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ASSESSMENT OF *TRYPANOSOMA EVANSI* INFECTION IN CAMELS HERD FROM GEDARIFF AND KORDOFAN STATES

Hamid I. M. N. Croof[2]; Hamid S. Abdalla[1] ; Nahla O. M. Ali [1*]

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, SUDAN.
2. Ministry of Animal Resources, Gedarrif State. Gedarrif, SUDAN.

المستخلص

اجرى البحث الحالى لدراسة تقييم البوليميريز التفاعلى التسلسلى (PCR) كإختبار تشخيصى حقلى للعدوى بالتربانسوما ايفانساى فى الابل فى كل من شرق وغرب السودان مقارنة مع الاختبارات بالطرق الباراسيتولجية والسيرولجية.وهو من أهم الامراض التى تصيب الابل فى السودان. أهم اهداف هذه الدراسة تقييم مقدرة كفاءة البوليميريز التفاعلى التسلسلى فى تحديد طفيل التربانسوما و معرفة الخصائص الجزيئية للطفيل المستخدم من القضارف شرقا و كردفان غربا. اجريت إختبارات تشخيصية مجهرية للمرض شملت المسحة الرطبة و المسحة المصبوغة و الغشاء الابيض. اجرى المسح بغرض التعرف على الطفيل المسبب للعدوى بمرض التربانسوموسس في المضيف الطبيعى (الإبل) و قد أختيرت العينات عشوائيا و جمعت فى اوراق الترشيح ليتم استخلاص الدى ان اى (DNA) ليستخدم فى تحليل البوليميريز التفاعلى التسلسلى. و يتم تمييز خصائص طفيل التربانسوما ايفانساى باستخدام انواع متخصصة من البوادى. و هنا يتم تفكك عشوائى للتركيب الشكلى (لل الدى ان اى) ليستخدم للمقارنة في التركيبة الوراثية للتربانسوما ايفانساى التى تم عزلها و المستجلبة من عدوى طبيعية للأبل . وقد اجري التشخيص لعدد 600 من الإبل و قد اظهرت النتائج حساسية عالية و تخصص نوعى لفحص البوليميريز التفاعلى التسلسلى (90%) و بدرجة اقل حساسية للاختبارات

[*] ***Corresponding author:** Nahla O. M. Ali**,** Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, Shambat Campus, P. O. Box 32, Khartoum North 13314, SUDAN

Tel: (+) 249 922 579271, Fax: (+) 249 185 312638, E-mail: dr_nahla2004@yahoo.com

السيرولجية (CATT) والمجهرية: المسحة المصبوغة (TSF) والغشاء الابيض (BC) والمسحة الرطبة (WSF).
تناولت الدراسة أيضاً تقصى حقلى (إستبيان) شمل حركة هذه الحيوانات - العلامات التشخيصية العيارية - الأمراض الأخرى المتواجدة فى المنطقة - الحيوانات الأخرى التى ترعى مع الابل - الخدمات البيطرية المتوفرة ومستواها والأدوية المستخدمة بالاضافة للمشاكل التى تواجه الرعاة و مقترحات حلولها. هذا البحث يعطى صورة عامة عن مستوى حدوث المرض خاصة وانه لا توجد اى دراسة جينية مؤسسة و مستحدثة عن الطفيل فى السودان. كما إنه يؤطر لصياغ استراتيجية وبرنامج مكافحة كيموثيربيوتيكية (دوائية). وعموماً فإن هذه الإختبارات الجزيئية تمكن من التشخيص للعدوى بالتربانسوما وهذا ما لا تستطيعه طرق التشخيص الطفيلية المجهرية و السيرولوجية. كما ان هذا الاختبار لايستخدم للتشخيص الجزيئى فحسب بل بصورة مثلى فى الدراسات الوبائية، تصميم البرامج العلاجية وبرامج السيطرة على المرض.

