HEMATOLOGICAL AND MOLECULAR STUDY OF *TRYPANOSOMA EVANSI* IN EXPERIMENTALLY INFECTION IN RABBITS

R. Khalid Ali **I. Badr Falih**
Lecturer **Prof.**
Coll .Vet. Med.,University of Fallujah **Coll. Vet. Med.,University of Baghdad**
Email:dr.rafidkhalid@uofallujah.edu.iq

ABSTRACT

The lack of a sensitive diagnostic test for trypanosomasis in animals may hamper epidemiological data and control program implementation. This study aimed to detection of *Trypanosoma evansi* in rabbits that experimentally infected by this parasite which identified from camels of iraq by polymerase chain reaction. A study was conducted on 24 males rabbit divided into two groups (12 rabbits for each group). The first group was injected intra peritoneal (i/p) with 0.5 ml containing $1x10^5$ trypanosome. The second group injected with distilled water and considered as control. At 7 and 10 days post infection, blood sample were collected from these rabbits. White blood cells count was conducted after 14 and 28 days post infection. The results showed that the *18S RNA* and *RoTat* 1.2 genes related to *T. evansi* were identified .Furthermore ,there was significant elevation of Neutrophils ,Monocytes ,Lymphocytes ,Eosinophil and Basophils in infected rabbits compared with the control group. From this study we can conclude that PCR is a sensitive and effective technique for diagnosis of *T.evansi* through using *18S RNA* and *RoTat* 1.2 genes. In addition ,WBC count was significantly elevated in an infected rabbits compared to the control. Whereas ,Lymphocyte was significantly higher in control group compared with infected group.

Key words: Hemoprotozoa, PCR, *18SRNA* gene, trypanosomiasis, good health

دراسة دمية وجزيئية للإصابة التجريبية بمثقبيات ايفانسي في الارانب

رافد خالد علي انعام بدر فالح
مدرس أستاذ
كلية الطب البيطري، جامعة الفلوجة كلية الطب البيطري، جامعة بغداد

المستخلص

ان قلة وجود اختبارات التشخيص الحساسة لداء المثقبيات في الحيوانات المصابة تؤدي الى اعاقة البيانات الوبائية وتنفيذ برامج المكافحة ,حيث هدفت الدراسة الى تشخيص داء المثقبات في الارانب المصابة تجريبا بهذا الطفيلي والمعزولة من الابل في العراق بواسطة استخدام تفاعل البلمرة المتسلسل .شملت الدراسة استخدام 24 ارانب وتم تقسيمها الى مجموعتين وبواقع 12 ارنب لكل مجموعة حيث حقنت المجموعة الاولى ب من طفيلي المثقبيات داخل غشاء الخلب بينما اعتبرت المجموعة الثانية كمجموعة ضابطة والتي حقنت بالماء المقطر و تم جمع عينات الدم في اليوم السابع والعاشر من الاصابة. كما تم حساب كريات الدم البيضاء بعد 14 و28 يوم من الاصابة اظهرت النتائج وجود اصابة موجبة تم تحديدها باستخدام جين *18SRNA*و1.2 *RoTat* مع وجود ارتفاع ملحوظ في اعداد خلايا العدلات , وحيدة النواة ,الخلايا اللمفاوية , الحمضات وخلايا القعدات مقارنة باعداد هذة الخلايا في المجموعة الضابطة ,نستنتج من الدراسة الحالية ان تفاعل البلمرة المتسلسل طريقة فعالة وحساسة في تشخيص الاصابة بداء المثقبات باستخدام جين *RoTat*و*18SRNA* كما اظهرت النتائج وجود ارتفاع معنوي في اعداد الكريات البيض في المجموعة المصابة مقارنة بالمجموعة الضابطة بينما كانت اعداد الخلايا اللمفاوية مرتفعة في المجموعة الضابطة مقارنة بالمجموعة المصابة .

الكلمات المفتاحية :الاوالي الدمية ,تفاعل البلمرة المتسلسل ,جين 18SRNA, داء المثقبيات، صحة جيدة.

