Available online at www.scientiaresearchlibrary.com

Scientia Research Library

ISSN 2348-0416
USA CODEN: JASRHB

Journal of Applied Science And Research, 2017, 5 (2):12-23

(*http://www.scientiaresearchlibrary.com/arhcive.php*)

Prevalence and Risk Factors Associated with Bovine Trypanosomosis Using Conventional Methods and Polymerase Chain Reaction (PCR) in the Blue Nile State, Sudan

Khalda Abbass Elkhazeen[1], Rihab Ali Yaji[2],Diyaa Eldeen Ahmed Salih[2], Rihab Ali Omer[3] and Atif Elamin Abdelgadir[4*]

[1]*Ministry of Agriculture and Animal Resource, Blue Nile State, Sudan*

[2]*Central Veterinary Research Laboratory, Soba, Sudan*

[3]*National University, Khartoum, Sudan*

[4]*Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, Sudan*

ABSTRACT

This study was conducted to determine the prevalence and associated risk factors of bovine trypanosomosis in cattle using conventional methods and Polymerase Chain Reaction (PCR) in the Blue Nile State, Sudan. The study was conducted in five localities (Ad-Damazin, Al- Rosayris, Qissan, Baw and AL- Tadamon) during rainy season and winter in the year of 2014. A total of 300 cattle were selected using random sample method. Hundred samples from resident group (Kennana) (70 females and 30 males), while 200 samples were collected from nomads group (Kennana: 10 females and 20 males and Fulani: 107 females and 63 males). All samples were collected from age groups 1-3 years and greater than 3 years. Blood samples were collected and examined for the presence of bovine trypanosomosis using PCR techniques and parasite detection tests (wet smear, thin smear, thick smear and buffy coat). The apparent prevalence was as follow, 3 (1%) using wet smear, 3(1%) thin smear film, 2 (0.67%) thick smear film , 3(1%) hematocrit centrifugation technique. T. vivax was the only spies recorded using conventional methods. Higher prevalence rate was recorded using PCR 35 (11.67%) and T. vivax and T. conglense were recorded by using this method. During the study, the prevalence rate of bovine trypanosomosis by age was 1.67% for age group (less than 3 years) and 10% for the second group (more than 3 years). Sex revealed that 2.33% were positive for males while 9.33% were positive for females. The prevalence rate by breed were found to be 11% for Flata (Ambararo) and 0.67 for Kenana, the data were analyzed using Statistical Package for Social Science Programme (SPSS) and the difference was significant for above mentioned factors as follow, the age (x^2 =7.019, p_ value .006), andbreed(x^2 =16.472, p_ value .000), but no significant for sex (x^2 =2.110, p_ value .104). The study revealed that the PCR is more sensitive than microscopic techniques. Hence,more studies are needed to determine the types of trypanosomes species in the study area, particularly in tsetse infested areas as well as more

research is required to find out the effect of the Rosaries Dam heightening and its effect on trypanosomes vectors occurrence.

Keywords: Trypanosomosis, Conventional methods**,** Polymerase Chain Reaction (PCR), Blue Nile State, Sudan.

._____

INTRODUCTION

Trypanosomosis is one of the important constraints to livestock in tropical Africa. The parasites are transmitted mechanically by biting flies or biologically by the tsetse flies.The parasite can be diagnosed parasitological by direct blood examination under the microscope, serologically by detection of antibodies or antigens in the serum and by molecular biological techniques for detection of the DNA of the organism (OIE, 1996).The disease causes different pathological effects in animals but the most important were are nervous signs, myocardial damages and anaemia(Urquhart, 1980). Human infections of animals species have been reported in India making it a potential human pathogen (Joshi *et al*,. 2005). Practical control strategies of the disease included vector control, parasite control using chemotherapy and chemoprophylaxis in addition to usage of trypanotolerantlivestock breed. Unfortunately, development of practical means of immunization by vaccines were failed due to the phenomenon of antigenic variation of trypanosomes i.e. their ability to vary the antigenicity of their glycoprotein surface coats in the host (Walker, 1986).