Abstract

Trypanosoma evansi infection is the most important disease of Camel in the Sudan. The objective of this research was to give an account of the Camel current situation in the two study areas. However, Comparison between conventional (parasitological), serological (Card agglutination test) and molecular (PCR) techniques in diagnosis of *T. evansi* in camels, were carried out from a total of 600 natural hosts (Camels). The obtained results showed that PCR has higher sensitivity and specificity (90%) than others techniques. This work has provided information on the prevalence of the disease; therefore, certain steps should be taken for treatment and designing rational Trypanosomiasis control program as well as it will be useful for any epidemiological study. The Questionnaires results showed the current situation of Camels in Al-Showak and Al-Obeid areas, concerning the migration of animal, movement pattern, sings of clinical

diagnosis, other diseases, other grazing animals, veterinary services, problems facing the owners and their solutions. To improve the health and productivity of Camels in Sudan, these findings should be taken in consideration by the authorities.

Key words: *Trypanosoma evansi* – Camels - Gedariff - Kordofan

Introduction

Trypanosomosis due to *T. evansi* is a chronic wasting disease characterized by intermittent fever $(38.5 - 40)^0$C, anaemia, fluctuating parasitaemia, emaciation, weakness with pale in the mucous membranes and dry coat (Syakalima, 1992). The animal stands with its head hanging forward. Its eyes turn dull and are closed with considerable amount of tears (Karram *et al*., 1991). Diagnosis of *T. evansi* infection usually starts with clinical symptoms or the detection of antibodies to *T. evansi*. Conclusive evidence of *T. evansi* infection, however, relies on detection of the parasite in the blood or tissue fluid of infected animals. Unfortunately, parasitological techniques cannot always detect ongoing infections as the level of parasitaemia is often low and fluctuating, particularly during the chronic stage of the disease (Nantulya, 1990).

Camel Trypanosomosis has been reported to be transmitted mechanically by a number of species of haematophagous biting flies including the genera *Tabanus*, *Stomoxy*, *Lyprosia* and *Haematobia* (Diptera) (Rutter, 1967; Scott, 1973). In the Sudan tabanid flies play an important role in

the mechanical transmission of animal Trypanosomosis (Karib, 1961). They were also considered as major cause of the seasonal migration of cattle from the South to North during the rainy season (Kheir, *et al*., 1995).

The aim of this study was to assess *T. evansi* infection in camels herd in Gedariff and Kordofan states by comparing conventional and molecular methods of detection, to assess the situation of the disease in the two geographically different regions of the Sudan.

Materials and Methods

Study area:

El-Showak is a research station that belongs to the Camel Research Centre of the Faculty of Veterinary Medicine University of Khartoum (CRC). It is a focal point for Camel pastoralists in Butana area. Being a collection point, it becomes an important Camel market in the region. Butana is situated well within the arid zone of the Sudan and occupies an area of approximately 120000 km^2 and lies between latitude 13^0 4' N to 17^0 50' N and longitude 32^0 to 36^0 E. Most of the Butana is series of flat easily flooded plains interspersed by few hills. The prevailing climate is warm in summer which extends most of the year (March-October) and includes the rainy season (June-September). The vegetation composed of *Aristida spp*. (Gow) *Cymbopongon nervatus* (Nal); *Acacia mellifera* (Kitir); *Calatropis procera* (Usher); *Capparis deciduas* (Tunduub) and a variety of grasses (Abdalla, 1985). Normally the Camels and their owners

move / migrate in search of water and grasses eastward to the Ethiopian borders, where the Tsetse flies *Glossina fucipes* is reported (Kheir *et al.,* 1995). Figure (1) shows the Map of the El-Showak study area.

El-Obied is the capital of Northern Kordofan. It is about 400 Km West of Khartoum. It is one of the largest animal resources centers in the Sudan. The region is dry with sandy soil and the *Acacia spps*. are the dominant trees. The nomads in the dry season (summer) migrate southward in search of water and grasses and in the wet season (autumn) with the muddy conditions and the flies they move northward. Therefore, in the hot season they are forced to enter tsetse area south of Bahr el Arab, rendering animals to tsetse challenges (Abdalla, 1999). Figure (2) shows the Map of the El-Obied study area.

Study Subjects:

The survey was conducted in camels owned by pastoralists. From the beginning they knew that their animals would be examined for diseases and that they would be treated without charges, so they readily cooperated. The local authorities had given full support and all possible facilities were made accessible throughout the whole study.

