein 1988). In chronic and subclinical cases, despite the absence of obvious clinical signs, infected horses can carry the piroplasms in their blood for many years and these carrier animals are thought to be responsible for the maintenance of infection (Schein 1988). In general, *T. equi* is more virulent than *B. caballi* and also the cost of *B. caballi* infection to the racing industry is less severe than that of *T. equi* because infection in carrier animals can be sterilised by suitable drug treatment, whereas *T. equi* infection may be suppressed by chemotherapy but not eliminated (De Waal and Van Heerden 2004). The prevalence and risk factors of equine theileriosis caused by *T. equi* have been the focus of epidemiological studies in recent years. Studies from countries surrounding Iran such as Saudi Arabia (Alanazi et al. 2012), Kuwait (Donnelly 1980a), Oman (Donnelly et al. 1980a), Turkey (Sevinc et al. 2008; Karatepe et al. 2009), Iraq (Al-Saad 2009) and United Arab Emirates (Jaffer et al. 2010) as well as from other countries, found varying degrees of parasite prevalence. There is a considerable population of Arab horses in the Khuzestan province, in south-western Iran, and the province is the centre of Persian Arab horse breeding and training. Also, Khuzestan has been hosting

Supported by the Shahid Chamran University of Ahvaz, Iran.

races for several years with the number of participants increasing yearly. Since it seems that equine theileriosis is endemic in Iran (Bahrami et al. 2014), the identification of risk factors associated with *T. equi* infection may play a role in the adoption of control measures. Moreover, epidemiological evidence may facilitate a better understanding of the mechanisms by which *T. equi* is spread in host populations. However, more information on the distribution of EP and its endemicity is required to build a national control strategy against the disease. There is very little information on equine theileriosis in Iran and no information is available on the prevalence and risk factors related to *T. equi* in Persian Arab horses; therefore, the present study was designed to establish the prevalence of *T. equi* in equids of the Khuzestan province where Arab horse populations are high, to determine whether there exists enzootic stability for *T. equi* in the southwest of Iran, and to identify factors associated with the infection in equids.

MATERIAL AND METHODS

The study was conducted in Khuzestan, a southwestern province of Iran from December 2011 to July 2012. Khuzestan province has an area of about 64 236 km^2 (Statistical book of Khuzestan province, 2006). The province has hot and wet summers, a mild spring and cold winters (Figure 1).

Figure 1. Map of Iran, Khuzestan province. Sampling locations are marked with numbers. 1 = Ahvaz, 2 = Abadan, 3 = Ramhormoz, 4 = Baghe-Malek, 5 = Shush; 6 = Dezful, 7 = Shushtar

Blood samples. In this study, blood samples were taken from 165 Arab horses based at stables. Samples were collected between September 2013 and March of 2014. Analysed samples originated from seven geographical regions within Khuzestan province (18.1% were from Ahvaz, 15.8% were from Shush, 14.5% were from Dezful, 14.5% were from Shushtar, 13.9% were from Abadan, 11% were from Ramhormoz and 12.2% were from Baghe-Malek). Twenty four randomly selected farms participated in this study. On average, 20% of the total numbers of animals in the farms were sampled. The farms had acceptable management (animals reared in stalls, fed with forage and concentrate rations, systematic control of ticks with frequent veterinary care). There were 118 (71.5%) mares and 47 (28.5%) stallions. Blood samples were obtained from a total of 165 animals which consisted of 42 horses less or equal to two years old and 123 horses more than two years old. The blood samples were collected from the jugular vein into sterile vacuum tubes containing EDTA and kept at –20 °C pending analysis. For statistical analysis, the following factors were included: collection area; sex; age (less or equal to two years and more than two years); equid farm activity (sports, exhibition, farming and breeding) and close contact with cattle (yes or no). All horses were healthy at the time of blood collection.

DNA extraction and polymerase chain reaction (PCR) amplification. DNA was extracted using a genomic DNA purification kit (Cinna Gen, Iran). For detection of *T. equi*, primers targeting the 18S rRNA gene were selected from the literature (Alhassan et al. 2005). The forward primer (BEC-UF2) had the sequence 5'-TCGAAGACGATCAGATACCGTCG-3' and the reverse primer Equi-R had the sequence 5'-TGCCTTAAACTTCCTTGCGAT-3', yielding a 392 bp product. PCR reactions included a negative control, consisting of the reaction mix and 2 µl of DNase/RNase-free water and a positive control that consisted of a DNA sample from the blood of a horse with clinical theileriosis. All PCR reactions were performed in a 20 µl mixture consisting of 10 µl Taq master mix, 1µM primers and 5 µl DNA template. PCR cycling included an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 50 s and extension at 72 °C for 60 s. This was followed by a final extension step at 72 °C for 5 min. PCR products were electrophoresed in 1.5% agarose in Tris-acetate-EDTA (TAE) buffer, and stained with ethidium bromide to visualise the

amplified DNA fragments under ultraviolet light. Positive samples showed a band of approximately 400 bp.

