Turkish Journal of Veterinary & Animal Sciences

Volume 44 | Number 2 Article 25

1-1-2020

Detection of Trypanosoma evansi in camel abortions (Camelus dromedarius) in Iran using polymerase chain reaction

MOHAMMADREZA NAZEM

MARYAM KARIMIDEHKORDI

MEHRAN FARHOODI MOGHADAM

TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 358-363

doi:10.3906/vet-1908-76

Detection of *Trypanosoma evansi* in camel abortions (*Camelus dromedarius*) in Iran using polymerase chain reaction

Mohammad Reza NAZEM[1], Maryam KARIMI DEHKORDI[1,*], Mehran FARHOODI MOGHADAM[2]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Alborz, Iran

Received: 27.08.2019 • **Accepted/Published Online:** 19.02.2019 • **Final Version:** 06.04.2020

Abstract: *Trypanosoma evansi* is a blood parasite protozoan that causes trypanosomiasis or surra in a variety of economically valued animals such as cattle and camels. This infection causes abortion in camels. The present study was conducted to evaluate the presence of *Trypanosoma evansi* in aborted fetuses of Iranian camels (*Camelus dromedarius*) by polymerase chain reaction (PCR) method. In this study, 244 abomasal contents of aborted fetuses were collected from an Iranian camel herd in the east of Iran. The results showed that 41 of 244 (16.8%) aborted fetuses were infected with *Trypanosoma evansi* DNA. The results showed that a high percentage of abortions in Iranian camel herds were due to *Trypanosoma evansi* infection. There was a significant difference in positive *Trypanosoma evansi* DNA in aborted fetuses between sampling locations ($P < 0.05$).

Key words: *Trypanosoma evansi*, abortion, camel, Iran, polymerase chain reaction

1. Introduction

Trypanosoma evansi is a kinetoplastid hemoprotozoan that causes enormous economic losses [1,2] via a disease known as surra [3]. It occurs in different geographical areas and involves a large variety of animals (e.g., camels, domestic animals, buffalo, cattle, horses, and feral dogs) [1,4]. Its geographical core area is located in the north of the tsetse belt and it transferred to the Arabian Peninsula, Iran, and other Asian countries [5]. Hematophagous flies cause mechanical transmission, which leads to the transmission of the disease to other parts of the world [2]. It should be noted that vampire bats have been identified as carriers of the disease in South America [4,6]. *Trypanosoma evansi* occurs in acute and chronic forms because the suppression of the immune system causes secondary infections [7]. In the acute form, there are fatal parasites in the blood [8]. Clinical symptoms often vary in each region; thus, the diagnosis is difficult in the host [7,9]. The main symptoms include recurrent fever, anemia, emaciation and diarrhea, atrophy of the thigh muscles, lacrimation, corneal opacity, edema, abortions, and premature births. *Trypanosoma evansi* can cause low reproduction in herds of camels [4,10,11]. Although the mechanism of reproductive problems is not entirely explicit, intrauterine infection and the stress of infection are known as the causes of abortion [12,13]. Several methods have been used for the identification of *Trypanosoma evansi* in the fetus, among which PCR is recommended as a sensitive, appropriate, and accurate method [7]. This paper investigates the prevalence of abortion associated with *Trypanosoma evansi* using PCR in Iran.

2. Materials and methods

2.1. Sample collection

In this study, 244 abomasal contents of four-month aborted fetuses from camel herds were collected in the eastern provinces of Iran (Figure 1) between February 2013 and July 2015. All samples had only abomasal contents, which we collected under sterile hygienic conditions and transported immediately to the laboratory at 4 °C. All abomasal contents samples were kept at –20 °C until experiments. We also estimated the ages of the aborted fetuses [14].

2.2. DNA extraction

We collected 10 mL of abomasal contents of aborted fetuses by 16-G sterile needles from each sample. Genomic DNA was extracted from abomasal contents of aborted fetuses using a DNA isolation kit according to the instructions (CinnaGen, Iran). The concentration of DNA was determined by spectrophotometric measured at 260 nm optical density.

* Correspondence: ma_karimivet58@yahoo.com

Figure 1. Map of the sampling localities in Iran.

