Diagnosis and Epidemiological Studies of Bovine Trypanosomiasis in Kaliobia Governorate

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Abstract: This investigation was performed on 131 animals (cattle and buffaloes) from farms located in different places in kaliobia aged from 1.5-5 years the samples were collected from clinically infected animals that suffer from "surra" disease and animals apparently healthy in contact with infected animals (subacute or chronic Infection). This investigation reported that 51animals showed the clinical signs of illness as pyrexia, parasitaemia,progressive emaciation, generalized edema and recurrent episodes of fever occur during course of disease .The microscopic examination of blood film revealed (Trypanosoma evansi) in 5 out of 80 apparently healthy animals (6.3%).While PCR examination recorded the infection in 35 out of 80 animals positive (43.6%).So PCR is considered the most suitable diagnosis for early diagnosis of Trypanosoma and consequently controlling programs and considered the confirmatory test.

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1. Introduction:

Trypanosomiasis is a disease complex caused by several species of Flagellate protozoan parasites (Trypanosomes) that live in body fluids. It occurs through the tropical and subtropical regions of Africa. It affects cattle, buffalos and human. , the fact that the disease is transmitted mechanically as well as cyclically has certainly expanded the disease distribution out of the tsetse belt area. Trypanosomiasis,also known as (surra) is caused by T.evansi and is quite common among horse, cattle, buffaloes, and camels (Sehgal et. al.2006) in this study, trypanosomosis was examined in suspected to infected using PCR as confirmatory test for early diagnosis andconsequantly controlling programs .The disease has economic important due to loss of condation,reduction in milk yield (Reghu Ravindran et.al.2008) decrease capacity of work and may affect on quality of semen in bulls and cause irregular estrus, abortion and stillbirth in cows (El Sawlhy 1999). PCR is high sensetive method that could detect T.evasi infection bovine three day earlier than miroscpically (Wasana et al. 2000). Prevalence of disease depends on rate of exposure, availability of infected animals, the insect reservoir and seasons (Mottilb et al. 2005). The conventional parasitological methods lack sensitivity and serological techniques ,which detect antibodies or antigen lack specificity or sensitivity, respectively. Therefore molecular technique, especially (PCR) has been developed in order to overcome the problems faced with conventional and serological technique. In addition ,it was reported that PCR is a reliable method for diagnosis and epidemiological studies (El-Metanawey et. al.2009)

2. Materials and methods

Animals

Total number of animals 131aged from 1.5-5years old from different location in Kalubaia was clinically examined with special attention to signs related to trypansoma evansi infection .Samples also collected from apparently healthy animals in contact to infective animals.

Samples

The blood samples were collected from jugular vein by sterile sharp needle with wide pore samples were collected in clean and dry test tube containing EDTA as anticoagulant for blood smear and PCR analysis.

Blood film:

Three thin blood film were prepared and left to dry and then fixed in absolute methyl alcohol for 1-2min. Staining with freshly filtrated and diluted Giemsa stain for 30-45 min, washed with distal water to remove excess of stain after that the slides were left to dry and then examined under oil immersion lens according to(Coles,1986) .

Examination of blood film for trypanosoma:

It begins 1/4-1/2 inch from end of the blood film and transferring from one part of the film to the other (cross-section method) to give constant and representive examination of the film according to (Barrent,1965) animals is considered negative if three slides are negative.

DNA extraction:

Two hundred and fifty microlitres of EDTA blood was mixed with 250 µl lysis buffer (0.32 M Sucrose, 0.01 M Tris, 5 mM MgCl2, 1% Triton X-100, pH 7.5). The mixture was centrifuged at 13,000 g for 25 sec. The supernatant was removed and the pellet washed with 500 µl lysis buffer. The centrifugation and washing were repeated twice. The final pellet was resuspended in 250 µl 1 x PCR buffer (10 mM Tris-Hcl (pH 8.3), 50 mM KCl, 0.1 % Triton X- 100). 1.5 µl of proteinase-K (10 mg/ml) was added and vortexed. The samples were incubated at 56 °C for 1 hour and at 95°C for 10 min. (to inactivate the proteinase-K) and stored at -20°C until use. DNA was extracted as described by Higuchi (1989).

DNA amplification:

PCR were carried out in 25 µl reaction volumes containing 10 mM Tris-HCl (pH8.3)Polymerase chain reaction (PCR) assays were further performed to confirm the Trypanosomiasis in buffaloes were T. evansi. Forward 5-ACA TTC CAG CAG GAG TTG GAG-3 primer and reverse 5-CAC GTG AAT CCT CAA TTT TGT-3 primer, which are trypanosome specific (Holland et al., 2001), were used for amplification of the 239-base pair (bp) fragment from T. evansi genomic DNA. Amplification was carried out in a thermal cycle .