The disease surveys in the Sudan indicated high prevalence of bovine Trypanosomosis outside the tsetse belt which was attributed to mechanical transmission by other biting flies(Rahman, 2005). Four economically important animal Trypanosome species were reported in the Blue Nile State, these are *T. vivax, T. congolense,* and *T. brucei*.(Salim, *et al*., 2011) .

Objectives of the study:-

1. To determine the prevalence of bovine Trypanosomosis using conventional methods and Polymerase Chain Reaction (PCR) in the Blue Nile State, Sudan.
2. To determine the risk factors that associated with bovine Trypanosomosis in the Blue State, Sudan.

MATERIAL AND METHODS

Study area:

The study was conducted in Blue Nile State in the Sudan. It covers an area which is estimated at 38.500 km^2 extending between latitudes 9° 30' -12° 35' N and longitude 53° 80' -35° 80' E. The state borders Sinnar State to the north, South Sudan to the south and west, and its shares international borders with Ethiopia to the east. The Blue Nile River transects the state in the north eastern parts for a length of 200 km.

The human population of the state was estimated at 850.750 in 2004with an average annual growth rate at 3.1%.The ethnic groups are comprised of nomadic pastoralist including Fulani groups, Kenanna, Ruffaa and Engasana. These pastoralists own most of the livestock in the breed state.The state is divided into five localities. These are: Ad-Damazin, Al- Rosayris, Al-Kurmuk, Qissan, Baw and AL- Tadamon (Figure, 1).

Identification of animal husbandry and breeding:

The study included animals from the belonging to the two livestock in the study area.These were grouped as follow:-

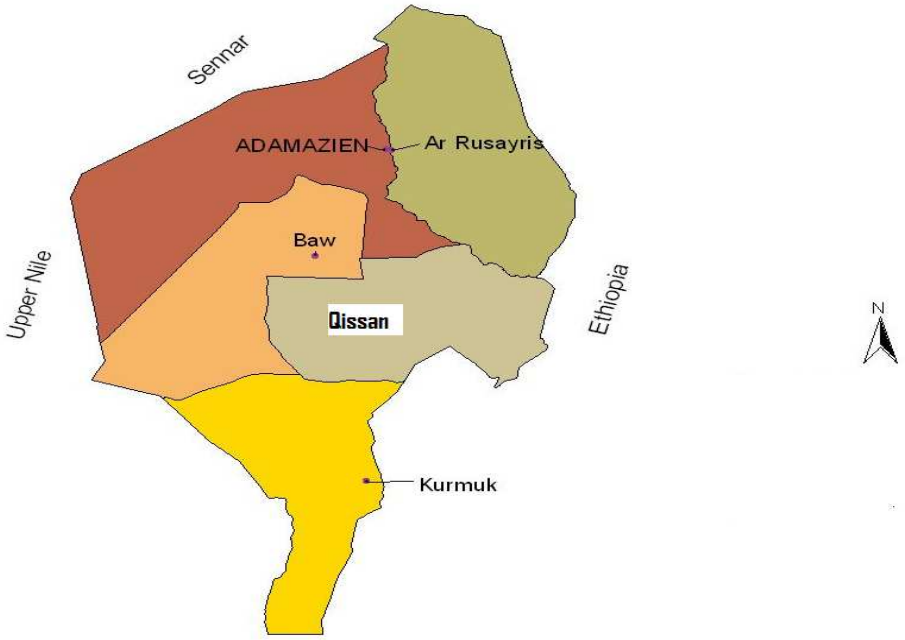
Sedentary system:

A number of 100 heads (70 female, 30 male) from this group were included in the study. All of which were belonging to Kennan and located around the northern and central parts of the state. The livestock owners graze their animals around the lake of Al-Rosayris dam and the areas surrounding the town. These animals were found in some farms in Damazin were kept under zero grazing conditions.

Nomads system:

From this group, a number of 200 heads were included. Kennan (10female, 20male)and Fulani(107female, 63male) herds of this group were having movement to the to the South eastern parts of the state during the dry season until reaching KhorYabus, Ethiopia and some group go up to the Nasir in the South Sudan and along the Sobat river. This movement occurs in (late October and early November).