2.3. PCR procedures

In this study, oligonucleotide primers (F: 5'-GCGCGGATTCTTTGCAGACGA-3' and R: 5'-TGCAGACACTGGAATGTTACT-3') were used to determine the presence of the *ISG75* gene of *Trypanosoma evansi* in samples. PCR was performed on reaction mixtures of 25 µL containing 10X reaction buffer (Fischer Biotech), 1.5 mM $MgCl_2$, 200 µM of each of the four deoxynucleoside triphosphate (dNTPs) (Promega), primers at 1 µM, and 0.5 U of Taq DNA polymerase (Fischer Biotech). The cycles included an initial step at 94 °C for 4 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. PCR elongation was continued at 72 °C for 5 min. The amount of DNA template added per reaction was 3 µL [3]. A nontemplate control (water blank) and a positive control DNA from *Trypanosoma evansi* (ISG75 strain) were included in each PCR run. Amplification products were analyzed by electrophoresis through 1% agarose gel and stained with ethidium bromide.

2.4. Statistical analysis

The one-way analysis of variance (ANOVA) test was applied for the frequency of aborted fetuses in samples. $P < 0.05$ was considered statistically significant. These analyses were performed using SPSS 22 (IBM Corp., Armonk, NY, USA).

3. Results

The results showed that of 244 abomasal contents of aborted camel fetuses, 41 (16.8%) samples contained verified *Trypanosoma evansi* DNA. The primers used were derived from the *ISG75* gene of *Trypanosoma evansi*.

Agarose gel electrophoresis of positive samples revealed a 257-bp fragment. An example of a PCR amplification of samples is shown in Figure 2.

Table 1 shows the distribution of samples in different parts of Iran. The greatest number of abortions occurred in South Khorasan Province, while most of the positive *Trypanosoma evansi* DNA infections were observed in Sistan and Baluchestan (Table 2).

Table 3 demonstrates that due to *Trypanosoma evansi* infection, the age of aborted fetuses was between 4 and 12 months, while most of them were at the age of 8–9 months. The results show a high frequency of trypanosomiasis infection in aborted fetuses of Iranian camels.

4. Discussion

The reproductive problems of trypanosomiasis among female camels are abortion, weakness of newborns, and premature births [11]. However, there is incomplete information about the incidence of abortion [15]. In Iran, *Trypanosoma evansi* was diagnosed for the first time in 1932. Since then, the population of camels has been reported to be 160,000. *Trypanosoma evansi* is endemic in the world [16]. In many countries, trypanosomiasis is controlled and eradicated with surveillance and prevention [5], and it is sometimes accompanied by the mass slaughter of infected animals [17]. Although the PCR method is more sensitive and accurate than other methods in the identification of *Trypanosoma evansi* [7], it is recommended that several methods be simultaneously employed to identify *Trypanosoma evansi* [18].

Sazmand et al. [1], in a literature review, reported that the prevalence rates of *Trypanosoma* infections in camels were between 0% and 19.47%. In another study, Sazmand et al. [19] reported that *Trypanosoma evansi* was

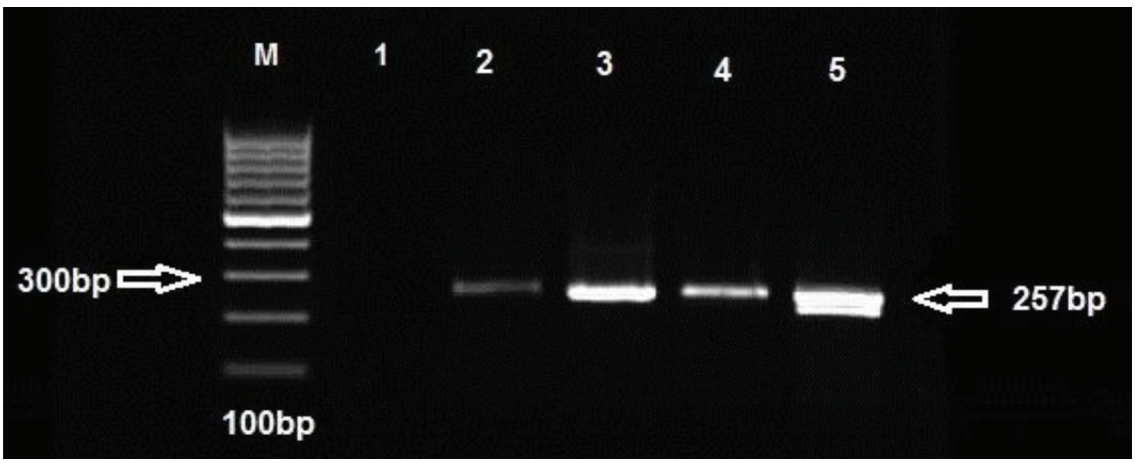

Figure 2. Gel electrophoresis for detection of trypanosomiasis infection in aborted fetus samples. Line M is molecular mass ladder (100 bp) from Fermentas, Germany; line 1 is PCR negative control; lines 2, 3, and 4 are positive samples; and line 5 is positive control (257 bp).