Received:12/3/2022, Accepted:22/6/2022

INTRODUCTION

Trypanosomosis of *camel* (Surra) is the most frequent disease in the world and its caused by *Trypanosma evansi*. The disease transmitted mechanically by haemotophagus biting flies such as *Stomoxys* and *Tabanus* spp. (4). Salivaria branch, which originates in front portion of the insects digestive system, is the route through which *T. evansi* is spread. (6). *Trypanosma evansi* uses glucose catabolism to produce adenosine triphosphate (ATP) which is necessary for parasite movement and survival. Anemia is a characteristic pathology of infections with *T. evansi* due to the peroxidation of the erythrocytes lipid which causes membrane damage, osmotic fragility, and RBC death (13). Males are more susceptible to infection than female (22). But *T. evansi* infection leading to abortion of pregnant animals and generating economic loss as a result of its propensity to generate negative feedback (2). In camels, Trpanosomiasis causes severe morbidity (16). Camels become infected with trypanosomiasis in two forms (acute and chronic). Chronic form is the most frequent, and it may be related with secondary infections and this may be due to the fact that trypanosomiasis infection causing immunosuppression and complicating clinical diagnosis (27). Fever, emaciation, anemia, ocular opacity, lacrimation, diarrhoea and edema of the dependent parts are insufficient for diagnosis and identification of this parasite in the blood is problematic due to the intermittent nature of parasitaemia (8,15).Alternative diagnostic techniques that are more delicate are therefore needed. For the identification of camel's disease, several diagnostic methods such as PCR have been utilized, including COX1 and ITS2 region (12). In a variety of hosts, PCR has been shown to be more sensitive and accurate than traditional techniques of parasitology, with the benefit of being able to identify parasites at the species level (1). Present study aimed to detection of *Trypanosoma evansi* in rabbits that experimentally infected by this parasite which identified from camels in Iraqi.

MATERIALS AND METHODS

From January to October 2020, twenty four adult healthy rabbits with 7-9 weeks of age were used for the present study. The rabbits divided into two groups, 12 rabbits for each. The first group was injected with 0.5 ml of blood from infected *camel* containing $1x10^5$ trypanosome intraperitoneally (i/p). The second group injected with distilled water and considered as control. Blood sample were collected from these rabbits and the genes of *18S RNA* and *RoTat 1.2* related to *T. evansi* were identified by polymerase chain reaction (PCR). The protocol of genes detection was used according to manufacturing company (19). Blood was conducted after 14 and 28 days post infection for hematological analysis.

Microscopic finding

10 µL of blood was used , a clean slide was prepared for examination,general smear protocol applied to obtain a clear smear veiw using Giemsa stain(1:9) with (pH 7.2).followed by washing with clear water .

DNA extraction The DNA of *Trypanosoma evansi* was extracted from infected rabbite according to the manufacturer instructions(G-spin DNA extraction kit,intron biotechnology, cat.no. 17045). All extracted DNA were kept at -20C until examined. Molecular identification of *Trypanosoma evansi* using specific primer of *UNI18s* gene (5'-GCGAAACGCCAAGCTAATAC-3') (5'-ACGGCACAAAACTACGTG-3'),integrated DNA Technologies company,Canada).For PCR reaction utilized 25µl and qualified in Table 1, the progarm of amplification is specifice in Table 2.

Table 1.The PCR reaction for detection of *Trypanosoma evansi*

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table 2. Amplification program for polymerase chain reaction

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1 cycle
2-	Denaturation	95°C	45sec	
3-	Annealing	58°C	45sec	35 cycles
4-	Extension	72°C	45sec	
5-	Final extension	72°C	7 min.	1 cycle

Statistical analysis

All variable results were analysis by (one-way ANOVA) and the significant differences for the result was determined at (P≤0.01) (17).

RESULTS AND DISCUSSION

Microscopical examination of blood smear showed the protozoa of *Trypanosoma. evansi* ,which appeared as slender shape with either tapered or blunt ends and with central nucleus position and free flagellum(Figure 1).

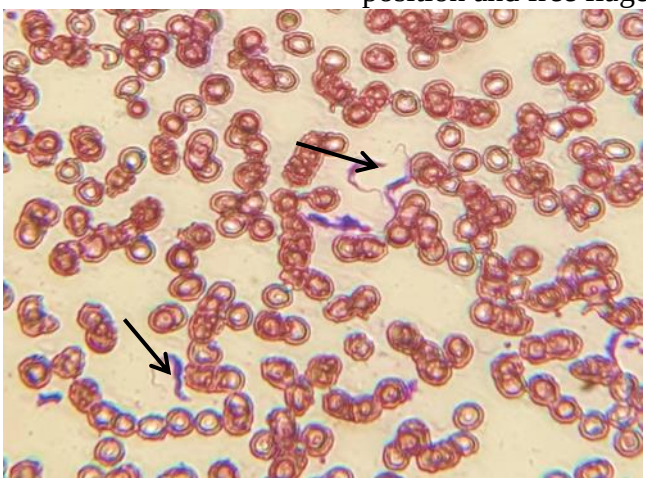

Figure 1.Numerous *Trypanosome evansi*(black arrow) in blood smear of infected rabbits stained by Giemsa stain(100x).

