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Original papers

Molecular evidence of *Trypanosoma evansi* infection in Iranian dromedary camel herds

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ABSTRACT. *Trypanosoma evansi* is a pathogenic African animal protozoan, affecting livestock and wild animals worldwide and Iran. The present study was carried out to detect the infection of *T. evansi* in one-humped camels (*Camelus dromedarius*) of southeastern Iran. Over six months, a total numbers of 370 dromedary camels were randomly selected from three sub-areas located in southeastern Iran in 2015. Blood samples were taken from jugular vein and examined by using micro-hematocrit centrifugation (MHCT) and PCR techniques. Genomic DNA was extracted and PCR was performed to amplify a fragment of the mini-chromosome satellite DNA TBR1/2 of *T. evansi*. The overall prevalence was 31.35% (116/370). The highest *T. evansi* infection was significantly in adult camels (24.05%, 89/370) with chronic clinical signs (11.89%, 44/370). There was significant difference between prevalence and sex (9.46% male and 21.89% female). Only 19.19% (71 out of 370) of the infected camels were from the plain areas. The highest *T. evansi* infection rate was significantly recorded in the camels of north (19.19%, 71/370) part of the region. The molecular analysis was uncovered high level of infection with *T. evansi* in camel herds which can help to establish effective control programs in the region.

Key words: *Trypanosoma evansi*, camel, PCR, Iran

Introduction

Cameline surra disease, also called Tibersa is an animal pathogenic protozoan infection throughout the world and Iran, caused by *Trypanosoma* (*T.*) *evansi* subspecies or even strains of *T. brucei* with no intermediate hosts and insect developmental cycle [1]. The parasite mechanically is transmitted by biting flies of the genus *Tabanus*, *Stomoxys*, *Haematobia* sp., *Lyperosia* and vampire bats in South America [2]. This pathogenic animal trypanosome affects cattle, buffaloes, camelids (*Camelus dromedarius* and *C. bactrianus*), sheep, goats, horses, donkeys, mules, pigs, cats and dogs throughout the world [2–7]. Surra is basically a disease of camelids (*Camelus dromedarius* and *C. bactrianus*) and equids. In Africa, *T. evansi* is a parasite of camelids as definitive host and reservoir. Recently, human infections have been reported in India making it as zoonoses. Humans are normally resistant to the infection, as well as to infection with the African trypanosomes [8–9].

During the past decades, several parasitological examinations were the frequent methods to detect *Trypanosoma* infection. However, the sensitivity and/or specificity of these methods were low because of difficulties in detection and differentiation of *Trypanosoma* infection during the chronic stage of the disease, in particular when level of parasitemia was low. Recently, DNA-based techniques have been developed for detection of *Trypanosoma* infection with low level of parasitemia in chronic stage. Molecular tools like conventional PCR especially are useful when large number of animals need to be sampled during field studies [10–13].

Camel husbandry has important role in southeastern Iran and are considered as multipurpose animals. According to Iranian Veterinary Organi-

zation (I.V.O., 2015), an average population of 162,371 camelids distributed over 10 provinces in many flocks and camel-raising areas. Approximately 33.8% of this population exists in Sistan-va-Baluchestan province. Camel trypanosomosis is a problem in camel husbandry and the main factor for the decline in production and economic losses [18]. The first occurrence of *T. evansi* infection reported in Iranian livestock by Rafiee [14]. The traditional parasitological methods were previously employed in Centre (3.96–15.45%) and South (1.6–2.1%) parts of the country [15–23], while there was only one molecular report [24]. Thus the present study was aimed to investigate molecular evidence of *T. evansi* infection in one-humped camels (*Camelus dromedarius*) of southeastern Iran.

Materials and Methods

Study area. The area of study was in Sistan-va-Baluchestan Province where located in southeastern Iran (25°3'–31°28' in latitude and 58°47'–63°19' in longitude) about 1300 m above sea level and inclusive two parts of Sistan (plain area, 8117 km^2) and Baluchestan (mountainous area,179,385 km^2) [18]. Ecologically, this area is classified as a warm and dry zone with average annual maximum and minimum temperature of 40°C and 12–13°C, respectively. Annual precipitation varies between 51mm in the north and 121mm in south.

