INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

COMPARATIVE STUDIES ON DIAGNOSIS OF *TRYPANOSOMA EVANSI* IN CAMELS IN AL-NAJAF PROVINCE, IRAQ

Jihad T. Aboed & Azhar Ali Faraj
Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq.

ABSTRACT
The present study was designed to compare the molecular diagnosis and light microscopy for detection of *Trypanosoma evansi* in camels in the AL-Najaf province. Blood samples of 100 camels were collected during the period from October 2016 to April 2017. Results revealed that PCR using *T. evansi* specific primers was the highest test of *T. evansi* diagnosis (90%) followed by the light microscopy (28%). Amplification of DNA from animals infected with *T. evansi* allows the diagnosis of parasite levels far lower than detectable by microscopic microscopy diagnosis.

KEYWORDS: Microscopic examination, Molecular diagnosis, *Trypanosomaevansi*, Camels, Iraq.

INTRODUCTION

Camel Trypanosomosis (surra), caused by the protozoan parasite *Trypanosoma evansi* (Eyob and Matios, 2013).The *Trypanosoma evansi* infects different hosts species and transmitted mechanically by biting flies (Tabanidae and Stomoxys species)also by Vampire bats (Desquesnes *et al*,. 2013). Surra disease that causes considerable economic losses due to weight loss, weakness, abortion , estrus cycle disorders, reduced productivity and death (Reid, 2002; Sazmand *et al.*, 2011). Currently, laboratorial diagnosis of Trypanosomasis is usually performed by direct parasitological examination and/orserological methods, which are time consuming and may lack accuracy (Ashour *et al.*, 2013). The polymerase chain reaction (PCR) is nowadays a simple and valid molecular test to diagnosis the parasite species, strains and genotypes (Elhaig*et al*., 2013, Barghash*et al.*, 2014). The aim of this study is identify *T. evansi* infection in camels by direct microscopy examination and molecular assay.

MATERIALS & METHODS

One hundred venous blood samples were collected from camels during the period from October 2016 to April 2017 in AL-Najaf province. Five ml of blood sample were collected from each animal and kept in anticoagulant ethylene diamine -tetra acetic acid (EDTA) tubes. Samples were transferred in cooling box to the laboratory to conduct the necessary tests to determine the infection with *T.evansi*.

Microscopic examination

Blood films were prepared from each sample, stained by Giemsa and subjected to light microscopic examination to investigate the parasite (Beaver and Jung, 1985).

DNA isolation and PCR assay

DNA was extracted from blood samples using the whole blood DNA extractions as described by Sazmand *et al*. (2016).

Primers

Two primers were used for detection of the *T. evansi* in this study (Table 1).

TABLE 1: The primers with their sequences and product size

Primers	Primer sequence (5′ to 3′)		Product size (bp)	References
RoTat 1.2	F	GCG GGG TGT TTA AAG CAA TA	205	(CLAES *et al*.,2004)
	R	ATT AGT GCT GCG TGT GTT CG		
TBR1.2	F	GAA TAT TAA ACA ATG CGC AG	164	(Muieed *et al.*, 2010)
	R	CCA TTTATT AGC TTT GTT GC		

Statistical Analysis

Data were analyzed by using SPSS software version 15. Chi-Square test was used to assess the significant differences. P<0.01 is considered significant,

RESULTS

The percentage of infection was showed that among (100) *T. evansi* suspected cases were examined microscopically 28%, whereas 90 % by PCR (Table 2), (Figure 1, 2).

TABLE 2: Showed the examined camels and positive cases.

Diagnostic methods	Total number	positive	%
Microscopic examination	100	28	28%
PCR examination	100	90	90%
Chi-Square	---	---	**13.562 ****