Figure (1): Blue Nile State Provinces



Source: Ministry of Agriculture and Animal Resource, Blue Nile State, Sudan

Sampling

A total of 300 Blood samples were collected from the jugular vein of cattle in the morning (7. -11. AM) or the early evening (4.30-6. PM) After and disinfecting the area , Blood samples were also taken from the ear vein in filter paper of the same animal In both group age and sex of examined cattle were recorded.

Parasitological methods

Wet smear

A drop of fresh blood was placed in a clean slide and covered with cover slip and then examined under light microscope using medium magnification (objective 40X). Diagnosis of the Trypanosomosis was based on motility of trypanosomes between the blood cells.

Thin blood smears:

A thin smear film was prepared by having a drop of fresh blood on a clean slide.This was then spread by the corner of another slide at 45 C° angle and let to dry in the air. This slide was then fixed by absolute methanol for 4 min and then stained with 10% Gemsa for 45 min. Excess stain was removed with phosphate buffer saline or distilled water, the slide was dried by air and examined under light microscope (objective 100 with immersion oil). This method was used for the identification of different species of trypanosomes depending on their morphology.

Hematocrit centrifugation technique (HCT)

Heparinisedmicrohaematocrit capillary tubes, containing blood samples were centrifuged for 5 min at 12,000 rpm. After the centrifugation, trypanosomes were usually found in or just above the Buffy coat layer.

Dark ground Buffy coat technique

The capillary tube was cut using a diamond tipped pen 1 mm below the Buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed onto a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for the movement of parasite (Paris et al., 1982). Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations provided by OIE (2010).

Packed cell volume (PCV)

Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected directly into a pair of heparinised capillary tubes. The

tubes were then sealed at one end with crystal seal. PCV was measured in a micro-haematocrit centrifuge (Hawksley and Sons, UK). The capillary tubes were placed in microhaematocrit centrifuge with sealed end outer most. Then the tube was loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 min. After centrifugation, the capillary tubes were placed in a haematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic (OIE, 2010).

DNA extraction from filter paper blood samples

Using a 3 mm micro punch, was cut from the filter paper a dried blood spot and placed in a clean 1.5ml ependorf tube. According to the manufacturer's instructions, 180 µL of Buffer ATL were pipetted into 1.5 ml eppendorf tube and incubated at 85C for 10 min. 20 µL proteinase K were added the mixture and was incubated at 56 C for 1h. 200µL AL Buffer after that were added to the mixture and incubated at70 C for 10 min. amount of 200 µL ethanol (96-100%) to the sample the mixture from step 5 was carefully applied to Qlamp Mini Spin column closed the cap and centrifuged at (8000 rpm) from 1 min . The spin column was washed twice using 500 µL of washing buffers W1 and W2, respectively The column was then placed in a clean 1.5 ml eppendorf tube and the DNA was eluted with 150 µL AE or distilled water centrifuged at maximum speed for 1.5 minutes at room temperature, the column was discardedand the purified DNA was kept at -20 oC.

Primers

A list of the primers and the expected size of amplification product obtained with each specific pair of oligonucleotides is given in Table (1). These primers were kindly provided by Bioneer Company.

PCR cycling

PCR amplifications were performed in a 20 ul reaction mixture containing o.5uM FW primer,o.5uM Rev primer, 14 ulH2O, GoTaq (5u/ul The reaction mixtures) . PCR cycles for ITS1 CF and BR primers were: initial step at 94LC for 5 min, followed by 35 cycles of 94LC for 40 s, 58LC for 40 s, 72LC for 90 s, and final extension at 72LC for 5 min

DNA electrophoresis

-2% gel was prepared for small fragments (0.2–1kb) in 1%TBE required (around50-100 mL depending on the tray size and number of samples).

- The first, last or middle well was loaded with a suitable marker according to the expected sample size, usually one base pair

A marker (100 bpFermentas ladder) in addition to the number of samples as well as negative and positive controls were included in the electrophoresis. 14- 16 µL of each sample was loaded in the gel, starting from left to right-Samples were run using a power supply volt of 80-90 for 35-40 minutes.