Study design:

Since little information is available on the situation of Trypanosomiasis in camels in Western Sudan, thus a cross-sectional survey was performed to determine the prevalence of the disease and to provide baseline data. In additional to that, treatment of infected herds was performed and

interviews with owners were carried out to gain insight in their socio-economic system and the problems they face under the prevailing conditions of drought and desertification.

Sample Size:

The Eastern and Western regions hold about 2/3 of the animal wealth in Sudan. Out of 3 million Camels in Sudan, there are about 2 million in these two areas. Consequently sample size was sufficient enough to cover large number. A number of 500 Camels (*Camelus dromedaries*) from Eastern region (El-Showak – Gedarif State) and 100 Camel from Western region (El-Obeid – North Kordofan State) were examined in this survey during the period 2005 - 2007 for *T. evansi* infection. Those camels were of different age and sex groups. The animals were selected on the willingness of owners to participate in the study.

Methods used:

The tests used in this study were Wet Smear Film (WSF), Thin Smear Film (TSF) as in figure (3), Buffy Coat (BC) in addition to Packed Cell Volume test (PCV), sero-diagnostic CATT and Molecular diagnosis DNA amplification by Polymerase Chain Reaction (PCR). All these techniques were compared for their sensitivity. The number of samples tested by each method is shown in Table (1). The DNA was extracted as described by Walsh and co-workers (1991) and Wooden and his colleagues (1993) and PCR performed as previously described (Ali, 1998; Ali *et al.*, 2003). Samples collected from eastern region (E15, E16, E18, E19, E21 and E26) were loaded on 1.5% TBE agarose gel and visualized with 1 mg/ml

ethidium bromide under UV light (Figure 5). Samples collected from western region (W40, W41, W42 and W47) were loaded on the same gel for comparison. DNA extracted from experimentally infected rat blood (originally from E20) was used as positive control.

Packed Cell Volume (PCV):

Fresh blood samples from ear vein of Camels were drawn into heparinized capillary tubes (70mm) long and centrifuged in a micro-hamatocrit centrifuge for 5 minutes. The PCV percent was read on the micro-haematocrit reader.

Questionnaire:

Interviews with owners were carried out to gain insight in their socio-economic system and the problems they face under the prevailing conditions of drought and desertification. For this purpose a questionnaire form was made for each camel to be included in the survey. Information such as date – study area – record number – owner's tribe – movement pattern – diseases – diagnosis – treatment – problems, were all contained in the form.

Figure (1): Map of El-Showak study area.

Figure (2): Map of El-Obied study area

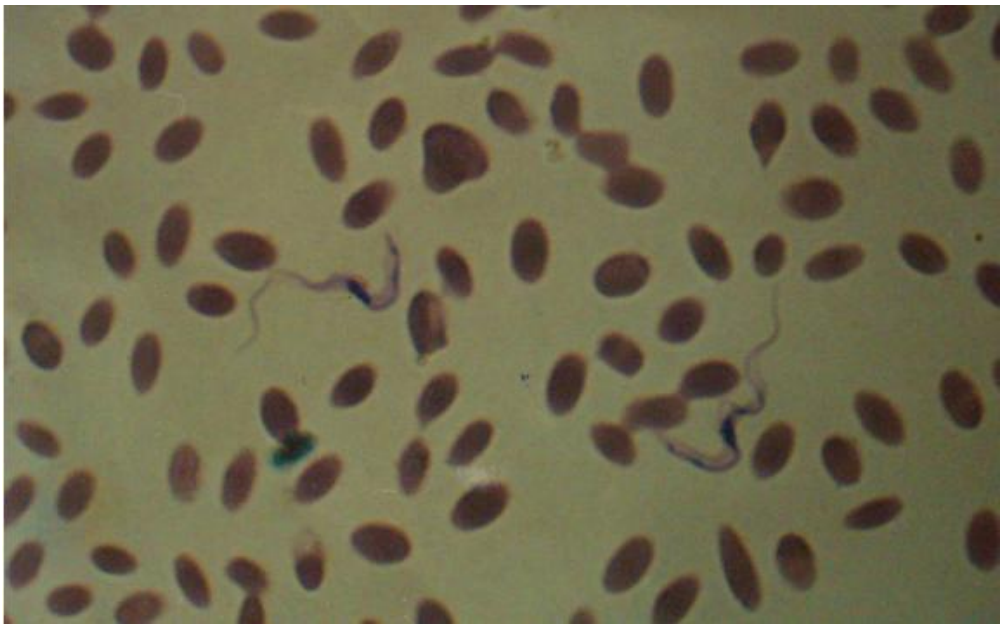

Figure (3): The Geimsa stained smear of Camel infected blood.