The *18S RNA* and *RoTat* 1.2 genes of *T. evansi* were amplified at the size of 550bp and 205 bp, respectively (Figures 2 and 3). The 18S RNA gene was registered in GenBank at NCBI by the following code: KY114580.1.

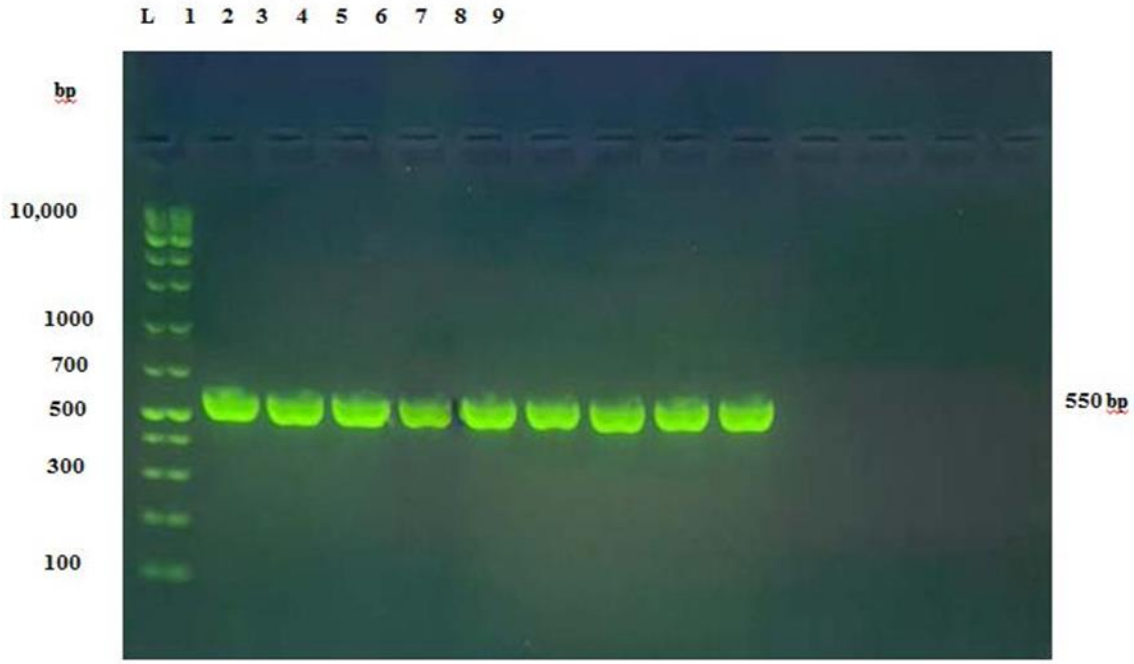

Figure 2. Fragment size(bp)of gene *18S RNA* gene=550pb

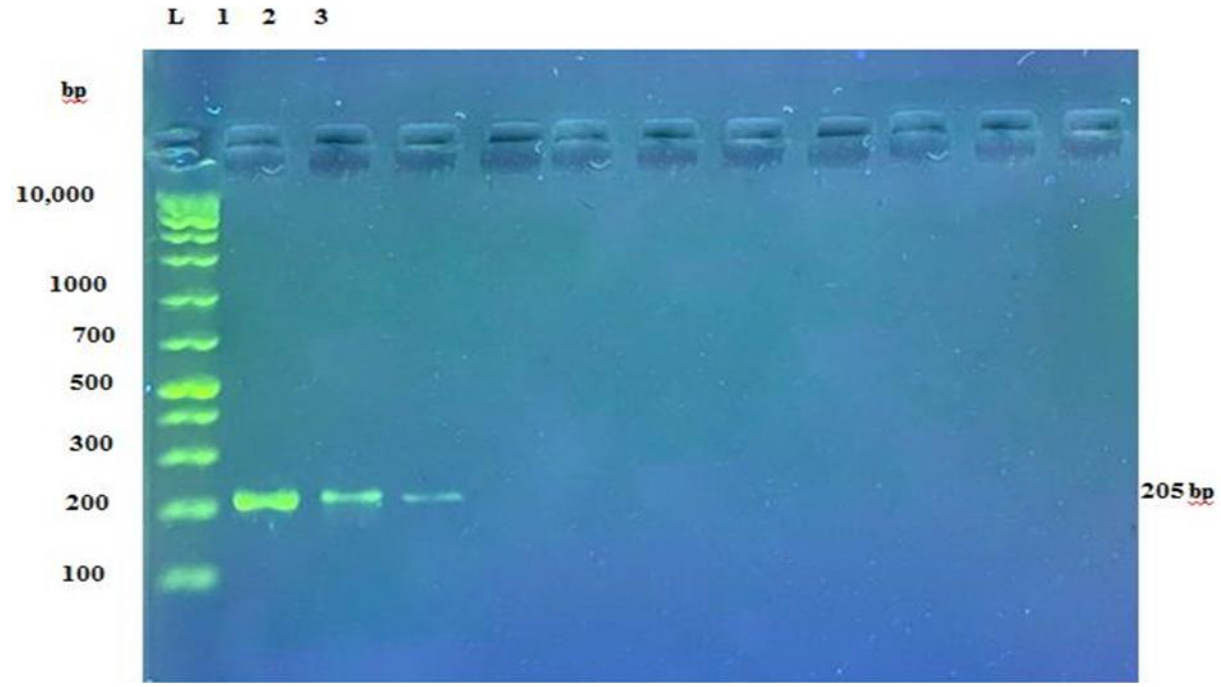

Figure 3.Fragment size(bp)of gene RoTat 1.2 gene =205pb

White blood cell count (WBC)

Our finding showed a significant differences($p \leq 0.01$) in WBCs count mean between infected and control group, except 14 days and 28 days. While the lymphocyte mean also show significantly higher number in control group comparing with the infected group Table 3.

Table 3. WBC count (mean ± S. D.) at 14 and 28 days post infection in infected and control rabbit groups

Type of cell	Control group	infected group at 14 with $1x10^5$ *T. evansi*	Infected group at 28 days
Neutrophils%	**48.6 ±1.8**	**67.4±2.3**	**61.4 ±10.7**
Monocytes%	**4.7 ± 0.3**	**7.8 ± 1.6**	**6.8 ±1.9**
Eosinophils %	**0.7 ± 0.1**	**2.6 ± 0.4**	**2 ± 0.4**
Basophils %	**0.9 ± 0.1**	**3.5 ± 0.3**	**3.9 ± 0.4**
Lymphocytes %	**41.7 ± 1.5**	**31±3.4**	**25.5 ±3.5**

***Horizontal column means that there is a significant differences at P ≤0.01. Mean ± S.D.**

The important of trypanosome infection in camels is attributed to the pathogenesis and clinical signs of this disease in world wide . Numerous research on the characterization of trypanosomes in general and *T. evansi* in particular have been published (20,25). Despite the fact that camels are generally lived in different environmental (desert),compared with livestock camels still has some cases of infection with *T.evansi* . Transmission via biting flies is conceivable due to the presence of vector around the susceptible camel (19). PCR methods is successfully used for identification of *T .evansi* in horses and buffaloes (5). Application of PCR for detection *T. evansi* in camels is limited, PCR contamination is also an issue. Use of clear aerosol resistant tip(nucleic acid free)and different places for both DNA extraction