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ORIGINAL ARTICLE

Prevalence of *Trypanosoma evansi* in camels using molecular and parasitological methods in the southeast of Iran, 2011

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Abstract Surra is caused by infection with the protozoal parasite, *Trypanosoma evansi*. This parasite was transmitted mechanically by biting flies which is widespread in camels in the world. The aim of this study is to determine the prevalence of *T. evansi* in camels in Rafsanjan, Kerman province, southeast of Iran. In this study, 95 suspected camels were randomly selected in 2011. Blood samples were taken from deep blood vessels. Thin and thick blood smears were prepared in laboratory. Blood smears were stained by Giemsa and studied under a light microscope. The positive blood samples were also used for further molecular analysis. Data were analyzed using SPSS 17.0 software and $P \leq 0.05$ was considered as statistical difference. A total of 95 camels were examined for infection with *T.evansi* using parasitological and molecular methods. The overall prevalence of infection was 2.1 %. It was found that the frequency of infection was significantly higher ($P < 0.05$) in age group >6 years old than the corresponding younger camels. However, there was no significant difference when the gender was considered. PCR technique confirmed the two infected cases were *T. evansi*. Results of the present study indicated that surra is present in Rafsanjan county, Kerman province in an infection rate of 2.1 % in camels. To our knowledge, this is the first study reported from this province. Further investigations are needed to focus on vectors and to evaluate the risk factors.

Keywords *Trypanosoma evansi* · Camel · Surra · Iran

Introduction

Camel trypanosomiasis known as surra, is a disease of camels caused by *Trypanosoma evansi*, the family of Trypanosomatidae, with high mortality rate (Egbe-Nwiyi and Chaudhry 1994; Pacholek et al. 2000). *Trypanosoma evansi*, has a wide range of geographic distribution and affects a broad range of domestic animals, including camels, equines, cattle, dogs, buffaloes, small ruminants, carnivores and pigs. Transmission of *T. evansi* is mechanically by haematophagous flies mainly of the genus *Tabanus* (Brun et al. 1998; Chaudhary and Iqbal 2000; Losos 1980). The disease occurs in Asia, Africa, South and Central America and causes significant economic damages (Reid 2002).

The form of infection is generally chronic with periodic increase in body temperature, anorexia, depression, pale mucous membranes, dullness, a very thin hump and drop to one side, abortions in pregnant females, and death in untreated camels (Esievo and Saror 1991; Logan-Henfrey et al. 1992; Murray and Dexter 1988).

Several methods have been developed for detection of *T. evansi* infection. One of the laboratory methods for detection of this parasite is achieved by direct microscopic smear preparations of blood; however, this method has a poor sensitivity. Also, polymerase chain reaction (PCR)

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has been widely applied with infinitely high positive predictive values.

In some of the provinces of Iran such as Fars, Bushehr and Yazd, there are the reports of surra in camels (Moghaddar and Diantpour 2009; Sazmand et al. 2011; Zarif-Fard and Hashemi-Fesharki 2000). The aim of this study was to determine the frequency of *T. evansi* in camels by direct microscopic smears in Rafsanjan, Kerman province, southeastern Iran. The positive blood samples were also employed for further molecular analysis.

Materials and methods

Sampling

The survey was carried out during February to December in 2011. Blood samples were taken from 95 randomly selected camels in Rafsanjan county (29° 45′N, 54° 50′–56° 45′E), Kerman provinces in southeast of Iran. A questionnaire was completed for each camel, recording sex, age and clinical signs. Blood samples were taken from the jugular vein from each camel using 5 ml disposable syringes. The blood samples were kept in ice and processed on the day of collection. The blood samples with EDTA were used for parasitological diagnosis and PCR experiments.

Thin and thick smears

Two drops of the freshly collected blood were placed on a clean slide. One drop of blood was spread all around the top quarter of the slide making thick smear. The second drop was placed into the middle of the same slide and spread gently using another slide to make a thin smear. The slides were marked and allowed to dry. Thin smears were fixed with absolute methanol. Both thin and thick smears were stained by Giemsa based on standard procedures and studied under a light microscope. The positive blood samples were also employed for further molecular analysis.

Molecular technique

DNA extraction

The positive blood samples were also employed for further molecular analysis. DNA was prepared from direct smears. Smear scrapings were transferred to 1.5 ml micro tubes and centrifuged three times in physiological saline solution. DNA was extracted by proteinase K using the high pure PCR template purification kit (Roche, Germany), according to the manufacturer's instructions.

DNA amplification

The primer sequences used were as follows: nuclid repeat primer 1- (NRP1): 5′-CGAATGAATATTAAACAATGCG CAGT-3′ nuclid repeat primer 2- (NRP2): 5′ AGAACCA TTTATTAGCTTTGTTGC-3′ (Artama et al. 1992). PCR was performed by incubating the samples at three temperatures corresponding to three steps (denaturation, annealing and extension) in a cycle of amplification. DNA amplification were performed using Taq DNA Polymerase 2× Master Mix RED (Ampliqon-Biomol, Hamburg, Germany) denaturation step at 94 °C for 10 min, followed by 35 cycles consisting of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C. The last extension step was 10 min longer. The PCR products were visualized by electrophoresis using a 2 % agarose and ethidium bromide.