Results

Result of the Packed Cell Volume (PCV) for the surveyed Camels:

All the camels examined had PCV above 20%. 50% of the camels examined had a PCV value that lies between 35 and 40. The results were shown in figure (4).

Result of the Polymerase Chain Reaction (PCR) for surveyed Camels:

As shown in figure (5) a 200 bp DNA fragment was amplified by PCR from DNA extracted from suspected samples collected from both Eastern and Western regions. TB-01 (Forward) and TB-02 (Reverse) set of oligonucleotides primers were used in this PCR analysis. A small size DNA fragment approximate 100 bp appeared nearly on all analyzed samples these gave positive result with PCR. All trials of PCR optimization were unable to get rid of it, thus it may not be a non-specific binding or primers dimer.

Result of Questionnaire:

El-Showak and EL-Obied are the two localities where the surveys were carried out. With respect to the type of migration of the studied animals, the condition and situation of herd were illustrated in figure (6), and the direction of movement is shown in figure (7).

The drugs given to treat Camel Trypanosomiasis were 66.7% quinapyramine sulphate and 33.3% dimenasine aceturate as concluded from the questionnaire.shown. Other animals grazing with Camels

according to the owners' were shown in figure (8). According to the owners', there are different types of Veterinary services available for them, this shown in figure (9). These services level as stated by the owners' either fair (83.3%) or good (16.7%). Main problems facing Camel breeding are: Lack of settled herds (camp), insufficient health care, confiscation of grazing lands, lack of water resources, taxes and decree, firm regulation that obstacle movement, lack of grazing route and ticks infestation that represent problem in all of the surveyed herds.

Table (1): *T. evansi* infection rates among camels examined by parasitological, serological and molecular techniques.

Technique	Examined Camels	Infected Camels	Percentage (%)
Wet Smear Film (WSF)	600	10	01.7
Buffy Coat (BC)	600	22	03.7
Thin Smear Film (TSF)	600	36	06.0
CATT	210	100	47.6
PCR	40	36	90.0

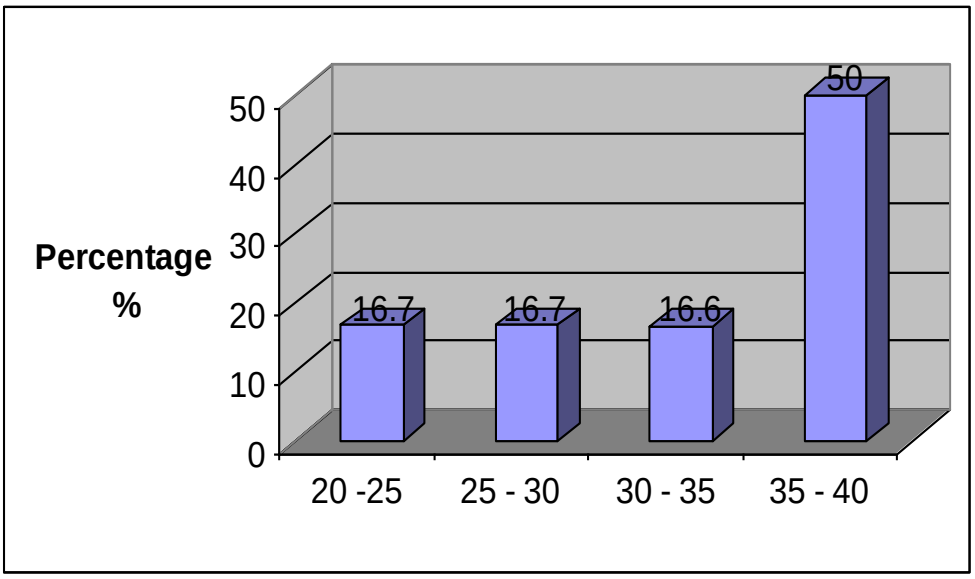

Figure (4): The PCV values of the camels selected for this study.