and amplification could help to reduce contamination .To keep high degree of sensitivity at the same time reduce the specificity, each PCR experiment should include negative and positive control. The nested PCR assays speed, specificity and sensitivity would make these test more easier to detect *T. evansi* infection in different species of animals (10,17). But these test is a higher sensitivity than microscopic analysis of blood smears (18,19). Epidemiological molecular analysis result range from 33.9 percent to 42.1 percent (11). PCR claimed the highest rate of infection. Indicating high sensitivity level which was 90% and these results are corresponding with Elhaig (7). *18 SRNA* and *Rotat*1.2 are the molecular targets genes used in this study. 18S RNA results showed dromedary camels in scrupulous area were

infected with Trypanosomes ,especially *T. evansi* which were also positive with *RoTat* 1.2 genes during this experiment ,some samples were found to be negative by optical microscopy but positive by PCR. This method has a low false-positive rate and is unaffected by other blood parasite species (20). It is worth noting that the level of blood parasites was highest in the dry season, which leading to increase activity of these flies and facilitated transmission and infection due to the gathering of camels herds near water well, in addition to other predisposing factors such as dry rain and low level of animals nutrition in the last part of the dry season (24). Neutrophils, monocyte, eosinophil and subsequent basophile which observed at the present study associated with the host immune response against parasitic infection and leading to activation of mononuclear phagocytes as well as neutrophils leukocytes may be associated with the stress of Trypanosome infection result in the production of corticosteroid and these result are corresponding with Aradaib and Majid (3), Leukocytosis and lymphocytosis were recognized in the acute phase, followed by leukopenia in the chronic phase (9,14) leukocytosis is not a reliable marker of infection as revealed by different studies.(23,26).