All PCR were performed at the Central Veterinary Research Laboratory, Soba, Sudan.

Statistical analysis

The data were analyzed using Statistic Package for Social Science Programme (SPSS version 16.0)

Table (1): ITS CF and ITS BR PCR and Nested ITS PCR

Species	Primer sequence	Amplification conditions	Product size
ITS CF and ITS BR PCR (Njiru*et al* , 2005)	ITS1 CF: 5' CCG GAA GTT CACCGA TAT TG' 3 ITS1 BR: 5' TTG CTG CGT TCT TCAACG AA' 3.	94°C for 5min, 94°C/40sec **, 58°C/40sec** Step4- 72°C/90 sec 72°C/5min Step6- Stop reaction(**45cycles)**	*T. congolense*Savannah 700 bp *T. congolense*Kilifi 620 bp Trypanozoon members (*T.brucei*)480bp *T. vivax*250 bp
ITS Nested PCR (Cox *et al*, 2005)	ITS1 (5'-GAT TAC GTC CCT GCC ATTTG-3') ITS2 (5'-TTG TTC GCT ATC GGT CTT CC-3'), ITS3 (5'-GGA AGC AAAAGT CGT AAC AAG G-3') ITS4 (5'-TGT TTT CTT TTCCTC CGC TG-3')	95 °C for 7min (94 °C for 1min, 55 °C for 1min, and 72 °C for 2min) (**35cycles)** 72°C/5min	obtained (bp) *T. congolense*(Savannah) 1413-1408 *T. brucei*1207–1224 -1215 *T. vivax*611- 620 *T. theileri*988- 998

RESULTS AND DISCUSSION

Prevalence of Bovine Trypanosomosis:

The prevalence of bovine Trypanosmosis in study area was found a number of 3 (1%) cattle females all belonging to nomads group were found to be positive for Trypanosomosis by using wet smear thin smear and Buffy coat, this number was reduced t0 2 (0.67%) using thick smear method. Using PCR the percentage of positive animal was increased to 35 (11.67%) from these 22 (7.33%) were genetically allocated to *T.vivax* .Figure (2)**.** and 13 (4.33%) to*congolense*Figure (3). *Spreading* this number to breed of cattle included in the study 130 herd were form to Kennan (80female, 50male) and 170 were from Fulani (107female, 63male)**.**this result are summarized in table (2)

The general condition of the herd reflected by PCV was good during the study where the PCV varied between 21%- 25%. , the general condition was poor and the mean herd PCV varied between 18.5%-20%. The mean PCV in the infected animals is 21.5, while that of non-infected animals was 22.5. There was no significant different between mean PCV of infected and non-infected animals. The results are shown in table (3).

Risk FactorAnalysis:

The prevalence of Bovine Trypanosomosis which were found to be positive for the conventional methods results for sex gave 0% for male and 1% for female; statistical analysis showed that the difference between the two results was not significant (=1.216 p_ value .388) **.**The result of the age groups using thin blood smear test revealed 0% for age 3year and less while it gave 10% positive for animal above 3 years old (=2.858 p_ value .170) (no significance) The prevalence rate for the breed was 0.33% for kenana while, and 0.67% for Falata breed (=2.633 p_ value 0.187) (no significant).this result are summarized in table(4) **.**

The PCR technique results revealed that only 2.33% were positive for male while, 9.33% were positive for female and statistically. The difference was not significant (=2.110, p_ value .104). Age was divided into two groups, the first group was comprised of animals of three years age and less which tested1.67%positive the second group was comprised of animals of more than 3years age which gave 10% positive. The difference statistically was significant (7.019 p_ value .006).The prevalence were found to be 11% for flata (ambararo) and 0.67 for kenana while statistically, the difference was significant (=16.472 p_ value .000).this result are showed in Table (5).

Table (2) Prevalence of Bovine Trypanosomosis usingconventional methods and Polymerase Chain Reaction

Used Method	No. of Percentage Positive
Wet smear , thin smear , Buffy coot	3 (1%)
Thick smear	2(0.67)
PCR technique	35(11.67)

Table (3): Mean packed cell volume (PCV) levels of animals in the Blue Nile State.