Statistical analysis. Fischer's Exact and Chi-squared tests were used to compare infection percentages among different age, sex, location, and equids farm activity groups. *P*-values of < 0.05 were considered statistically significant.

RESULTS

Samples were considered positive if a band of approximately 435 bp was amplified, similarly to that of the positive control, which had been verified by sequencing as *T. equi*. The overall prevalence of *T. equi* in Arab horses was 28.5% (47/165). Of the 47 positive samples, five were sequenced in order to verify the positive result. All sequenced samples were found by BLAST analysis to be closest to the *T. equi* 18S rRNA gene in GeneBank with a similarity of ≥ 98%. Based on the results, 13 out of 47 stallions (27.6%) and 34 out of 118 mares (28.8%) were found to be positive (CI, 0.5-2.25). Therefore, in terms of sex, there was no significant difference between the prevalence percentage of infection in stallions and mares ($P = 0.88$). The prevalence of infection in horses under two years of age was 30.95%, while a prevalence percentage of 27.64% was detected in horses older than two years (CI, 0.55–2.52). Thus, based on these results, age was not identified as a risk factor for *T. equi* infection in this study ($P = 0.68$). The odds of racehorses being infected with *T. equi* were approximately two times greater than in animals from the stud farm (38.2% vs. 23.63%; CI, 0.99–4.02), but the difference was not significant ($P = 0.06$). The odds of the infection in equids reared without contact with cattle was 1.75 times greater than in animals kept in close contact with cattle (CI, 0.66–4.59) but again the difference was not significant ($P = 0.26$). There was, however, a significant geographical variation in the prevalence of *T. equi* infection, ranging from 8.3% (2/24) in Shushtar to 55.6% (10/18) in Ramhormoz (CI, 2.46–76.82). The odds of infection in horses in Ramhormoz were 13.75 times greater than in animals kept in Shushtar (Table 1).

Table 1. Prevalence of *T. equi* in equids in Khuzestan province, southwest of Iran and related factors

Factor	No. examined	Positive	%	*P*-value	OR	CI 95%
Prevalence	165	47	28.48	–	–	–
Sex						
Male	47	13	27.65	0.88	–	–
Female	118	34	28.8		1.06	0.5–2.25
Age						
≤ 2 years old	42	13	30.95	0.68	1.17	0.5–2.52
> 2 years old	123	34	27.64		–	–
Activity						
Race	55	21	38.2	0.06	2	0.9–4.02
Reproduction	110	26	23.63		–	–
Contact with cattle						
Yes	30	6	20	0.26	–	–
No	135	41	30.37		1.75	0.66–4.59
Locality						
Shushtar	24	2	8.3		–	–
Baghe–Malek	20	3	15	0.49	1.94	0.29–12.95
Shush	26	6	23.11	0.17	3.3	0.6–18.27
Dezful	24	6	25	0.14	3.67	0.66–20.42
Ahvaz	30	11	36.7	0.03	6.37	1.25–32.4
Abadan	23	9	39.1	0.02	7.07	1.33–37.65
Ramhormoz	18	10	55.6	0.003	13.75	2.46–76.82

DISCUSSION

Equine theileriosis is a notifiable disease (OIE 2010) with a wide distribution. Subclinical infections have particular relevance to the horse-racing industry where the geographical movement of presumably healthy horses may aid in the spread of *T. equi* or where subclinical infection may negatively affect the animal's performance. It has also been shown that strenuous exercise, such as that experienced by racehorses, can cause subclinical infections to become acute (Hailat et al. 1997). Due to concerns about importing *T. equi* into non-endemic regions, the World Organization for Animal Health (OIE) implemented a mandatory screening process for the international movement of horses. Several molecular techniques for the detection of *T. equi* and *B. caballi* have been described. These methods are based on species-specific PCR amplification of mainly the 18S rRNA gene (Tenter and Freidhoff 1986; Bashiruddin et al. 1999; Criado-Fornelio et al. 2003). The Khuzestan province is the main centre of Persian Arab horse breeding and training in Iran and the province has been hosting international and national races for several years. Therefore there was an urgent need to investigate the presence of infections such as *T. equi* among the Persian Arab horses. The present study shows *T. equi* to be prevalent in all areas sampled, with an overall prevalence rate of 28.5%. No apparent tick infestations were observed on horses during the sampling time. However, potential tick vectors may be present in the sampled region. In our previous study the molecular prevalence of *T. equi* was 22.86% in horses in central Iran (Bahrami et al. 2014). Abedi et al. (2014) found 45% of horses on the north-eastern border of Iran to be infected with *T. equi*. Thus, overall, it has been demonstrated that *T. equi* is present in horses in Iran. Similar studies conducted in countries neighbouring Iran described *T. equi* prevalence rates among horses of 10.4% in Saudi Arabia (Alanazi et al. 2012), 77.1% in Kuwait (Donnelly et al. 1980b); 97.75% in Oman (Donnelly et al. 1980a), 12.8–16.21% in Turkey (Sevinc et al. 2008; Karatepe et al. 2009), 81.11% in Iraq (Al-Saad 2009) and 32.45% in the United Arab Emirates (Jaffer et al. 2010).

