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EPIDEMIOLOGICAL STUDIES OF *T. EVANSI* IN CAMELS IN SAUDI ARABIA USING REAL TIME POLYMERASE CHAIN REACTION TECHNIQUE

(With One Table and Two Figures)

By

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دراسات وبائية على التريبانوسوما ايفانساى في الإبل بالمملكة العربية السعودية باستخدام تفاعل البلمرة المتسلسل ذو الزمن الحقيقي

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مرض التريبانوسوما (سارا) في الجمال ما زال يسبب مشاكل على مستوى العالم. ويصاحبه خسائر اقتصادية في المناطق التي تنتشر فيها تربية الجمال. ونظرا لقلة البيانات المنشورة عالميا باستخدام تفاعل البلمرة المتسلسل ذو الزمن الحقيقي كاختبار سريع للكشف عن طفيل التريبانوسوما ايفانساى في الجمال. فقد استهدفت هذا الدراسة استخدام اختبار البلمرة المتسلسل ذو الزمن الحقيقي للكشف عن الحامض النووي الخاص بطفيل التريبانوسوما ايفانساى وذلك باستخدام بادئ تتابع وراثي خاص بهذا الطفيل باستخدام صبغة السيبر الأخضر الفلوريسنت. حيث تم جمع عدد 242 عينه دم من جمال يحتمل إصابتها بالتريبانوسوما ايفانساى من محافظات مختلفة من السعودية (الرياض- القصيم- الخرج – الدوادمى) وبإجراء الاختبار كانت نقطة التعاظم للعينات الايجابية للإصابة بطفيل التريبانوسوما ا ايفانساى تظهر اتحادها عند درجة حرارة 85 درجة مئوية والذي ينطبق مع عينات ايجابية ضابطة لطفيل التريبانوسوما ايفانساى. وقد وجد آن عدد 102 عينه ايجابية من عدد 242 عينة وذلك بنسبه 42.15% ، كما أشارت الدراسة إلى مدى دقة هذا الاختبار للكشف عن التريبانوسوما ايفانساى في الجمال وتعد هذه أول دراسة وبائية للتشخيص الجزيئي لطفيل التريبانوسوما ايفانساى في الجمال في السعودية والتي تعكس مدى انتشار هذا الطفيل بين الجمال في المناطق المختلفة بالمملكة مما يستوجب مزيدا من الدراسات لمحاولة استكشاف مدى احتمال وجود أنواع أخرى من هذا الطفيل وكذلك محاولة السيطرة على الإصابة بهذه الطفيليات بالمملكة.

SUMMARY

Trypanosomasis (surra) still a serious problem in camel husbandry world widly. It causes considerable economic losses in many camel-rearing regions. A little date for Real Time polymerase chain reaction (PCR) for rapid detection *Trypanosoma evansi* of blood from naturally infected camels were puplished. In the present study 242 blood samples from clinically suspected camels to surra from four province of Saudia (Al-Riyadh, Al- Qassim, Alharig, and Al-Dawadmi) were collected and used for further examination. Real Time PCR-based assay for blood samples were conducted in direct detection of *T. evansi* DNA from the collected blood samples using specific primer set (TR3/TR4) derived from nuclear repetitive gene of *T.evansi* and SYBER Green1 fluorescent dye have been used. The melting peak chart of the positive samples showed one single peak at an average Tm of 85.0^{o}C. The performed real time PCR technique was found accurate method for diagnosis of trypanosoma infection of camels where; 102 (42.15%) out of 242 were positive. It is concluded that camel trypanosomiasis in Sudia apparently caused by the present single parasite species *T. evansi* and detection of other typanosomes species are recommonded. In addition, the disease is highly prevalent in the country, which strengthens the need to change control policies and institute measures that help prevent the spread of the parasite. To our knowledge, this is the first molecular diagnosis report, which gives a picture of camel *trypanosomiasis* covering large geographical areas in Saudi Arabia.