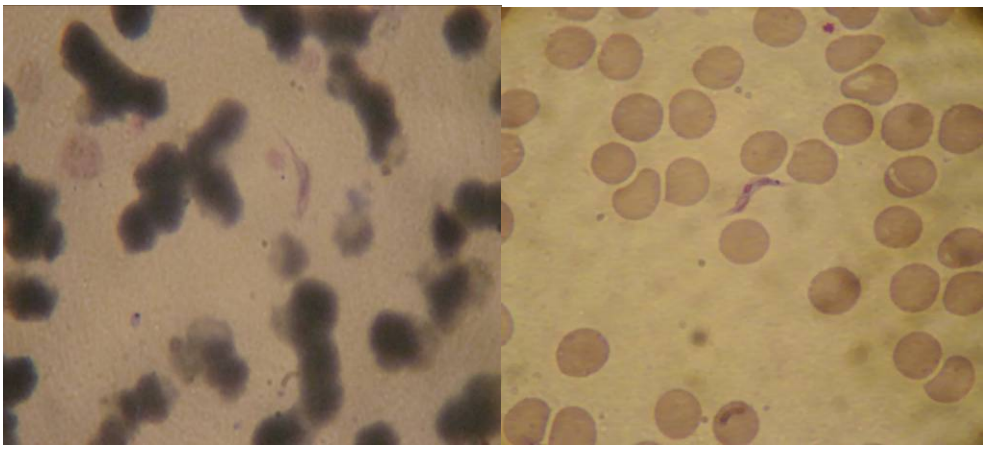
The final reaction volume was 25 µl. For detection of amplified product, 5 µl of the PCR product was electrophoresed on a 1.5% agarose gel with Tris borate-EDTA as the running buffer (30 min at 50 V) and a 100-bp DNA. The gels were stained with ethidium bromide (5 µl/100 ml of gel) and analyzed on a UV transilluminator to visualize the expected size (239- bp) product.

3. Results

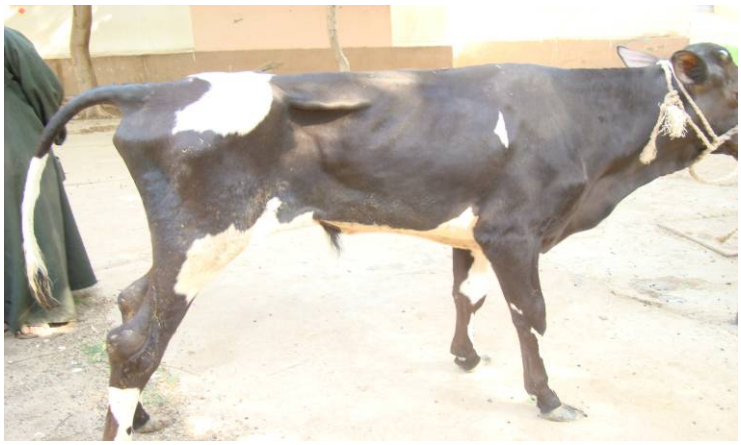
table (1) showed that total number of 131 animals from cattle and buffaloes1.5:5 years old were clinically and microscopically examined with special attention to signs related to T.evansi infection as pyrexia, parasitemia, progressive emaciation , and recurrent episodes of fever which were recorded in 51 animals as shown in fig (2). Microscopic examination of blood films revealed that T.evansi is an extra cellular motile with rapid twisting motion that stain bluish with red nucleus by Giemsa stain fig (1). Among contact animals (80 animals), 5 animals were infected and the others were micoscopically negative.On the other hand when performing PCR for these animals, 35 animals confirmed to be infected with T.evansi as in figure (3) where they lacking the clinical signs of Trypanosomiasis and were recorded microscopically negative .

Table(1)Comparative study of techniques between infected animals with T.evansi

Total number of examined animals(cattle and buffaloes)	Infected animals showing clinical signs		Animals apparently healthy in contact with infective animals (suspected infection) (80 animals)			
			Microscopic examination		PCR examination	
Examined animals	No.of +ve	Rate	No.of +ve	Rate	No.of +ve	Rate
131	51	38.9%	5	6.3%	35	43.6%



Fgure (1) Giemsa stained blood film showed trypanosome bluish with red nucleus



Figure(2) showed clinical signs of trypanosomiasis :
1-emaciated ,pyrexia and anemia 2-off food weakness and edema in different places