Animals. Over six months, a total numbers of 370 Iranian one-humped camels (55.68% male and 44.32% female) were randomly selected using cluster sampling method from suburban of 13 municipalities (Fig. 1). The data pertaining to the camels, *i.e*. age, sex, breed, management system, daytime, tag number and geographic distribution recorded. The camels were indigenous and raised following traditional practices. The animals were also divided in three age groups, namely camel calves (less than 2 years-old), young (2–4 years-old) and adult (more than 4 years-old); based on the owner information and eruption of permanent incisor teeth [25] (Table 1). All camels were also subjected for a clinical examination including general body condition and clinical signs. The place of study were divided into three sub-areas, *i.e*. north (n=168), center (n=84) and south (n=118) (Table 1). According to the owners, there were no prophylactic treatments against protozoa infection.

Sample processing. The blood samples were taken from jugular vein using venipuncture and aliquot in 5 ml tube with EDTA (ethylene-diaminetetra acetate) for whole blood examination and 5 ml coated tube for molecular examination.

Micro-hematocrit centrifugation technique (MHCT). The collected blood in capillary tube (coated with EDTA) was subjected for MHCT as described by Woo [26] and Karimi et al. [17]. Briefly, 70 µl of blood was centrifuged at 10,000 rpm for 5 minutes and observed for *Trypanosoma* parasitemia at the buffy coat layer under light microscope at magnification of 400–1000×.

Molecular procedures. DNA extraction. Genomic DNA was extracted from stored EDTA blood using a commercial kit according to the manufacturer's instructions. Each blood sample subjected for digestion by using lyses buffer (4 M sodium chloride, 10 mM tris-HCL, 2 mM EDTA, 20 mg/ml proteinase K) at 55°C for 10 minutes. Isopropanol (100 µl) was added and mixed. The genomic DNA was collected and stored at -20°C until PCR analysis.

Molecular procedures. Polymerase chain reaction (PCR). The conventional PCR employed to amplify a 164 bp fragment of the highly repeated sequence of mini-chromosome satellite DNA TBR1/2 of *T. evansi* in a Bioer XP thermal cycler described by Pruvot et al. [27]. Briefly, PCR performed in 25 µl reaction mixture containing 2 µl (100 ng) of genomic DNA (diluted 1:30), 1.5U of *Taq* DNA polymerase (Fermentas, Germany), 50 mM of each dNTPs (CinnaGen, Iran), 2 mM of MgCl2, 2.5 µl of PCR reaction buffer (10×) and 0.2 µM of the specific primers TBR1 (5'-GAATATTAAACAAT GCGCAG-3') and TBR2 (5'-CCATTTATTAGCT TTGTTGC-3') [5]. In each PCR reaction, mixture with and without genomic DNA of *T. evansi* was used as positive and negative controls, respectively. The samples were subjected for an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, and a final extension step at 72°C for 5 min. PCR products analysed by electrophoresis on 1.5% agarose gel stained with ethidium bromide (1 µg/ml).

Statistical evaluation. Data were analysed by using non-parametric Chi-square ($\chi 2$) test with a confidence interval of 95% (SPSS 16.2, SPSS Inc., Chicago, IL, USA). Probability of ≤ 0.05 was regarded as significant.

Table 1. The prevalence of *Trypanosoma evansi* infection in Iranian camels based on sex, age, and geographic distribution in examined camels (n=370)

		No. of examined animals	Prevalence (n/N, %)	
			MHCT	PCR
Age	Calf	27	0 (0.0)	5 (1.35)
	Young	90	0 (0.0)	22 (5.95)
	Adult	253	44 (11.89)	89 (24.05)[a]
Sex	Male	156	14 (3.78)	35 (9.46)
	Female	214	30 (8.11)	81 (21.89)[b]
Studied sites	North	174	18 (4.86)	71 (19.19)[c]
	Center	89	8 (2.16)	28 (7.57)
	South	107	18 (4.86)	17 (4.59)
Total		370	44 (11.89)	116 (31.35)

Explanations: MHCT – Micro-hematocrit centrifugation technique; n – animals infected with *Trypanosoma evansi*; N – total examined animals; [a]χ^2=14.082, *P*=0.001; [b]χ^2=19.215, *P*=0.001; [c]χ^2=18.446, *P*=0.001

Results

Clinical and molecular findings

A total of 44 out of 370 examined camels (11.89%) were positive for *T. evansi* infection using MHCT. While, based on molecular findings, prevalence of *T. evansi* infection was 31.35% (116/370). The infection was varied from 1.35% to 24.05% in different age groups. The highest prevalence was significantly found in adult camels (24.05%, 89/370) with chronic clinical signs (11.89%, 44/370), *i.e.* fever, epiphora, anemia, edema and debility (χ^2=14.082, *P*=0.001). There was significant difference between prevalence and sex (9.46% male and 21.89% female) (χ^2=19.215, *P*=0.001) (Table 1).