Table 1. The frequency of aborted fetuses in different provinces of Iran (%). *

Group	North Khorasan	Khorasan Razavi	South Khorasan**	Yazd	Kerman	Semnan	Sistan and Baluchestan	Fars
North Khorasan (13.1)[a,*]		13.9[a]	16.4[a]	8.6[a]	12.3[a]	9[a]	15.5[a]	11[a]
Khorasan Razavi (13.9)[a]	13.1[a]		16.4[a]	8.6[a]	12.3[a]	9[a]	15.5[a]	11[a]
South Khorasan (16.4)[a]	13.1[a]	13.9[a]		8.6[b]	12.3[a]	9[b]	15.5[a]	11[a]
Yazd (8.6)[a]	13.1[a]	13.9[a]	16.4[b]		12.3[a]	9[a]	15.5[a]	11[a]
Kerman (12.3)[a]	13.1[a]	13.9[a]	16.4[a]	8.6[a]		9[a]	15.5[a]	11[a]
Semnan (9)[a]	13.1[a]	13.9[a]	16.4[b]	8.6[a]	12.3[a]		15.5[b]	11[a]
Sistan and Baluchestan (15.5)[a]	13.1[a]	13.9[a]	16.4[a]	8.6[a]	12.3[a]	9[b]		11[a]
Fars (11)[a]	13.1[a]	13.9[a]	16.4[a]	8.6[a]	12.3[a]	9[a]	15.5[a]	

*Different letters in the same row indicate significant differences ($P < 0.05$).
**Greatest number of abortions.

Table 2. The frequency of *Trypanosoma evansi* DNA positivity in different provinces of Iran (%).

Group	North Khorasan	Khorasan Razavi	South Khorasan	Yazd	Kerman	Semnan	Sistan and Baluchestan**	Fars
North Khorasan (21.8)[a,*]		17.6[a]	20[a]	4.7[a]	16.6[a]	9.1[a]	26.3[a]	7.4[a]
Khorasan Razavi (17.6)[a]	21.8[a]		20[a]	4.7[a]	16.6[a]	9.1[a]	26.3[a]	7.4[a]
South Khorasan (20)[a]	21.8[a]	17.6[a]		4.7[a]	16.6[a]	9.1[a]	26.3[a]	7.4[a]
Yazd (4.7)[a]	21.8[a]	17.6[a]	20[a]		16.6[a]	9.1[a]	26.3[b]	7.4[a]
Kerman (16.6)[a]	21.8[a]	17.6[a]	20[a]	4.7[a]		9.1[a]	26.3[a]	7.4[a]
Semnan (9.1)[a]	21.8[a]	17.6[a]	20[a]	4.7[a]	16.6[a]		26.3[a]	7.4[a]
Sistan and Baluchestan (26.3)[a]	21.8[a]	17.6[a]	20[a]	4.7[b]	16.6[a]	9.1[a]		7.4[a]
Fars (7.4)[a]	21.8[a]	17.6[a]	20[a]	4.7[a]	16.6[a]	9.1[a]	26.3[a]	

*Different letters in the same row indicate significant differences ($P < 0.05$).
**Greatest number of *Trypanosoma evansi* DNA positivity.

Table 3. The frequency of *Trypanosoma evansi* aborted fetus groups according to the time (month).

Group	4–5	5–6	6–7	7–8	8–9	9–10
No. of *Trypanosoma evansi* DNA-positive samples	3	2	2	6	12*	7

*Greatest number of abortions due to *Trypanosoma evansi* were at 8–9 months.

present in 15.45% camel blood smears in Yazd province, Iran. Ahmadi-Hamedani et al. [20] also concluded that trypanosomiasis was present in 4.76% camels of Semnan, Iran. Khosravi et al. [21] indicated that *Trypanosoma evansi* was present in 2.1% camels of Rafsanjan, Kerman province, Iran.