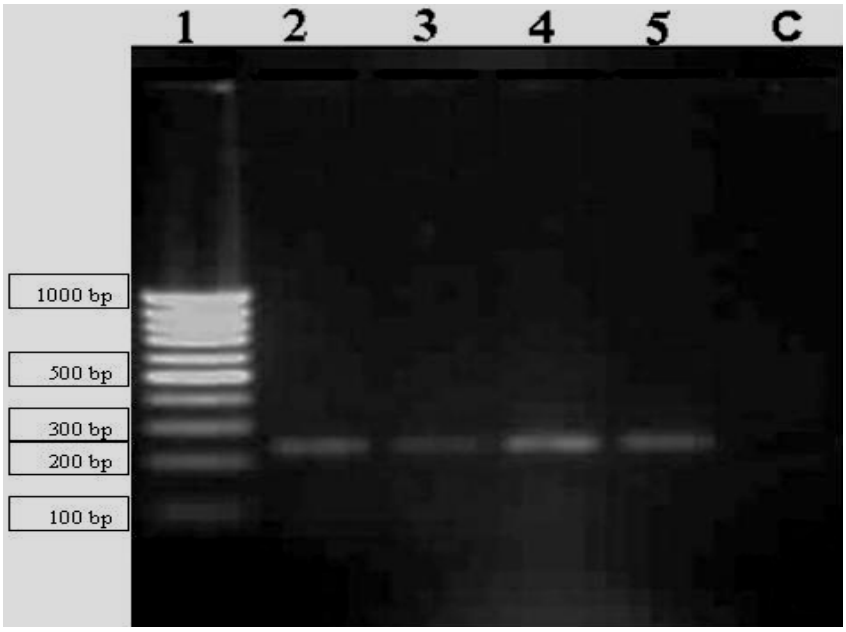


Figure (3) Electrophoresis gel(1.5% agarose,stained with ethidium bromide showing lanes from left to right 1.100-bp DNA ladder ; (2-5),Natural T.evansi infected blood of cattle(C), negative control .

4. Discussion:

Bovine trypanosomiasis is blood parasite disease that imposes large economic losses such as drop in milk yield, meat production, irregular estrous, abortion, stillbirth in cow and low quality semen in bulls (Wasana et.al.2000 and Ravindran et .al 2008).It also cause haematological and biochemical changes in calves (Hilali et al ; 2006)

Concerning the clinical signs, the affected cattle and buffaloes with T.evansi were suffered from pyrexia, parasitaemia, emaciation, generalized edema and recurrent episodes of fever occur during course of disease these results came agreement with (El Sawalhy 1999)

The method of choice to detect T.evansi in blood of infected animals especially in acute cases was blood film examination In the present work, examination of Giemsa stained blood film revealed T.evansi belong to the subgenus trypanozoon, the organism is an extra cellular motile (rapid twisting motion)that stain bluish with red nucleus by Giemsa or leishman stain (Aradaib and Majid,1990)

Blood smear examination proved to be of limited value in diagnosis of subacute on chronic cases. In this study only 5 animals were positively identified by microscopic examination out of 80 samples were apparently healthy in contact with infected animals this result in agreement with Herbart and lumsden (1976) who found that when parasites number less then2,500,000 parasites per ml present in blood samples ,microscopic detection is not feasible

The PCR technique as used in this study detected the low parasitaemia and suspected Trypanosomiasis infection using specific trypanosome primer (T.evansi) . PCR has some major advantages over the parasitological techniques; sample processing does not have to be done within minimum time after collection but can be delayed for some time after preservation at -20 °C. The PCR technique has been verified on blood samples of infected cattle, confirming its higher sensitivity and specificity when compared to parasitological techniques (Clausen, 1998). The PCR technique is accurate, more sensitive and specific method in diagnosis of trypanosomes infected cattle than other parasitological methods and overcome the problem of non specific reaction in case of serological tests; it can detect low parasitic cattle in the chronic cases. The strength of PCR was shown in detection of infection in a parasitaemic cattle showing clinical signs of diseases and was negative using parasitological tests.

The PCR test showed the best sensitivity compared with parasitological methods using classic investigation methods. Use of PCR as accurate and specific diagnostic technique, so treatment has to be carried out immediately in the field (Holland et.al.2004) facilitate control programming. PCR assays for diagnosis of trypanosome infection in cattle were evaluated for their ability to detect trypanosome DNA in blood spots samples collected from cattle in four different provinces from the Bolivian lowlands and the results compared with those obtained with standard parasitological Micro Haematocrit Centrifugation Technique (MHCT) and stained smears and serological methods (Card Agglutination Test for *T. evansi* and Antibody ELISAs for *T. vivax* and *T. congolense*). Kappa agreement analysis showed a significant agreement between PCR assays and results from parasitological methods. Results from PCR assays for *T. vivax* and *T. evansi* were combined with results from parasitological and serological assays to provide information on prevalence rates for the four provinces from where the samples were obtained. (Gonzales et al, 2003*).*

In this study out of 75 samples, PCR revealed that 35 individuals were confirmed to be infected with *T. evansi,* where they were lacking the clinical signs of Trypanosomiasis and negative by microscopic examination so this animal may be in early stage of subclinical and chronic infection.