No significant differences in the frequency of *T. equi* infections were observed between various groups (age, sex). However, the odds of racehorses being infected with *T. equi* were approximately two times greater than in animals with reproductive activities. Since the farms participated in the present study had acceptable management the difference may be related to stress factors. Racehorses participate in high-intensity exercise when they are getting ready for a race. Heavy exercise is a well-known physical stressor that may temporarily compromise the immune system and immunocompromised animals have been shown to be more susceptible to infection compared with their immunocompetent counterparts (Hodgson, 2002; Sevinc et al. 2008). In the present study the odds of infection in equids reared without contact with cattle was 1.75 times greater than in animals kept in close contact with cattle although this difference was not significant. The *Rhipicephalus microplus* tick is known to transmit *T. equi* (Guimaraes et al. 1998; Ueti et al. 2008). Cattle are the primary host for *R. microplus*, and infestation of horses with this tick is dependent on the presence of cattle in the same area (Labruna et al. 2001). However, the proximity of equids to cattle was not associated with positivity for *T. equi* in this study, probably due to the fact that this was an enzootic stability area for this agent. Furthermore, other forms of transmission are possible, such as an iatrogenic route through infected blood (Tenter and Friedhoff 1986), transplacentally (Allsopp et al. 2007) as well as congenital transmission (Santos et al. 2008). In the current study we found significant regional differences in prevalence. The highest prevalence, 55.6%, was found in Ramhormoz followed by Abadan (39.1%) while the lowest prevalences were in Shushtar and Baghe-Malek, respectively. In some studies marked differences in positivity between geographical areas were attributable to differences in the management of the horses including their nutrition and tick control (Salim et al. 2008), host activity (Kouam et al. 2010), and differing climates (Moretti et al. 2010). Since all the farms participating in the present study provided comparable conditions for their horses, it is likely that other factors are responsible for these regional differences. Many studies have implied that there is a negative association between tick population and altitude. Shushtar (8.3%) and Baghe-Malek (15%) had the lowest infection rates and are the regions with the highest altitude studied here. Since at high altitude the climate may interfere with the life cycle of the tick vector, increasing altitudes could reduce the likelihood of horse infection.

In conclusion, equine theileriosis has the potential to be very disruptive to Iran's Persian Arab

horse industry and should remain a major concern to the Arab horse community and regulatory agencies.

REFERENCES

Abedi V, Razmi G, Seifi H, Naghibi A (2014): Molecular and serological detection of Theileria equi and Babesia caballi infection in horses and ixodid ticks in Iran. Ticks and Tick Borne Diseases 5, 239–244.

Alanazi AD, Alyousif MS, Hassieb MM (2012): Seroprevalence study on Theileria equi and Babesia caballi antibodies in horses from central province of Saudi Arabia. Journal of Parasitology 98, 1015–1017.

Alhassan A, Pumidonming W, Okamura M, Hirata H, Battsetseg B, Fujisaki K, Yokoyama N, Igarashi I (2005): Development of a single-round and multiplex PCR method for the simultaneous detection of Babesia caballi and Babesia equi in horse blood. Veterinary Parasitology 129, 43–49.

Allsopp MTEP, Lewis BD, Penzhorn BL (2007): Molecular evidence for transplacental transmission of Theileria equi from carrier mares to their apparently healthy foals. Veterinary Parasitology 148, 130–136.

Al-Saad KM (2009): Acute babesiosis in foals. Journal of Animal and Veterinary Advances 8, 2585–2589.

APHIS (2008): Equine piroplasmosis and the 2010 World Equestrian Games. www.aphis.usda.gov./vsnahss/equine/

Bahrami S, Ghadrdan AR, Mirabdollahi SM, Fayed MR (2014): Diagnosis of subclinical equine theileriosis in center of Iran using parasitological and molecular methods. Tropical Biomedicine 31, 110–117.

Bashiruddin JB, Camma C, Rebelo E (1999): Molecular detection of Babesia equi and Babesia caballi in horse blood by PCR amplification of part of the 16S rRNA gene. Veterinary Parasitology 84, 75–83.

Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A, Barba-Carretero JC (2003): Molecular studies on Babesia, Theileria and Hepatozoon in southern Europe. Part I. Epizootiological aspects. Veterinary Parasitology 113, 189–201.