Statistical analysis

Data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). χ^2-test was used to compare the relative frequency of infection among different groups by age, sex and clinical signs. $P \leq 0.05$ was considered as statistical difference.

Results

A total of 95 camels consisting of 52 (54.7 %) and male 43 (45.3 %) were examined for infection with *T. evansi* using parasitological and molecular methods (Table 1). The overall prevalence of infection was 2.1 % (two cases). Infected camels showed progressive anaemia, cachexia, dullness, and marked depression and there was a direct relationship between the frequencies of infection in camels with clinical manifestations.It was found that the frequency of infection was significantly higher ($P < 0.05$) in age group <6 years old than the corresponding younger ones (>6 years) (Table 2). In contrast, there was no significant difference among the infected camels, when the gender was considered. The extracted DNA of 2 isolates displayed a fragment of 177 bp, corresponding to that of *T. evansi* (Fig. 1).

Discussion

Surra is a major veterinary disease with a serious morbidity and mortality among camels in Africa, Asia and South America including Iran (Enwezor and Sackey 2005). This study is a detailed investigation of the first surra report in camels in Kerman province, Iran.

Table 1 Prevalence of *Trypanosoma evansi* in camels in the southeast of Iran by sex, 2011

Sex	No.	(%)	Rate of infection (%)
Male	43	45.3	1 (50)
Female	52	54.7	1 (50)
Total	95	100	2 (2.1)

Table 2 Prevalence of *Trypanosoma evansi* in camels in the southeast of Iran by age, 2011

Age	No.	(%)	Rate of infection (%)
0–1	25	26.3	0
>1–3	21	22.1	0
>3–6	35	36.8	0
>6	14	14.7	2 (2.1)
Total	95	100.0	2 (2.1)

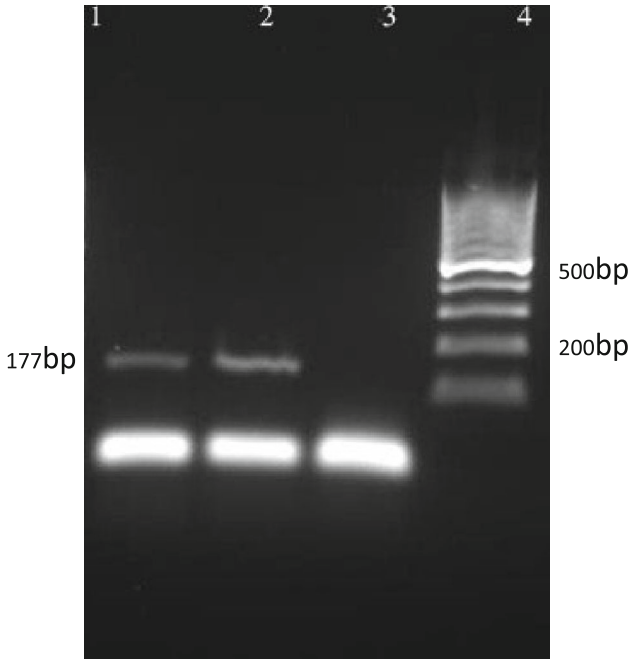

Fig. 1 Agarose gel electrophoresis of *Trypanosoma evansi* isolates. *Lane 1,2*; *T.evansi* isolates obtained in camels from Rafsanjan county, Kerman province, south-eastern Iran.; *lane 3*, negative control; *lane 4*, DNA size marker (Thermo Scientific Gene Ruler 100 bp DNA Ladder #SM0241)

The prevalence of *T. evansi* recorded during this study was comparable with earlier reports from Iran. However, previous studies from Yazd province (north of Rafsanjan county) and Fars province (west of Rafsanjan county) have reported higher prevalence of *T. evansi* (15.45 and 14 %, respectively) (Moghaddar and Diantpour 2009; Sazmand et al. 2011). Although, Kerman province is adjacent to these provinces, the frequency of infection was lower than those reported from Yazd or Fars province. Due to difficulties in working with camels, mainly because of semi-desert nature of them and blood sampling, the number of animals was limited.

The present study showed that the prevalence of camel trypanosomosis was higher in adults compared to the young camels. This finding is consistent with that found in Morocco, Canary Islands, Mauritania, Ethiopia and Iran (Atarhouch et al. 2003; Dia et al. 1997; Gutierrez et al. 2000; Moghaddar and Diantpour 2009; Tadesse et al. 2012). This is mainly due to heavy stress through their use for transportation of goods of larger scale movement and poor management, which increases the risk of infection, by the adult camels than the younger animals. Another possible explanation for such a higher infection in old camels than the younger ones could be due more exposure to the source of infection, therefore at higher risk of heamatophagous bites (Bhutto et al. 2010; Delafosse and Doutoum 2004).

According to study of Artama et al. (1992) that showed *T. brucei* and *T. evansi* had the same size band (177 bp) in the PCR method and if you increased the extension time you will also find longer band which is derived from template DNA of both of them, but due to host and *T. evansi* was the only species identified in Iran, in our investigation *T. evansi* verified.

In conclusion, results of the present study indicated that surra is present in Rafsnajan county, Kerman province and infection rate is 2.1 %. To our knowledge, this is the first survey reported from Kerman province. Further studies are needed to focus on vectors and to evaluate the risk factors for planning future control programs.

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