Key words: *Camels, Real time PCR, SYBER Green1, Trypanosoma evansi,.*

INTRODUCTION

Trypanosomasis (`surra') caused by *Trypanosoma evansi* is considered to be one of most important diseases of camels in KSA. *Trypanosoma evansi* is the most widely geographically distributed pathogenic trypanosome. It can infect several species of animals, including, camels, horses, cattle and buffaloes causing trypanosomosis commonly known as surra (Luckins, 1988). *Trypanosoma evansi* (*T. evansi*), the cause of trypanosomiasis (Surra), constitutes one of the major veterinary problems worldwide. The disease causes significant morbidity and mortality in camels (Pathak, and Khanna, 1995; Omer *et al.,* 2004). Surra, manifests itself usually as a chronic infection characterized by weight loss, anemia, infertility and abortion (Lohr *et al.,* 1986; Luckins, 1988). *Trypanosoma evansi* is a blood-borne

parasite that is transmitted mechanically by the bites of haematophagous flies. Initially, trypanosomes may be seen readily in the bloodstream, but in chronic infections parasites are difficult or impossible to find (Luckins, 1988) and in chronic infections is likely to be associated with secondary infections due to immunosuppression (Njiru *et al.,* 2004). Clinical signs and pathological lesions caused by *T. evansi* in camels are unreliable for definitive diagnosis (Chaudhary and Iqbal, 2000) in addition, detection of parasites in the blood is difficult because parasitaemia is intermittent (Mahmound and Gray, 1980). Serological tests have been developed and evaluated for diagnosis of trypanosomiasis in camels. They include card agglutination test and enzyme-linked immunosorbent assay (Ab-ELISA) (Nantulya, 1994; Davison *et al.,* 1999). In general serological techniques are useful for detection of a past infection but not for detection of an active infection with *T. evansi* (Viseshakul and Panyim 1990; Hopkins *et al.,* 1998). To address these problems; Real time polymerase chaine reaction technique have been developed and evaluated for detection of *T. evansi* directly from blood (Wuyts *et al.,* 1995; Omawa *et al.,* 1999; Imadeldin *et al.,* 2006; Yousef *et al.,* 2010). In Saudia, camels a principal mean of meat and milk production, they are used for transportation of crops and do other farm works; also, their hair, wool and hides are used. Trypanosomosis in camels causes considerable economic losses due to a decrease in milk and meat, premature births and abortions (Boid *et al.,* 1985). In-sight the aim of this study was planed to explore the rate of *T. evansi* among camel population in giving four different provinces in KSA by the most resent molecular technique; real time PCR as a preliminary step for designing rational trypanosomiasis control program.

MATERIALS and METHODS

Animals

This study was Realized from May 2010 to April 2011; a total number of 242 suspected clinical camels (*Camelus dromedarius*), 3-8 years old, were examined clinically: body temperature, mucous membrane, muscles of thigh and hump. These animals were located in four province in Saudia (Al- Riyadh, Al- Qassim, Alharig and Al-Dawadmi). The main complaints of the camel owners were loss of appetite, decrease of productivity, and reduce of the body weight.