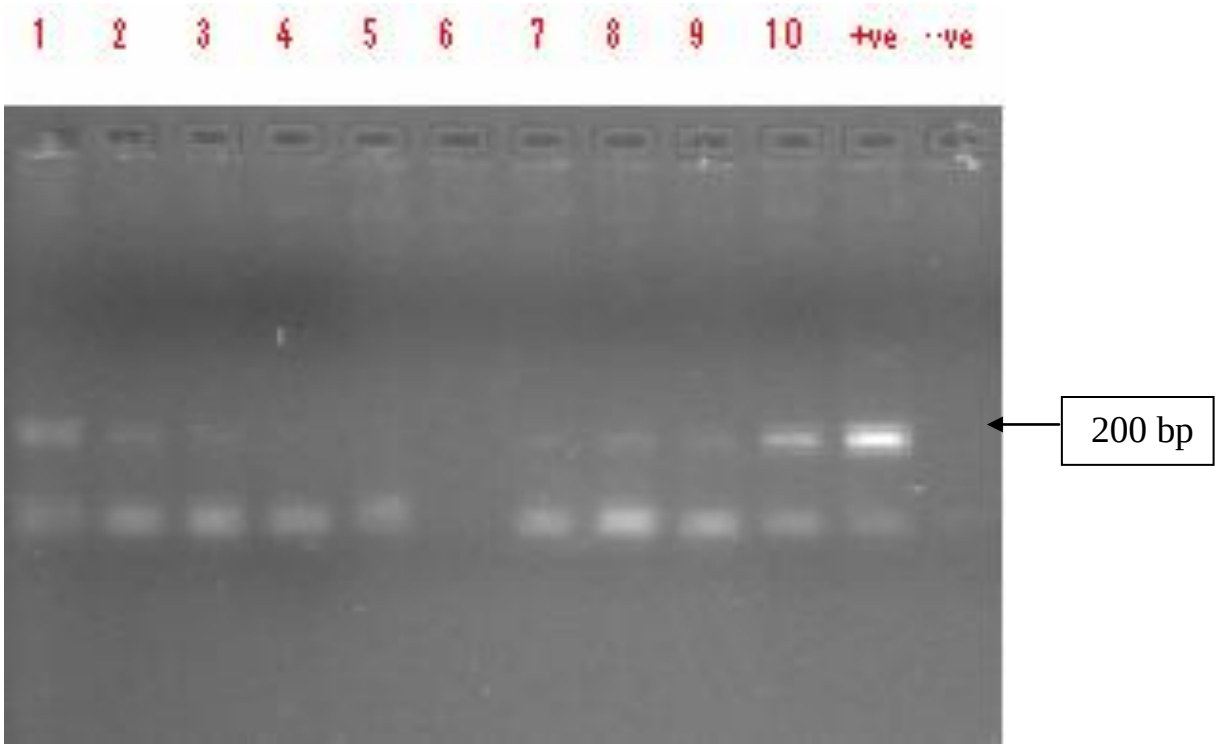

Figure (5): PCR analysis of some of the suspected samples

Samples were loaded as follows: Lanes (1-6) were (E15-E16-E18-E19-E21-E26) from Al-Showak region; lanes (7-10) were (W41-W42-W40-W47) from Al-Obeid region; lanes (11-12) were positive and negative control, respectively.