CONCLUSSIONS

PCR is an sensitive and effective technique for *T. evansi* diagnosis by using *18S RNA* and *RoTat* 1.2 genes. In addition to, WBC count (mean ± S.D.) was significantly elevated in infected rabbit compared to control groups, except mean of Lymphocyte which was significantly higher in control group in contrast to the infected group.

REFRENCES

1.Al-Fatlawi,M.A. and H.H. Al-Fatlawy.2021. COX1 gene and ITS-2 region: A comparative study of molecular diagnosis of *Parabronema skrjabini* in camels (*Camelus dromedaries*), Al-Najaf Province, Iraq. Iraqi J .Vet .Sci .25:36(1):77–84.
doi.org/10.33899/ijvs.2021.129228.1634.

2. Al-Rubaie, L. S. 2020. Study iron homeostasis of infected and non-infected iraqi camels with Trypanosomiasis. Iraqi J. Vet.Med.44(2):35-44.
doi.org/10.30539/ijvm.v44i2.975

3. Aradaib, I. E. and A. A. Majid.2006. A simple and rapid method for detection of *Trypanosoma evans*i in the dromedary camel using a nested polymerase chain reaction. Kinetoplastid biology and disease.*5*(1):1-6.
doi.org/10.1186/1475-9292-5-2

4.Benaissa, M.H., N. Mimoune, Y. Bentria, T. Kernif, A .Boukhelkhal ,C. R.Youngs and Y.Halis.2020. Seroprevalence and risk factors for *Trypanosoma* evansi, the causative agent of surra, in the dromedary camel (*Camelus dromedarius*) population in Southeastern Algeria. Onderstepoort J. Vet.Res.87(1).
doi:10.4102/ojvr.v87i1.1891.

5.Clausen,P.H.,S .Chuluun, R .Sodnomdarjaa, M. Greiner, K .Noeckler, C. K.H. Zessin and E .Schein.2003. A field study to estimate the prevalence of *Trypanosoma equiperdum* in mongolian horses. Vet.Parasitol. 115:9-18. doi:10.1016/S0304-4017(03)00160-2.

6.Desquesnes ,M.,P.Holzmuller,D.H .Lai, A.Dargantes,Z.R. Lun and S .Jittaplapong .2013. *Trypanosoma evansi* and surra: A review and perspectives on origin, history ,distribution,taxonomy, morphology ,hosts and pathogenic effects. Bio.Med.Res.Int.pp:1–22. doi.org/10.1155/2013/194176

7.Elhaig,M.M., A.I. Youssef and A.K. El-Gayar.2013. Molecular and parasitological detection of *Trypanosoma evansi* in Camels in Ismailia.Egypt.Vet.Parasitol.198(1-2):214–218.doi.org/10.1016/j.vetpar.2013.08.003

8.Eyob,E. and L .Matios.2013. Review on camel trypanosomosis (surra) due to *Trypanosoma evansi* epidemiology and host response. J. Vet. M. A .H.5(12): 334-343. doi: 10.1155/2013/194176

9.Eze ,J.I., L.J.E. Orajaka, N.C .Okonkwo, I.O. Ezeh, C .Ezema and G.N .Anosa.2012. Effect of probiotic (*Saccharomyces cerevisiae*) supplementation on immune response in *Trypanosoma brucei* infected rats. Exp. Parasitol.132(4):434–9.
doi.org/10.1016/j.exppara.2012.09.021

10.Faye, B. and M. Bengoumi.2018. Hematology. camel clinical biochemistry and hematology. Springer International Publishing.13–45.doi.org/10.1007/978-3-319-95562-9_2

11.Fikru, R., Y. Andualem, T. Getachew, J. Menten, E. Haske and B. Merga. 2015.