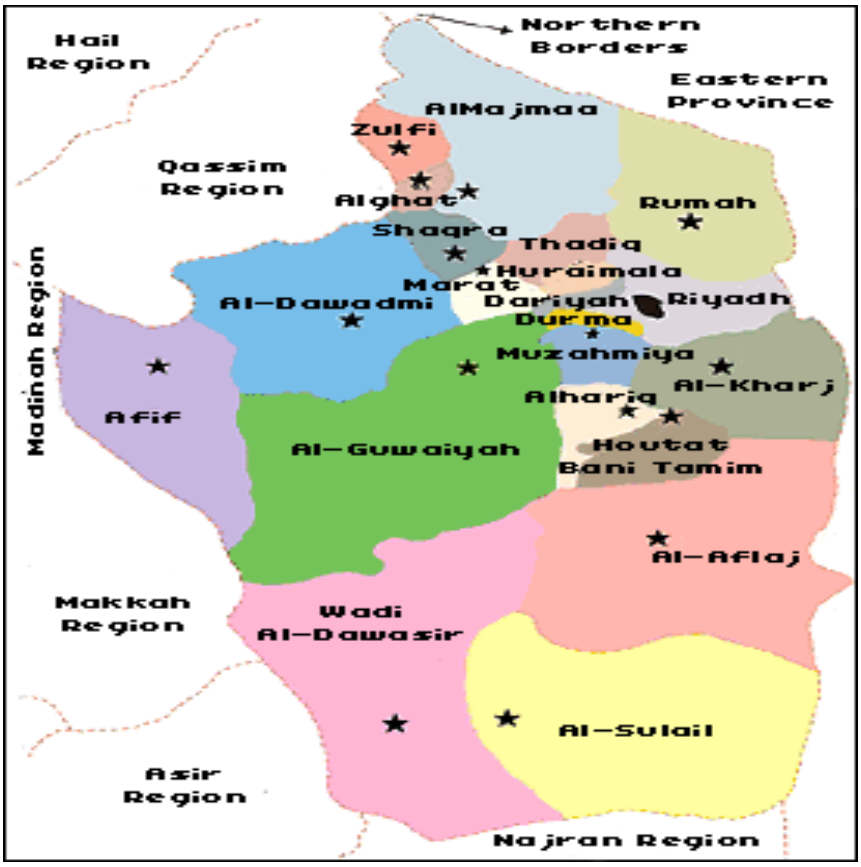

Fig. 1: Map of Al Riyadh showing study Provinces

Blood samples

Blood samples were collected in clean sterile vacationers, containing ethylene diamine tetra acetic acid (EDTA), from naturally suspected infected camels.

Extraction of DNA from blood samples:

Automated extraction of total genomic DNA from the whole blood by BioRobot EZ1 workstation (Qiagen®) using EZ1 DNA blood kit (Qiagen®) according to the manufacturer's instructions.

Primers:

T.evansi repetitive DNA primers was designed and synthesized in BiolegioBV®, Belgium to amplify a single band of 257 bp PCR product according to Chansiri *et al.* (2002); Yousef *et al.* (2010) for RT-PCR:

TR3 (5′ GCGCGGATTCTTTGCAGACGA 3′)

TR4 (5′ TGCAGACACTGGAATGTTACT 3′)

Real times PCR assay:

Polymerase chain reaction was carried out in LightCycler 2.0 (Roach®).The PCR mixture (20 µl) contained 15 µl of reaction mixture containing Fast start DNA Master ^{plus} SYBER Green 1(Roach®) and 10 *pmol*/*µ*l concentration of each primer and 5 *µ*l of genomic DNA. The PCR profile was performed as following; pre-heated for 1 cycle at 90 ºC for 10 min and then denatured at 94 ºC for 5 sec, annealing at 55 ºC for 10 sec and extension at 72 ºC for 10 sec. The PCR amplification was performed for 45 cycles. Fluorescence data were acquired at the end of each cycle in a single step. Once the plateau phase of the PCR had been reached, amplification was stopped and a standard melting curve analysis was performed (95ºC for 0 second, 65ºC for 10 seconds, and a 0.1ºC/second rise to 95ºC) with continual fluorescence measuring. PCR data were analyzed using LightCycler2 software version 4.05.

RESULTS

Table 1: The percentages of positives from four provinces in Saudi.

Province	No. of examined animals	No. of positive	% of positive to total examined
Al- Riyadh	80	25	10.33
Al- Qassim	70	41	16.94
Alharig	60	31	12.81
Al-Dawadmi	32	5	2.07
Total examined	242	102	42.15

The melting peak chart of the positive samples showed one single peak at an average Tm of 85.0ºC. (Fig.2).

The results of examined camels using real time PCR for detection of DNA of trypanosomes in suspected camels, showing a 102(42.15%). positive camels out of 242. The percentages of positives in different provinces as shown in Table (1) in Al- Riyadh, Al- Qassim, Alharig, and Al-Dawadm are 10.33%, 16.94%, 12.81% and 2.07% respectively**.**