** (P<0.01).

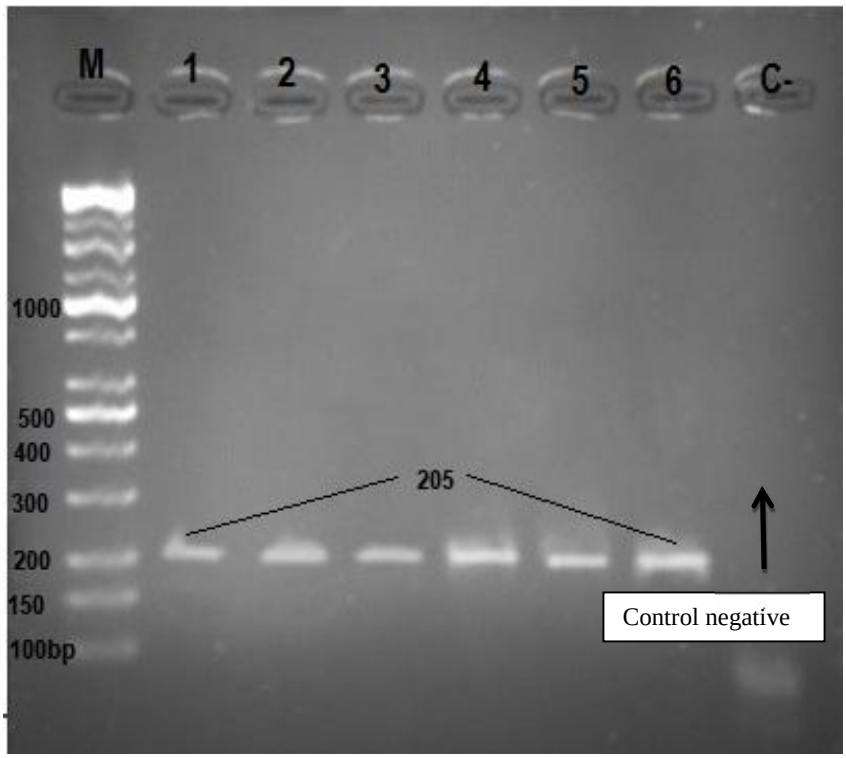

FIGURE 1: Gel electrophoresis of PCR product of *Rotat (*205bp), for *T.evansi*using 2% agarose gel. Lane 1- 6: PCR product positive for *Rotat* genes.

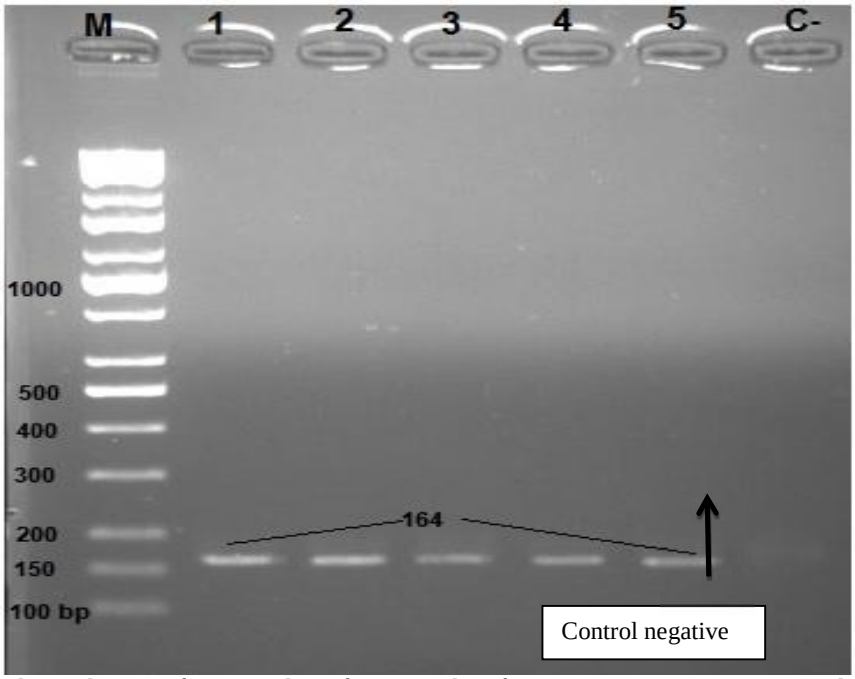

FIGURE 2: Gel electrophoresis of PCR product of *TBR* (164bp), for *T. evansi* using 2% agarose gel. Lane 1- 5: PCR product positive for *TBR* genes.

DISCUSSION

Molecular methods for diagnosis of *T. evansi*, as more sensitive and specific diagnostic tools, have been increasing used to detect *T. evansi* in carrier animals. It is recognized even relative amount of DAN of the parasite (Clausen *et al*., 2003; Masiga *et al*., 2001). There was no comprehensive data on the use of PCR for diagnosis of infection in Iraqi breed of dromedary Camels. Parasitological prevalence, assessed by Giemsa stained thin smear was much lower (28%) than molecular prevalence (90 %). The result of this study agreed with some studies, Clausen *et al.* (1998) who showed that the detection rate of PCR was about x 2 higher compared with (HAT) and (MAECT) for diagnosis of *Trypanosoma* infections in cattle and also Ravindran *et al.* (2008) found a higher prevalence of *T. evansi* in dogs (7.7%), camels (34.4 %), and in donkeys (6.8 %) during PCR than blood film examination. In Kenya Njiru *et al.* (2004) reported the PCR the considered the most sensitive technique than other methods to detect trypanosomiasis. Aslam *et al.* (2010) reported that PCR test be a superior assay over ELISA and MHCT for the diagnosis of trypanosomasis in horses. During this study, number of samples was negative by light microscopic but were found positive when using PCR. The ability of this tool are very low false-positive and insensitive to other blood parasite species(Sazmand *et al.*, 2016).