No. of examined animals	No. of positive	Tryps spp.			PCV average
		T.vivax	*T. congolense*	*T. brucei*	
100	00	00	00	00	25.6
80	02	02	00	00	18.1
120	01	01	00	00	20.2

Table (4): Association between some factors and Presence of Bovine Trypanosomosis using conventional method

Risk Factors	Number examined	Result of Thin Smear Test		Chi-squire x^2 (P-value)
		Positive	Negative	
Sex 1.Male 2. Female	 113 187	 0(0%) 3(1%)	 113(37.67%) 184(61.33%)	 1.216 .388
Age group 1. 0-3 years 2. More than 3years	 124 176	 0(0%) 3(1%)	 124(41.33%) 173(57.67%)	 2.858 .170
Breed 1. Kenana 2. Fallata	 130 170	 1(.33%) 2(.67%)	 129(42.67%) 168(56.33%)	 2.633 .187
Total	300	3(11.67%)	297(88.33%)	

Table (5): Association between some factors and presence of bovine trypanosomosis using PCR

Risk Factors	Number examined	Result of PCR Test		Chi-squire x^2 (P-value)
		Positive	Negative	
Sex 1.Male 2. Female	 113 187	 7(2.23%) 28(9.33%)	 106(35.33%) 159(53%)	 2.110 .104
Age group 1. 0-3 years 2. More than 3years	 124 176	 5(1.67%) 30(10%)	 119(39.67%) 146(48.67%)	 7.019 .006
Breed 1. Kenana 2. Fallata	 130 170	 2(0.67%) 33(11%)	 128(42.67%) 137(45.67%)	 16.472 .000
Total	300	35(11.67%)	265(88.33%)	