Trypanosome infection in dromedary camels in Eastern Ethiopia: Prevalence, relative performance of diagnostic tools and host related risk factors.Vet.Parasitol.211(3-4):175.81. doi.org/10.1016/j.vetpar.2015.04.008

12.Gaur, R. S., D. Shanker, V .Sudan,S. Paliwal, S. Singh, and A .Jadaun.2020. Associative genetic diversity of RoTat 1.2 VSG in different *Trypanosoma evansi* isolates. Acta. Parasitologica . S.S.B.M. 17:66(1):199–204.doi.org/10.1007/s11686-020-00273-4

13.Habila, N., M. H. Inuwa, I.A. Aimola, M.U. Udeh and E . Haruna. 2012. Pathogenic mechanisms of *Trypanosoma evansi* infections. Res.Vet.Sci.93(1):13-17. . doi.org/10.1016/j.rvsc.2011.08.011

14.Hassoon, A. F. and A.H. Farag. 2007. Blood picture and hepatic changes in rabbits experimentally infected with Trypanosoma evansi. Iraqi strain. Medical Journal of Babylon.4(3-4):358-364.

15.Hussein, S. M.2002.Histological changes of adrenal gland in rabbits during the experimental infection with Trypanosoma evansi and after treatment with Naganol. The Iraqi Journal of Veterinary Medicine. 26(2):135-142.

16.Kaaboub,E.A.,N.Ouchene,N.A.Ouchene, A. Dahmani, I. Ouchtati and A .Haif.2021. Investigation of the principal vectors of abortive diseases in one-humped camels (*Camelus dromedarius*). Iraqi J. Vet. Sci.15:35(3):411-5 doi.org/10.33899/ijvs.2020.126914.1415

17.Kolte,S.W., S.D. Larcombe, S.G .Jadhao, S.P. Magar, G .Warthi and N.V .Kurkure.2017. PCR diagnosis of tick-borne pathogens in maharashtra state, india indicates fitness cost associated with carrier infections is greater for crossbreed than native cattle breeds. PLoS.30:12(3):e0174595.doi.org/10.1371/journal.pone.017459

18.Njiru,Z.K., C.C.Constantine, S.Guya, J. Crowther, J.M. Kiragu, R.C.Thompson and A.M. Dávila.2005. The use of ITS1 rDNA PCR in detecting pathogenic african trypanosomes. Parasito. Res. 95: 186-192. Doi.10.1007/s00436-004-1267-5.

19.SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

20.Scherczinger,C.A., M.T .Bourke, C .Ladd and H.C .Lee.1997. DNA extraction from liquid blood using QIAamp. J . forensic Sci.1:42(5).14226. doi.org/10.1520/jfs14226j.

21.Shahatha,S.S.2020. Epidemiological, diagnostic and therapeutic study for mange in sheep of anbar province- iraq. Iraqi J. Vet.Sci.1:34(1):17.doi.org/10.33899/ijvs.2020.163587

22.Sheet,O.H., D.M .Jwher, R.A. Al-Sanjary and A.D .Alajami.2021.Direct detection of *Staphylococcus aureus* in camel milk in the Nineveh governorate by using the PCR technique. Iraqi J. Vet. Sci.26:35(4):669–72. doi.org/10.33899/ijvs.2020.127725.1524

23.Sivajothi,S., V. C. Rayulu and R. B. Sudhakara. 2013. Haematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits. J. Parasit.Dis.SSBM.19:39(2):216–20. doi.org/10.1007/s12639-013-0321-6.

24.Sobhy,H.M., S.M .Barghash, T.S. Behour and E.A .Razin.2017.Seasonal fluctuation of trypanosomiasis in camels in North-West Egypt and effect of age, sex, location, health status and vector abundance on the prevalence. IJBAS.6(1):64–8. doi.org/10.1016/j.bjbas.2017.01.003

25.Tavares,R., R .Staggemeier, A. Borges, M. Rodrigues, L. Castelan and J. Vasconcelos.2011.Molecular techniques for the study and diagnosis of parasite infection. J. Venom. Anim and Toxins incl. Trop. Dis. 17(3):239–48. doi.org/10.1590/s1678-91992011000300003

26.Wassink,G.J., G .Fishwick, J.J. Parkins, M .Gill, D.L. Romney and D. Richard.1997. The patho-physiology of *Trypanosoma congolense* in Scottish Blackface sheep: influence of diet on digestive function. Animal Science .64(1):127–37. doi.org/10.1017/s1357729800015630

27.Weldegebrial, G. A., T. K.Samson, D. T. Kidanie, T. B. Woldegebriel, T.H.Sisay and Z.K.Fikre.2015.Parasitological and serological study of camel trypanosomosis (surra)and associated risk factors in Gabi Rasu. Zone, Afar, Ethiopia. J.V.M.A.H.30:7(6):234 40.doi.org/10.5897/jvmah2015.0374