CONCLUSION

There is a significant difference in the incidence rate when using different diagnostic tools. The molecular assay for detection of Trypanosomasis is a best test (sensitive and specific). This test is suited for detect of Trypanosomasis in both mammalian blood and insect vectors and beneficial for epidemiological survey.

REFERENCES

Ashour, A.A., Abou EL-Naga,T.R., Barghash, S.M., Salama, M.S. (2013)*Trypanosoma evansi*: Detection of *Trypanosoma evansi* DNA in naturally and experimentally infected animals using TBR1 & TBR2 primers. Exp. Parasitol. 134 : 109–114

Aslam, A., Iqbal, Z.C., Rehman, H., Ashraf, K., Ahmad, N., Yaqub, T., Maqbool, A., Shakoori, A. (2010) Comparative Evaluation of Parasitological, Serological and DNA Amplification Methods for Diagnosis of Natural Trypanosomal Infection in Equines. Pakistan J. Zool. 42: 371-376.

Barghash, S.M., El-Naga, T.R., El-Sherbeny, E.A., Darwish, A.M. (2014) Prevalence of *Trypanosoma evansi* in Maghrabi camels (*Camelus dromedarius*) in Northern-West Coast, Egypt using molecular and parasitological methods Acta Parasitol. Glob.5 : 125-132.

Beaver, P.C., Jung, R.C. (1985) Animal Agents and Vectors of Human Disease.5thed.Lea and Febiger, Philadelphia. pp249.

Claes, F., Radwanska, M., urakawa, T., majiwa, PAO, Goddeeris, B., Büscher, P. (2004) Variable Surface Glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. Kinetoplastid Biology and Disease, Heidelberg. 3: 1-3.

Clausen, P.H., Gebresel Assie, G., Additchos, A., Mehlilz, D., Staak, C. (1998) Detection of trypanosomes in horses suspected of dourine in Ethiopia. Lokai. J. Exp. Res. 23:303-308.

Clausen, P.H., Chuluun, S., Sodnomdarjaa, R., Greiner, M., Noeckler, K., Staak, C., Zessin, K.H., Schein, E. (2003) A field study to estimate the prevalence of *Trypanosoma equiperdum*in Mongolian horses. Vet. Parasitol., 115:9-18.

Desquesnes, M., Holzmuller, P., Lai, D.H., Dargantes, A., Lun, Z.R. (2013) *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. Biomed Res Int. 194176.

Elhaig, M.M., Youssef, A.I., EL-Gayar, A.K. (2013) Molecular and parasitological detection of *Trypanosoma evansi* in Camels in Ismailia, Egypt. Vet. Parasitol. 198: 214-218.

Eyob. E., Matios. L. (2013) Review on camel trypanosomosis (surra) due to *Trypanosoma evansi*: Epidemiology and host response. J. Vet. Med. Anim. Health. 5: 334-343.

Masiga, R.C., Nyangao, J.M. (2001) Identification of trypanosome species from camel using polymerase chain reaction and procyclic transformation test. Camel Pract. Res. 8: 17-22.

Muieed, M.A., Chaudhary, Z.I., Shakoor, A.R. (2010) Comparative studies on the sensitivity of polymerase chain reaction (PCR) and microscopic examination for the detection of *Trypanosoma evansi* in horses. Turk. J. Vet. Anim. Sci. 34:507–512.

Njiru, Z.K., Constantine, C.C., Ndung'u, J.M., Robertson, I., Okaye, S., Thompson, R.C.A., Reid, S.A. (2004) Detection of *Trypanosomaevansi* in camels using PCR and CATT/*T. evansi* tests in Kenya. Vet. Parasitol.124: 187–199.

Ravindran, R., Rao, J.R., Mishra, A.K., Pathak, K.M.L., Babu, N., Satheesh, C.C., Rahul, S. (2008) *Trypanosoma evansi* in camels, donkeys and dogs in India: comparison of PCR and light microscopy for detection. Vet. Arhiv. 78: 89-94.

Reid, S.A. (2002) *Trypanosoma evansi* control and containment in Australasia. Trends Parasitol. 18:219-224.

Sazmand, A., Eigner, B., Mirzaei, M., Hekmatimoghaddam, S., Harl, J., Duscher, G.G. (2016) Molecular identification and phylogenetic analysis of *Dipetalonema evansi* (LEWIS, 1882) in camels (*Camelus dromedarius*) of Iran. Parasitol Res. 115:1605–10.

Sazmand, A., Rasooli, A., Nouri, M., Hamidinejat, H., Hekmatimoghaddam, S. (2011)Serobiochemical

alternations in subclinically affected dromedary camels with *Trypanosoma evansi* in Iran. Pak. Vet. J. 31:223–6.

Yadvendra, S., Pathak, K., Verma, K., Harsh, D., Kapoor, M. (1998) Prevalence and diagnosis of *Trypanosoma evansi* infection in camels in Rajasthan. J. Vet. Parasitol. 55 : 133-136.