5. Conclusion:

Early detection of T.evansi play an important role in epidemiology, control program of disease and application of treatment

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6. References:

1. El Sawalhy A.A. (1999) Veterinary Infectious Diseases Second Edation ,July 22,.I.S.B.N 977-19-8617-1
2. Barrent,B.M. (1965)the chemotherapy of babesia bigemina infection in cattle . Res.Vet. Sci.,6: 397-415 .
3. Clausen, P.H. (1998): Use of a PCR assay for the specific and sensitive detection of Trypanosoma spp. in naturally infected dairy cattle in peri-urban Kampala, Uganda. In: Jongejan, F., Goff, W., Camus, E. (Eds.), Tropical Veterinary Medicine: Molecular Epidemiology, Hemoparasites and their Vectors, and General Topics. Ann. NY Acad. Sci. 849, 21–31.

4. Coles,C.A. (1986) . Vetrinary clinical pathology, 4th Ed-W.B.saunders companr Philadelphia and London.

5. Earadaib,I and Amajid ,A.(2006): Asimple and rapid method for detection T.evansi in the dromedary camel using a nested polymerase chain reaction. Kinetoplastid Biology and Disease, 5: 2.

6. El-metanawy,M.T.,Nadia,M.E.,Abdel-Aziz,M.M., Hassanane,M.S. andAbde Aziz,T.H. : (2009) Comparative studies on diagnosis of trypanosome evansi in experimentally infected goats Global veterinarian 3(4)348-353,2009

7. Gonzales J. L., Jones,W.T., Cozzi,P.K. and Cuellar H.R. (2003): Evaluation of a polymerase chain reaction assay for the diagnosis of bovine trypanosomiasis and epidemiological surveillance in Bolivia Kinetoplastid Biology and Disease 2:8.

8. Herbert W. J. and Lusden H.R (1976) Trypansoma brucei: arapid matching methods for estimatig the host parasitaemia .exp. Parasitol.40, 427-431

9. Higuchi, R. (1989): Simple and rapid preparation of samples for PCR. In: Ehrlich (Ed.), PCR Technology, Stockton Press, New York, 31-38.

10. Hilali,M.,Abdel-Gawad,A.,NassarA., Abdel-wahab,A. (2006) : Hematological and bochemical changes in water buffalo calves (Bubalus bubalis) infected with Trypanosoma evansi. Vet. Parasitol. 139 (1-3): 237-
43.

11.Holand,W.,Than,N.G.H.,MY,L.N.,Do,T.T.,Deeris,G. M. and .verckuysse,J. (2004) .Prevalace of T.evansi in water buffalos inremote areas in northern Vietnam using PCR and seriologically methods .Trop.Animals Health Prod 36,45-48 .

12. Holland,W. G.,Claes,F., My, L. N.,Than, N.G., Tam,P.T.,Verloo,D., Buscher, P., Godderis , B . and Vercruysse, J . (2001). A comparative evaluation of parasitological tests and aPCR for Trypanosma evansi diagnosis in experimentally infected water buffaloes.Vet.Parasitol. 97: 23–33.

13. Mottelib, A. A., Hosin, H.I., Mould,I., El-sherif, A.M.,Abo-Zeid (2005): comparative evaluation of various diagnostic techniques for T.evansi in naturally infected camel ISAH -Warsaw Poland vol 2 .

14.Ravndran,R.,Rao,R.,Mishra ,K.M., Path,M.L.K. ,Babu,N. , Satheesh,C.C. and Rhul,S. : (2008) . Trypasoma evansi in camel, Donkey anid dog in India .Comparison of PCR and light microscopy for detection –short communication; Vetrinarski Arhiv 78(1), 89-94.

15.Sehgal, R. N., Valkiunas,M.G.,Iezhova,T.A. and Smith,T.B. (2006). Blood parasites of chickens in Uganda and Cameroon with molecular descriptions of *Leucocytozoon schoutedeni* and *Trypanosoma gallinarum*. Journal of Parasitology 92: 1336–1343.

16.Sukhumsirchart,W.,Khucarcontaworn,S.Sarataphan, N., Viseshakul, N., Chansiri, K. (2000) Application of PCR based aassay for diagnosis of T.evansi in different animals and vectors.Trop Med. Parasitol, 23:1-6