De Waal DT (1992): Equine piroplasmosis: a review. British Veterinary Journal 148, 6–14.

De Waal DT, Van Heerden J (2004): Equine piroplasmosis. In: Coetzer JAW and Tustin RC, Editors, Infectious Diseases of Livestock, Oxford, Southern Africa, 425–434.

Donnelly J, Joyner LP, Graham-Jones O, Ellis CP (1980a): A comparison of the complement fixation and immunofluorescent antibody tests in a survey of the prevalence of Babesia equi and Babesia caballi in horses in sultanate of Oman. Tropical Animal Health and Production 12, 50–60.

Donnelly J, Joyner LP, Frank C (1980b): Quantitative epidemiological studies on the prevalence of babesiosis in horses in Kuwait. Tropical Animal Health and Production 12, 253–258.

Friedhoff KT, Tenter AM, Muller I (1990): Hemoparasites of equines: Impact on international trade of horses. Scientific and Technical Review of the Office International des Epizooties 9, 1187–1194.

Guimaraes AM, Lima JD, Ribeiro MF (1998): Sporogony and experimental transmission of Babesia equi by Boophilus microplus. Parasitology Research 84, 323–327.

Hailat NQ, Lafi SQ, Al-Darraji AM, Al-Ani FK (1997): Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. Veterinary Parasitology 69, 1–8.

Hodgson J (2002): Equine exotic diseases, a manual for horse owners. RIRDC Publication No. 02/054, Printed by Union Offset, Canberra, 47–49.

Jaffer O, Abdishakur F, Hakimuddin F, Riya A, Wernery U, Schuster RK (2010): A comparative study of serological tests and PCR for the diagnosis of equine piroplasmosis. Parasitology Research 106, 709–713.

Karatepe B, Karatepe M, Cakmak A, Karaer Z, Ergun G (2009): Investigation of seroprevalence of Theileria equi and Babesia caballi in horses in Nigde province, Turkey. Tropical Animal Health and Production 41, 109–113.

Kouam MK, Kantzoura V, Gajadhar AA, Theis JH, Papadopoulos E, Theodoropoulos G (2010): Seroprevalence of equine piroplasms and host-related factors associated with infection in Greece. Veterinary Parasitology 169, 273–278.

Kuttler KL (1988): World-wide impact of babesiosis. In: Ristic M (ed.): Babesiosis of Domestic Animals and Man. CRC Press, Boca Raton, Florida. 1–22.

Labruna MB, Kerber CE, Ferreira F, Faccini JL, De Waal DT, Gennari SM (2001): Risk factors to tick infestations and their occurrence on horses in the State of Sao Paulo, Brazil. Veterinary Parasitology 97, 1–14.

Moretti A, Mangili V, Salvatori R, Maresca C, Scoccia E, Torina A, Moretta I, Gabrielli S, Tampieri MP, Pietrobelli M (2010): Prevalence and diagnosis of Babesia and Theileria infections in horses in Italy: a preliminary study. Veterinary Journal 184, 346–350.

Salim BOM, Hassan SM, Bakheit MA, Alhassan A, Igarashi I, Karanis P, Abdelrahman MB (2008): Diagnosis of Babesia caballi and Theileria equi infections in horses in Sudan using ELISA and PCR. Parasitology Research 103, 1145–1150.

Santos TM, Santos HA, Massard CL (2008): Molecular diagnostic of congenital babesiosis in neonates foals from

State of Rio de Janeiro, Brazil (in Portuguese). Revista Brasileira De Parasitologia Veterinaria 17, 348–350.

Schein E (1988): Equine babesiosis. In: Babesiosis of Domestic Animals and Man. In: Ristic M (ed.): Babesiosis of Domestic Animals and Man. CRC Press, Boca Raton, Florida. 197–208.

Sevinc F, Maden M, Kumas C, Sevinc M, Ekici OD (2008): A comparative study on the prevalence of Theileria equi and Babesia caballi infections in horse subpopulations in Turkey. Veterinary Parasitology 156, 173–177.

Tenter AM, Friedhoff KT (1986): Serodiagnosis of experimental and natural Babesia equi and B. caballi infections. Veterinary Parasitology 20, 49–61.

Ueti MW, Palmer GH, Scoles GA, Kappmeyer LS, Knowles DP (2008): Persistently infected horses are reservoirs for intrastadial tick-borne transmission of the apicomplexan parasite Babesia equi. Infection and Immunity 76, 3525–3529.

Received: 2014–08–14
Accepted after corrections: 2014–10–07

Corresponding Author:

Somayeh Bahrami, Shahid Chamran University of Ahvaz, Faculty of Veterinary Medicine, Department of Parasitology, Ahvaz, Iran
E-mail: s.bahrami@scu.ac.ir