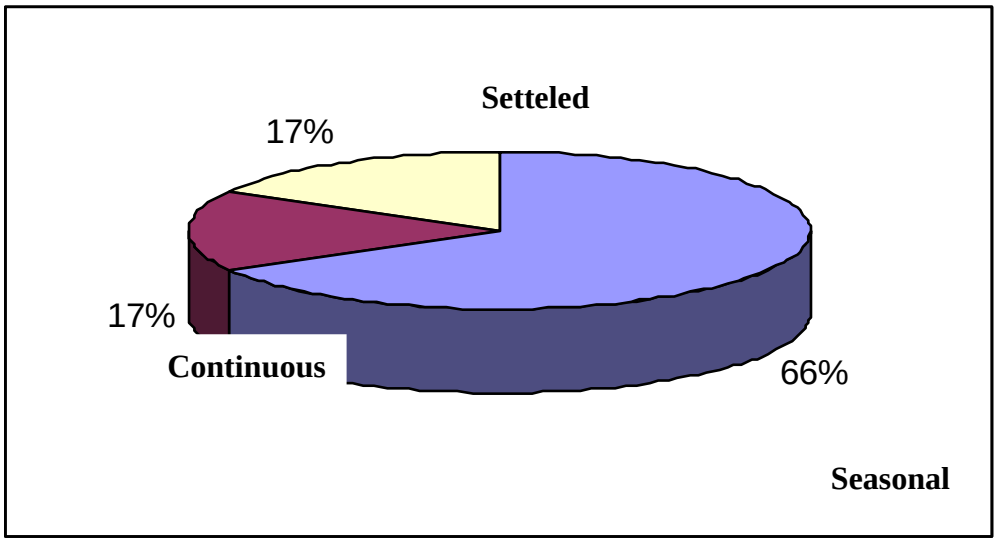

Figure (6): Migration pattern of the studied camels throughout the year.

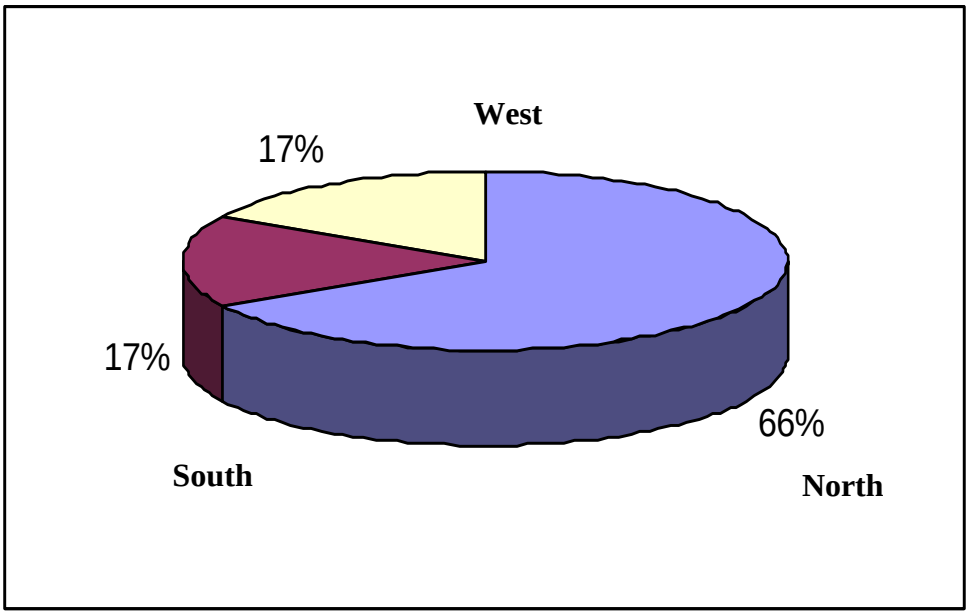

Figure (7): Movement pattern of the studied camels throughout the year.