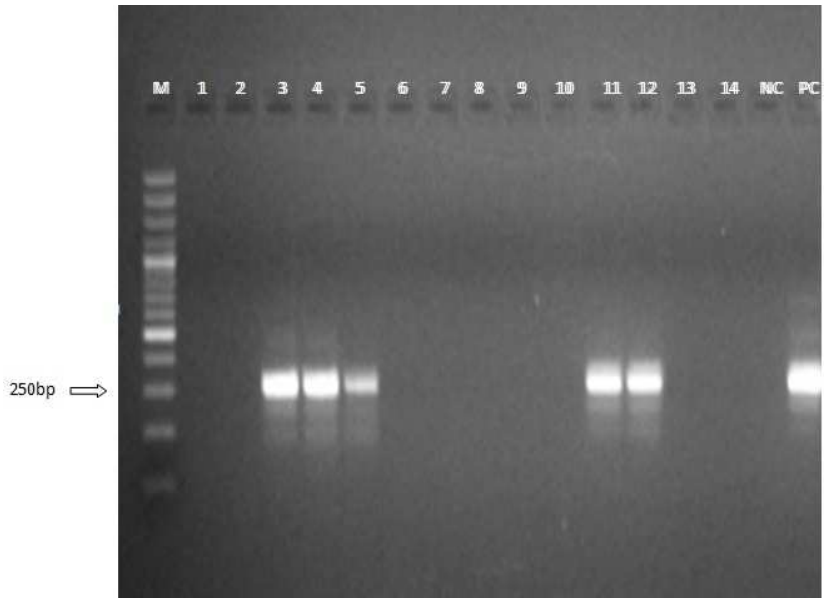


Figure (2): Detection of a 250bp band indicative for*T.vivax*

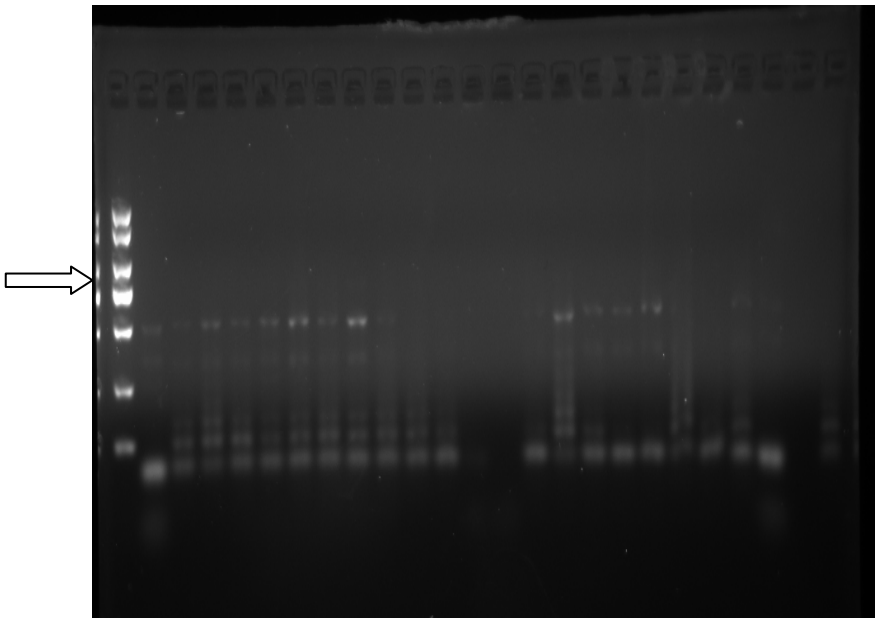


Figure (3): Detection of *T.congolansy* and *T.vivax*DNA amplified

Discussion

The current study aimed at identifying the prevalence of trypanosome species in five localities in the Blue Nile Area. These are namely: Ad-Damazin, Al- Rosayris, ,Qissan, Baw and AL-Tadamon. For the purpose of the study we used both conventional [parasitological method (wet smear, thin smear, thick smear and Buffy coat) and Polymerase Chain Reaction (PCR).Risk factors including age, sex and breeding system were also considered. The high infection rate is most likely due to the high tsetse challenge and other biting Deptra including Tabanids and Glossindae species in the study area in t addition to the fact that adult animals particularly, of nomadic tribes travel long distances for grazing which affect their general health condition and immunity.

In the current study, It is important to highlight that *T. brucei* was not founded in any of the cattle included in our study in spite to the presence of tsetse flies (main vector) in khorYabous area, almost An explanatory hypothesis could be the reduction of the lifespan of tsetse fly resulting from the disturbance of its habitat, favoring trypanosome species with a short development cycle, it is also wise mentioning that it became a regime for herd owners to treat their animals against trypanosomes also for prophylactic reasons.

In the current study we used different conventional methods which are usually used to identify trypanosotems infection under field conditions.The *T.vivax* was the only trypanosomes species found to infect cattle resident in tsetse free areas including cattle of the sentinel herd. The existence of *T. vivax* outside tsetse fly area indicates mechanical transmission. Regardless of including cattle from different breeding systems in study, we were only able to identify rate of 1% (3⁄300) in our study area. This value was increased to 11.7% (35⁄300) by using the PCR. This is indicating the real prevalence of the parasite may be higher that what can be identified using parasitological techniques.

In this study no significant differences were observed between the PCV of infected and non-infected cattle. This agrees with the finding of Hall et al. (1984) who noted that PCV is not a reliable indicator of bovine trypanosomosis in the field.

In this study most of the animals examined are females as the livestock owners tend to keep females

while males were usually sold. The prevalence of bovine trypanosomosis in this study indifferent age groups of cattle was notsignificant variation, may be due to an equal chance of exposure to the parasite. Similar findings were also reported by Cherenet et al.(2006) and Habtamu (2009).The study also revealed that infection rate was higher (56%, 170⁄300) among cattle of the nomadic breeding system this could be attributed to the fact that nomadic herds spend the dry season in the tsetse belt of khoryabus and Ethiopian border, which increase the risk of infection from these areas the resident cattle in Damazin town graze outside the town in the open grazing land around Damazin dam (Khazzan area). Some of the nomad's cattle coming from the tsetse bush pass through the area and get in contact with those cattle during the rainy reason.