In this study, DNA was successfully extracted from the abomasal contents of aborted fetuses. *Trypanosoma evansi* was detected in 41 (16.8%) of 244 samples aborted fetuses using the PCR method (Table 2). According to the results, most abortions occurred in South Khorasan (16.4%) while the highest percentage of *Trypanosoma evansi* was seen in Sistan and Baluchestan (26.3%). In the past, provinces such as South Khorasan, Razavi Khorasan, and North Khorasan were considered as affected areas. These results show that most positive cases were in the east of Iran, which could be due to the geographical location and the border with Afghanistan and Pakistan. Notably, the camel population is concentrated in the center and east of Iran.

In this study, aborted fetuses were between 4 and 12 months and the highest frequency was at 8 to 9 months of pregnancy, while previous studies found the highest rate in the last month of pregnancy. The results of this study were similar to reports from the Canary Islands in terms of abortion age (normal duration of pregnancy in dromedaries is 12.3 to 13.2 months) [15]. Thus, it seems that more studies are required to recognize the time of abortion.

The main laboratory finding of trypanosomiasis in dromedary camels is hemolytic anemia [15,22]. Researchers have also identified regenerative anemia, lymphocytic and monocytic leukocytosis, hyperproteinemia, and hypoglycemia with increased urea levels and decreased iron levels, although there was no report on its effects on the liver and kidneys [15]. As stated, most species can be infected with *Trypanosoma evansi* but they vary in sensitivity. For instance, horses are more sensitive [23]. Indian studies revealed that 34.4% of camels were *Trypanosoma evansi*-positive and showed a high prevalence of it [9]. In metropolitan France, an outbreak of trypanosomiasis was reported and melarsomine hydrochloride treatment controlled the disease successfully. Also, the animal's weight on the first day of treatment is important [18]. Studies showed that treatments with melarsomine at 0.5 mg/kg and also quinapyramine at 3.75 mg/kg were successful [17]. Due to the damage caused by abortion in camels, there is a need for control, prevention, and treatment of trypanosomiasis. In the control step, sick animals must be separated from others. Then wildlife and other animals close to camels must be examined [17]. Therefore, periodic testing of camel herds with improved diagnostic methods such as parasitology, ELISA, and PCR could eradicate the disease [18]. However, the results will be more significant if this process is accompanied by trypanocidal drugs. Examples of successful eradication have been reported in the Americas and Australia.

Today, the treatment of camels with *Trypanosoma evansi* is recommended by the World Organisation for Animal Health (OIE) and the Food and Agricultural Organization (FAO) [18]. Because the parasites stay in extravascular spaces such as the CSF, the most appropriate trypanocide is melarsomine hydrochloride, as at higher doses (0.5–1 mg/kg) only trypanocides can cross this barrier [18]. Before any massive treatment, identification of the sources and infected animals is necessary. For this purpose, massive treatment was performed in the Canary Islands for eradication and control [15]. According to the results of this study, *Trypanosoma evansi* is significantly responsible for abortion in Iranian camel herds (16.8%), which ultimately reduces their population. Therefore, researchers should continue monitoring, eradicating, and controlling the disease.

In conclusion, it is very important to control, treat, and monitor these animals. In order to maintain this major industry, regular meetings and consultations must be held by relevant agencies and private departments for improved diagnostic methods and treatment.