Geographic distribution of the infection

The infected camels were geographically observed in all three studied sites during the course of the study (Table 1). The highest and lowest prevalence was significantly found in north (19.19%, 71/370) and south (4.59%, 17/370) of the province, respectively (χ^2=18.446, *P*=0.001) (Table 1, Fig. 1). Only 19.19% (71/370) of the infected camels were from the plain areas, while the remaining infected camels (20.05%, 89/370) were found in the camels of mountainous area (Table 1).

Discussion

Cameline surra disease is a cosmopolitan hemoprotozoan infection and serious problem in camel husbandry and is a major threat to productivity and economic losses [13]. In Iran, the first cases of camel disease were recorded in 1979 and so far there were many reports on prevalence and economic losses [4,16,22,27,28]. Camel trypanosomosis may be introduced into Iran from Pakistan bordering southeastern Iran [18]. According to Zarif-Fard et al. [23] the prevalence of *T. evansi* infection in Iranian camelids was 10%. In this work, molecular and MHCT findings indicated overall prevalence was much higher than those reported from South (1.6–4.76%) [21,28,29], South-East (19.47–25.75%) [18,30] and Center (14–15.45%) parts of Iran [19,22,28] and other parts of the worlds [31–37]. The prevalence of *T. evansi* infection also reported in other neighbors countries of Iran, *i.e.* Oman, Iraq, the United Arab Emirates, and Pakistan [18,38]. In other countries, Sing et al. reported the prevalence was 20.80% in examined camels of rural parts [13]. While it was much lower than that reported from camels (80%) by Berlin et al. [4]. The main reasons of these differences may be due to semi-desert nature of camels, difficulties in blood sampling, sample size, sensitivity of diagnostic methods, feeding and herd management, awareness towards

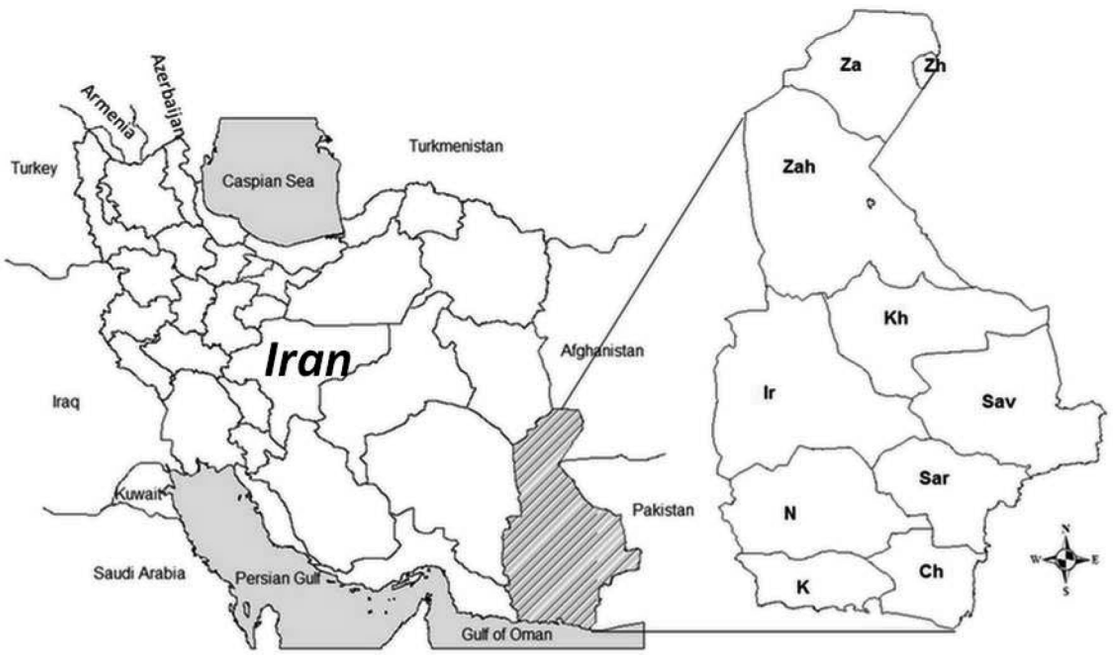

Fig. 1. Map of sampling sites in Sistan-va-Baluchestan Province, southeastern Iran (North: Zabol (Za), Zahak (Zh), Zahedan (Zah); Center: Iranshahr (Ir), Khash (Kh), Saravan (Sav), South: Chabahar (Ch), Konarak (K), Nikshahr (N), Sarbaz (Sar)).

chemoprophylaxis, and availability of medicines [13,24,39].