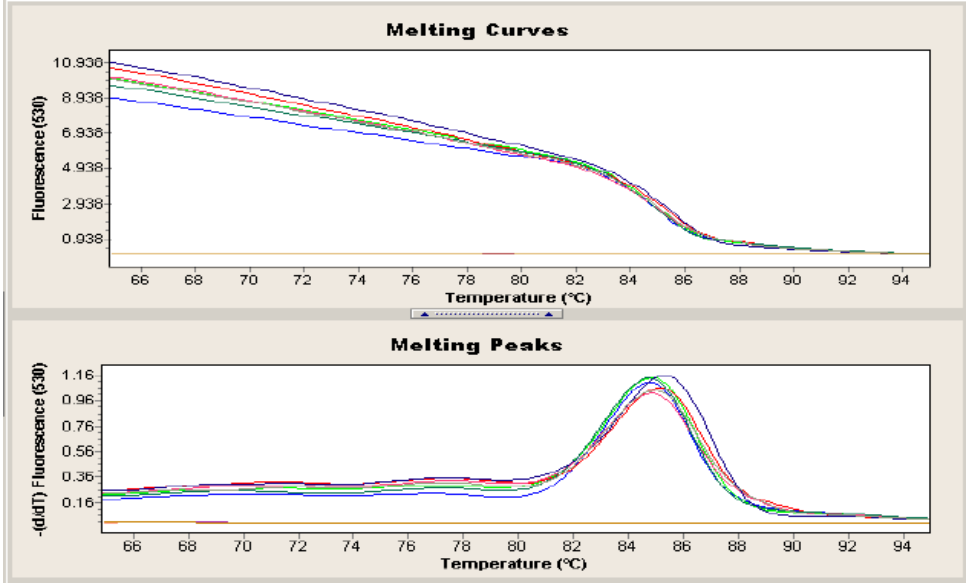

Fig. 2: Melting curve analysis of amplicon obtained from real time PCR.

DISCUSSION

The economic importance of *T. evansi* infection is mainly attributed to clinical disease in camels in many parts of the world (Dia *et al.*, 1997; Atarhouch *et al.*, 2003). The fact that PCR assays provided evidence of infection in animals that were classified as uninfected by parasitological techniques suggests that PCR could have an important role to play in the detection of infected animals harbouring a low parasitaemia that would be undetectable by smears (Chansiri *et al.*, 2002; Bashir *et al.*, 2011).

The better PCR sensitivity and the fact that samples can be taken as blood spots on filter papers open the opportunity to carry out epidemiological studies in remote regions in countries where accessibility and conditions to store samples are really difficult, Sample processing in PCR does not have to be done within minimum time after collection but can be delayed at least 180 days after preservation at -20ºC (Clausen *et al.*, 1998). The main advantages of different PCR assays are very low false-positives and insensitive to other haemoparasite species (Chansiri *et al.*, 2002). Conventional PCR (Omer *et al.*, 1998; Guterrez *et al.*, 2000; Masiga and Nyang'ao 2001; Bashir *et al.*, 2011) and real time PCR (Yousef *et al.*, 2010.) has been used successfully for detecting of infection with T. evansi in camels.

The real time PCR technique as used in this study was able to detect the suspected acute and chronic infection of *T. evansi* and may be the low parasitaemia consistently a new, sensitive and rapid diagnostic technique.

The obtained result (42.15%) was similar to that recorded in Kenya; 39.8% (Njiru *et al.*, 2004) and 34.40% in India (Reghu *et al.*, 2008) but higher than that recorded in India (17.05%) and Kenya (26.6%) (Singh *et al.*, 2004; Njiru *et al.*, 2004), but lower than that in Sudan (57.1%) (Bashir *et al.*, 2011). The improved detection rates found in the present study, was also reported in studies carried out in the Brazilian pantanal (Ventura *et al.*, 2001; Ventura *et al.*, 2002): these may be attributed to selection of assay conditions and primer sets being used. Our results also revealed that the real time PCR assay was beneficial for diagnosis of *T. evansi* among camels and could be useful for epidemiological, following of drug treatment study and designing rational trypanosomiasis control program in the endemic area.

It is concluded that camel trypanosomiasis in Sudai is apparently caused by a single parasite species *T. evansi* and there were no other typanosomes species detected. In addition, the disease is highly prevalent in the country, which strengthens the need to change control policies and institute measures that help prevent the spread of the parasite. To our knowledge, this is the first molecular diagnosis report, which gives a picture of camel *trypanosomiasis* covering large geographical areas in Sudai Arabia.

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