The current study indicated that Trypanosomosis is serious disease in the state. The same situation was also mentioned in other studies stating that animal trypanosomosis is a major obstacle to animal production in the State Kheir*et al.,* 1995, Rahman, 2002 and Mohammed-Ahmed 1989), even that their results were based mainly on surveys conducted among migratory herds. Being a major agricultural production State, Blue Nile has high potential for diary production and mixed farming. The results of this study showed that the prevalence of cattle trypanosomosis was grater in the south part of the State, which could be attributed to the abundance of the tsetse fly in the area.

CONCLUSION

The prevalence of Bovine trypanosomosis was high in Blue Nile State using PCR Technique. Hence, the PCR was found very sensitive than conventional methods.

Recommendation:

1. More studies are needed to reveal the types of trypanosomes species in the study area, particularly in tsetse infested areas.

2. More studies are needed to find out the effect of the Rosaires Dam heightening and its effect on trypanosomes vectors occurrence

REFERENCES

[1] Rahman, A.H. Observations on the trypanosomosisi problem outside the tsetse beltthe tsetse belts of the Sudan. Rev. Sci. Tech.. Int. Epiz, **2005**;24(3):pp 965-972.

[2] Rahman, A.H.Observations on the epidemiology of bovine trypanosomosis in the Sudan.Ph.D. Thesis, University of Khartoum, Khartoum, Sudan.**2002**;pp 144.

[3] Abdelrahman, M.M.; Frah, E.A.;Katiti, D.; Matovu, E. and Intisar, E. *African J. of Biotechnology*, **2011**; 10:4508-4512.

[4] Cherenet, T., Sani, R.A., Speybroeck, N., Panandam, J.M., Nadzr, S. & Van den Bossche, P., 'A comparative longitudinal study of bovine trypanosomiasis in tsetse-free and tsetse-infested zones of the Amhara region, northwest Ethiopia', Veterinary Parasitology; **2006**;140:pp 251–258.

[5] Ford, J.Control by destruction of larger fauna, In: The African trypanosomiasis, Mulligan, H.W. (ed.) George, Allen and Unwin, London, **1970**.

[6] Hall, M.J.R, Kheir, S.M, A/Rahman, A, H and Noga, S.Tsetse and Trypanosomiasis survey of Southern Darfur province.Sudan, 1.Bovine Trypanosomiasis. *Trop. Anim. Health. Prod.* **1984**;15:pp 191-206.

[7] Habtamu, G.,m'Current status of tsetse transmitted trypanosomes in Jawi district of Amhara region, north-west Ethiopia', DVM thesis, Faculty of Veterinary Medicine, Gondar University,

Ehiopia, **2009**.

[8] Joshi PP, Shegokar VR, Powar RM, Herder S, Katti R, et al. Humantrypanosomiasis caused by Trypanosomaevansi, in India: the firstcase report. *Am. J. Trop. Med.Hyg*. **2005**;73:pp 491– 495

[9] Kheir, S.M., abdalla, H.S. and A/Rahman, A.H. A study on tsetse andTabanid flies in southeastern Sudan. *The Sudan Journal of Veterinary Science and Animal Husbandry*. **1995**;4(1):pp 16-21

[10] OIE., Manual chapter X.6 , **1996**:pp 660-664.

[11] OIE. Manual of Standards for Diagnostic Tests and Vaccines.3rd edition.Office International Des Epizooties (World Organization for Animal Health), Paris.**2010**:pp723.

[12] Salim, B., Bakheit, M. A., Salih, S. E., Kamau, J., Nakamura, I., &Nakao, R. An outbreak of bovine trypanosomiasis in the Blue Nile State, Sudan. Parasites & Vectors, 4(1), 74.BioMed Central Ltd. **2011**; doi:10.1186/1756-3305-4-74

[13] Urquhart, G.M. The pathogenesis and immunology of African trypanosomiasis in domestic animals. *Trans. R. Soc. Trop. Med. Hyg*. **1980**;74(6)pp: 726-729.

[14] Walker, A.R. Veterinary Protozoology.Tropical Veterinary Science and Tropical Veterinary Medicine. Center for Tropical Veterinary Medicine. University of Edinburgh. Edinburgh. **1986**:pp.103.