The risk of infection significantly increased in adult (>4 years-old) and female camels. In

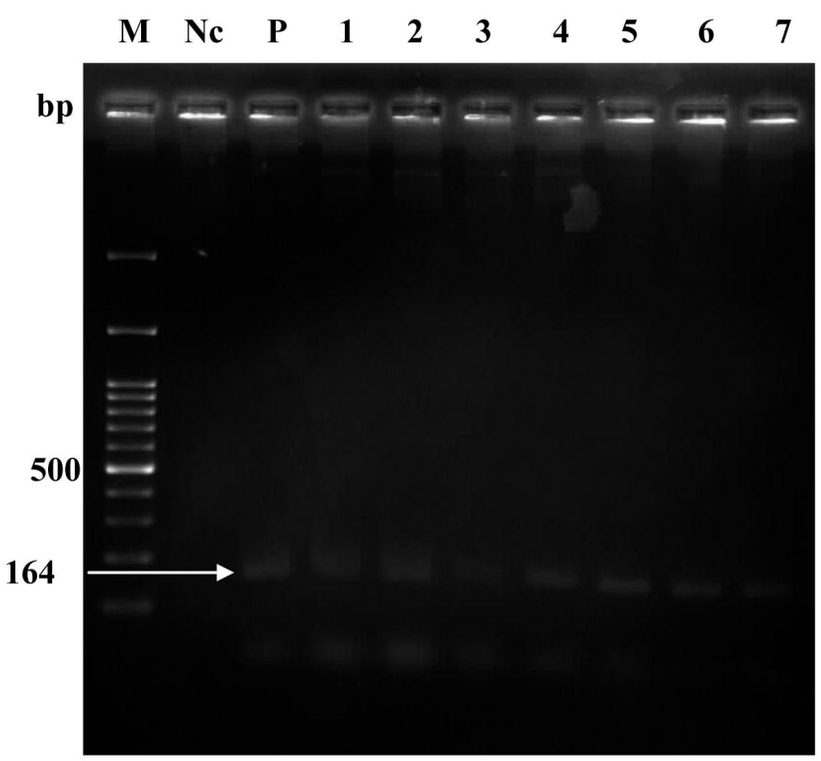

Fig. 2. Agarose gel electrophoresis (1.5%) of the PCR products representing the infection of *Trypanosoma evansi* in Iranian one-humped camels (Lanes 1–7, arrow head). Lane Nc: Negative control, Lane P: Positive control, Lane M: 100bp DNA size marker.

epidemiological studies, low prevalence in camel calves were frequently noted [31–33]. While, the highest infection reported in female dromedary camels in Iran and elsewhere [18–20,24,39]. Sing et al. [13] noted the highest incidence of *T. evansi* infection was in female camels (24.34%) up to 5 years-old (21.74%). The influence of sex may be related to the stress of lactations and successive pregnancies in female infected camels [20]. In addition, associated risk factor of age for adult infected camels may be due to more sensitivity of old camels and heavy stress through their use for transportation of goods with poor management, lack of maternally transferred immunity, being the fresh lot in the herd, more exposure to the source of infection like heamatophagous bites [13,39].

Surra disease is basically a disease of camelids with chronic clinical signs [40]. The fatality rate may reach up to 100% in untreated camels [15]. In this work, the highest prevalence of *T. evansi* infection was significantly in adult camels with chronic clinical signs. The high rate of asymptomatic infection of *T. evansi* in the examined camels was in line with other studies elsewhere [4,5,38,41,42].

During the course of the study, *T. evansi* infection was geographically found in all studied subareas with the highest prevalence in north part of the region. This finding was in accordance with Mirshekar et al. [18]. There is no exact reason why *T. evansi* infection was the highest in north part of the region. However, it is important to know that the investigated regions were difficult to access because of the lack of an adequate transport network, so that camel carriers could not easily access to veterinary services [18].

From the results of the current study, it was concluded that *T. evansi* infection was widely prevalent in southeastern Iran. Furthermore the results provided a baseline for more investigations and lunch control programs against *Trypanosoma* infection in camels of the region**.**

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