References

1. Sazmand A, Joachim A. Parasitic diseases of camels in Iran (1931-2017) - a literature review. Parasite (Paris, France) 2017; 24: 21-21. doi: 10.1051/parasite/2017024
2. Maharana BR, Tewari AK, Saravanan BC, Sudhakar NR. Important hemoprotozoan diseases of livestock: challenges in current diagnostics and therapeutics: an update. Veterinary World 2016; 9 (5): 487- 495. doi: 10.14202/vetworld.2016.487-495
3. Chansiri K, Khuchareontaworn S. PCR-ELISA for diagnosis of *Trypanosoma evansi* in animals and vector. Molecular and Cellular Probes 2002; 16 (3): 173-177. doi: 10.1006/mcpr.2002.0412
4. Chaudhary Z, Iqbal J. Incidence, biochemical and haematological alterations induced by natural trypanosomiasis in racing dromedary camels. Acta Tropica 2000; 77 (2): 209-213.
5. Desquesnes M, Bossard G, Patrel D, Herder S, Patout O et al. First outbreak of *Trypanosoma evansi* in camels in metropolitan France. Veterinary Record 2008; 162 (23): 750-752.
6. Bitome Essono PY, Dechaume-Moncharmont FX, Mavoungou J, Obiang Mba R, Duvallet G et al. Distribution and abundance of hematophagous flies (Glossinidae, Stomoxys, and Tabanidae) in two national parks of Gabon. Parasite (Paris, France) 2015; 22: 23-23. doi: 10.1051/parasite/2015023
7. Njiru Z, Constantine C, Ndung'u J, Robertson I, Okaye S et al. Detection of *Trypanosoma evansi* in camels using PCR and CATT/*T. evansi* tests in Kenya. *Veterinary Parasitology* 2004; 124 (3-4): 187-199.
8. Gatt-Rutter T. Diseases of camel. Protozoal diseases. Veterinary Bulletin 1967; 37: 611-618.
9. Ravindran R, Rao JR, Mishra AK, Pathak KML, Babu N et al. *Trypanosoma evansi* in camels, donkeys and dogs in India: comparison of PCR and light microscopy for detection–short communication. Veterinarski *Arhiv* 2008; 78 (1): 89-94.
10. Singh RP, Washishta M, Kala C. Diseases of Camels. Hyderabad, India: Seven Seas; 1980.
11. Yagil R. FAO Animal Production and Health Paper No. 26. Camels and Camel Milk. Rome, Italy: Food and Agriculture Organization of the United Nations; 1982.
12. Löhr K, Pholpark S, Siriwan P, Leesirikul N, Srikitjakarn L et al. *Trypanosoma evansi* infection in buffaloes in North-East Thailand. II. Abortions. *Tropical Animal Health* and *Production* 1986; 18 (2): 103-108.
13. Loses G, Ikede B. Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma brucei*, *Trypanosoma rhodesiense* and *Trypanosoma gambiense*. *Veterinary Pathology* 1972; 9 (1): 1-79.
14. Gazitúa FJ, Corradini P, Ferrando G, Raggi LA, Parraguez VCH. Prediction of gestational age by ultrasonic fetometry in llamas (*Lama glama*) and alpacas (*Lama pacos*). *Animal Reproduction Science* 2001; 66 (1-2): 81-92.
15. Gutierrez C, Corbera J, Juste M, Doreste F, Morales I. An outbreak of abortions and high neonatal mortality associated with *Trypanosoma evansi* infection in dromedary camels in the Canary Islands. *Veterinary Parasitology* 2005; 130 (1-2): 163-168.
16. Hoare CA. The Trypanosomes of Mammals. A Zoological Monograph. Oxford, UK: Blackwell Scientific Publications; 1972.
17. Gutiérrez C, Tamarit A, González-Martín M, Tejedor-Junco M. Control and eventual eradication of *Trypanosoma evansi* infection in dromedary camels after an episodic outbreak in mainland Spain: an example in a non-endemic area. *Veterinary Parasitology* 2014; 204 (3-4): 153-157. doi: 10.1016/j.vetpar.2014.05.004
18. Desquesnes M, Bossard G, Thévenon S, Patrel D, Ravel S et al. Development and application of an antibody-ELISA to follow up a *Trypanosoma evansi* outbreak in a dromedary camel herd in France. *Veterinary Parasitology* 2009; 162 (3-4): 214-220. doi: 10.1016/j.vetpar.2009.03.033
19. Sazmand A, Rasooli A, Nouri M, Hamidinejat H, Hekmatimoghaddam S. Serobiochemical alterations in subclinically affected dromedary camels with *Trypanosoma evansi* in Iran. *Pakistan Veterinary Journal* 2011; 31 (3): 223-226.
20. Ahmadi-Hamedani M, Ghazvinian K, Darvishi MM. Hematological and serum biochemical aspects associated with a camel (*Camelus dromedarius*) naturally infected by *Trypanosoma evansi* with severe parasitemia in Semnan, Iran. Asian Pacific Journal of Tropical Biomedicine 2014; 4 (9): 743-745.

21. Khosravi A, Hakimi Parizi M, Bamorovat M, Borhani Zarandi M, Mohammadi MA. Prevalence of *Trypanosoma evansi* in camels using molecular and parasitological methods in the southeast of Iran. *Journal* of Parasitic *Diseases* 2011; 39 (3): 422-425.

22. Yagoub I. Hematological studies in dromedary camels with single or concurrent natural infections of *trypanosoma-evansi* and haemonchus-longistipes. *Acta Veterinaria* 39 (2-3): 109-119.

23. Birhanu H, Fikru R, Said M, Kidane W, Gebrehiwot T et al. Epidemiology of *Trypanosoma evansi* and *Trypanosoma vivax* in domestic animals from selected districts of Tigray and Afar regions, Northern Ethiopia. Parasites & Vectors 2015; 8: 212-212.