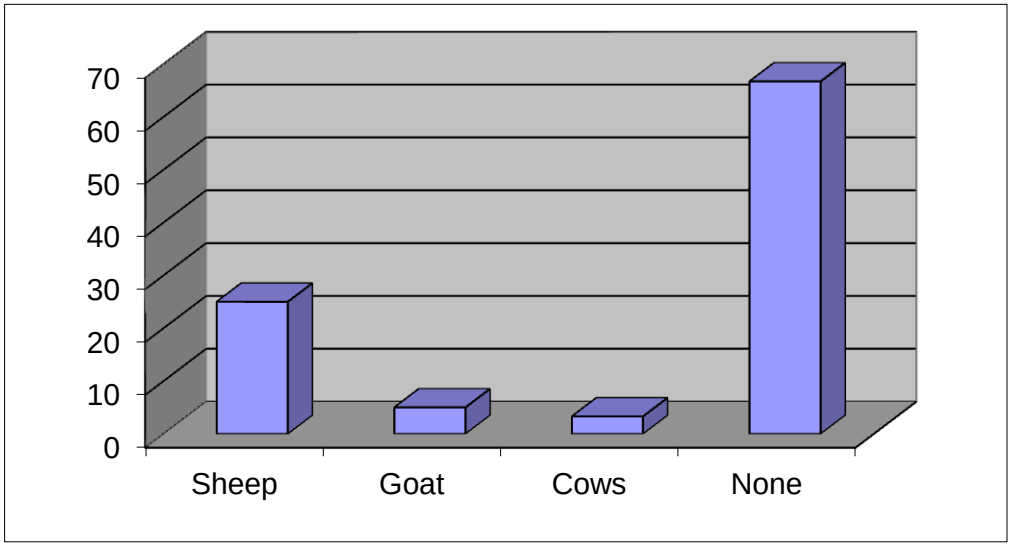

Figure (8): Types of Animals Grazing with Camels in the study areas.

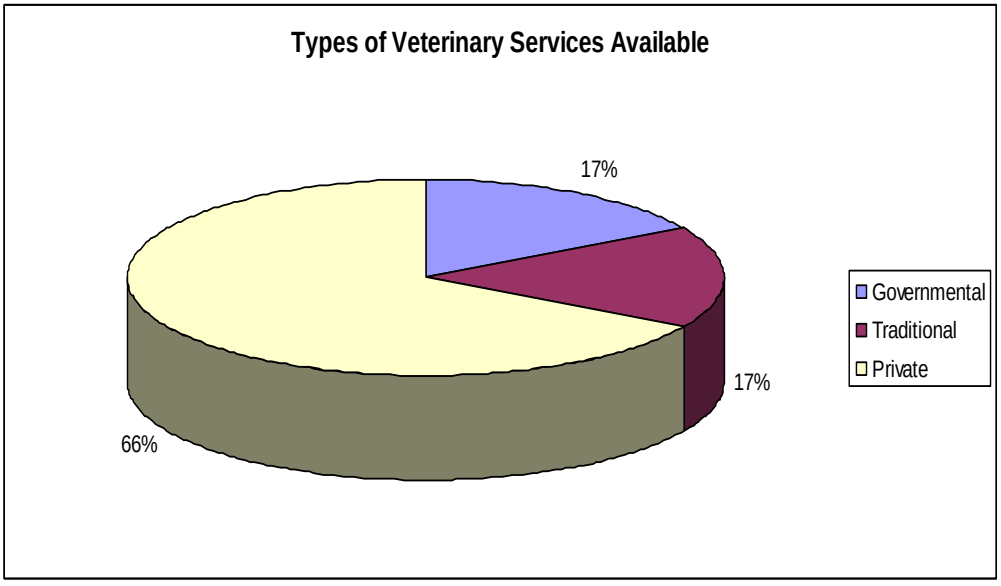

Figure (9): Types of Veterinary Services Available.

DISCUSSION

Camel Trypanosomiasis is regarded as a major constrain for Camel health and productivity in all camel rearing areas of the World (Gibson *et al*., 1983; Diall *et al*., 1993; Elsaid *et al*., 1998; Dia *et al*., 1997; Atarhouch, 2003). Diagnostic testing is the key for effective control of *T. evansi*. The results obtained during this study using parasitological techniques, showed *T. evansi* prevalence rate as follows: 1.7%, 3.7%, 6% were positive using Wet Smear Film (WSF), Buffy Coat (BC) and stained Thin Smear Film (TSF) respectively. This result is similar to the finding reported by El-Amin and co-workers (1998) in Butana using the same diagnostic techniques.

In the serological survey 210 Camels were tested, and 47.6% were found positive using CATT methods. This result is in agreement with that obtained by Njiru and his colleagues (2004) in Kenya, using CATT technique in a total of 549 Camels which randomly sampled. They found that the overall prevalence of "Surra" was positive at 5.3% using MHCT, 26.6% using PCR and 45.9% using CATT/T. evansi. Another study conducted by EL Said and co-workers (1998) in Sudanese Camel exported to Egypt using CATT technique showed that 28% of the Camels were positive for *T. evansi*. The result of PCV showed that all the surveyed camels had normal values that mean almost all camels were in good health condition. This observation suggests that the *T. evansi* infection detected by the different diagnostic methods was chronic. A

small size DNA fragment (approximate 100 bp) appeard nearly on all analysed positive samples by PCR. All trials of PCR optimization failed to delete it suggesting that it could not be a non-specific binding or primers dimer. It might be due to the fact that the amplified region exists in two copies in the genome of *T. brucei* or *T. evansi.* This idea could be tested by southern blot analysis, this is beyond the scope of this study.

This study shows that the majority migrations of owners and their animals are seasonally to all directions. The reasons for that could be lack of water and grazing, and existence of ticks and biting insects in certain season and area. Most of tribes studied were in the south region where the circumstances around them represent the main reason for migration into the north or west.

In this survey, and in the absence of other diagnostic technique, the camel owner and ethno-veterinarians rely on the smell of urine for diagnosis of *T. evansi* infections. This practice is based on the fact that specific urine smell is produced after break down of amino acids by the parasite in infected animals (Hunter, 1986). During this study, the majority of the owners recognized *T. evansi* infection through sings of stretching of neck, dullness and urine odour, while few of them observed other sings of Trypanosomiasis in addition to these signs.

In this study the common drugs used by the owners were only, Quinapuramine and Dimenazine Aceturate (Brenil). The dependence of owners on these two drugs for long time may lead to drug resistance. In the field, the existence of drug resistant Trypanosomes can be inferred

when Trypanosomes reappear in an animal blood following treatment. However, such inference may be compromised if animals are re-infected following treatment. At present, definitive diagnosis of drug-resistant infections depends on characterizing the Trypanosomes populations in the laboratory. For such purposes, both *in vitro* systems (Sutherland *et al*., 1993; Zhang *et al*., 1991; Zhang *et al*., 1993) and *in vivo* systems (Osman *et al*., 1992; Zhang *et al*., 1991; Zhang *et al*., 1993) can be used.

During this study some diseases were reported to be endemic with Trypanosomiasis. These were internal parasites like *Haemoncus contortus* and Hydatidiosis (Abufishaifish) especially in Western Sudan, the logical explanation could be that several animals species graze with camels including; sheep; goat and cattle. This may be a constant threat to camels and other animals especially when they are grazing with another group of animals on their movement. In other words, these grazing animals could serve as a reservoir for *T. evansi* and other wide host range diseases, or could acquire infection from camels and transfer it to other animals. This suggestion is in consistent with that reported earlier by Mahmoud and El Malik (1977), when they stated that as long as biting flies of Tabanids and Stomoxyins are abundant, one expects those goats, and sheep's harboring *T. evansi*; would constitute foci of infection (reservoir). Moreover, Boid and co-workers (1981) reported that, the existence of antibodies against *T. evansi* in sheep and goats pointed to their role as reservoirs for the disease.

The respondents answers showed the existence of several problems facing the owner as indicated in the questionnaire were considered as obstacles to the development of animal resource in Sudan especially Camel breeding. The expansion of the mechanized farming projects at the expense of the pastures and the grazing land, the heavy taxes are all serious problems. Heavy ticks infestation that affects domesticated mammals and tick born disease had their health and economic effect on man and his domestic animals. The competition over land for grazing and farming creates conflicts between farmers and pastoralists and it is becoming a major cause of civil unrest.

For the livestock in the Sudan there are, 80% of the national herd at risk of Trypanosomiasis. African animal Trypanosomosis affects the entire economy of livestock-agricultural production in vast zones of Tsetse-infested areas. The presence of the disease influences the sites for settlement of communities. Trypanosomosis has, therefore, cultural and socio-economic implications on the day-to-day life of the rural communities living in affected areas.

RECOMMENDATIONS

This study recommends that the PCR technique should be adopted as a routine method for diagnosis of *T. evansi* infection in Camels in all research centres of the Sudan. This implies that the research centres namely El-Showak and Al-Obeid should be supplied with all the facilities to conduct PCR analysis, those including trained people, reagents and

machines which are affordable either to the Government or to the private sector.

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