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Factors Affecting Seasonal Prevalence of Blood Parasites in Dairy Cattle in Omdurman Locality, Sudan

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Abstract: This study was conducted in Al-Rodwan project in Omdurman to investigate the prevalence of blood parasites in dairy cattle during different seasons and to assess the relationship between the occurrence of blood parasites and factors of seasonal variations, level of milk production, body temperature, packed cell volume, breed, age and sex. A total of 290 were during three seasons dry cool (100), dry hot (95) and wet hot (95). The sampling technique used was two stage cluster sampling method. The samples were examined in laboratory. Parasitological examinations included wet mount, buffy coat and thin blood film examinations were used. The results showed that the prevalence of blood parasites during different seasons was 8, 5.25 and 6.32% for dry cool, dry hot and wet hot seasons, respectively. The prevalence of *Theileria* sp. infection was found to be 7, 5.2 and 6.32% for dry cool, dry hot and wet hot season, respectively. While the prevalence of Babesia sp. infection was only recorded in the dry cool season as (1%). The results revealed that there was no effect ($\chi^2 = 0.6089$, p>0.05) of season on the occurrence of blood parasites. But a strong correlation (t-test = -43.6, p<0.05) was found between presence of blood parasites and milk yield. A positive correlation $(\chi^2 = 111.9, p < 0.05)$ was also recorded for body temperature with regard to the presence of blood parasites. Packed Cell Volume (PCV), breed, age and sex were found not to be associated with presence of blood parasites $(\chi^2 = 3.145, p > 0.05; \chi^2 = 0.005, p > 0.05; \chi^2 = 0.222, p > 0.05 \text{ and } \chi^2 = 0.483, p > 0.05, \text{ respectively}).$ The results also revealed there was no effect of breed, sex and age on occurrence of blood parasites infections ($\chi^2 = 0.005, 0.483$ and 0.222, respectively). Also, there was no relationship between the presence of blood parasites and PCV ($\chi^2 = 3.145$).

Key words: Omdurman, seasons, blood parasites, parasitological examinations, prevalence, relationship

INTRODUCTION

Theileriosis, Babesiosis, Trypanosomosis and Filariasis are diseases caused by blood parasites and occur throughout the world. Clinical and pathological changes due to infection of *Theileria* species in cattle show enlargement of lymph nodes, respiratory difficulty, anemia, pyremia, depressed milk yield, diarrhea, fever and body weight loss (Gill *et al.*, 1977; Michael *et al.*, 1989; Radostits *et al.*, 2000; Fukasama *et al.*, 2002). With *Babesia bovis* and *B. bigemina* sub-clinical infections occur fairly commonly especially in young cattle. The acute syndrome is characterized clinically by high fever, anorexia, depression, weakness, cessation of rumination, fall in milk yield, anemia and decrease in Packed Cell Volume (PCV) (Callow and Pepper, 1974; Smith and

Kilborne, 1893; Patarroyo et al., 1995; De Vico et al., 1999; Radostits et al., 2000). The general clinical picture of trypanosomiasis is fever, dullness, anorexia, ocular discharges, abortion in pregnant females, diarrhea, paled mucous membranes and progressive drop in PCV resulting in anemia (Soulsby, 1982; Seifert, 1995; Radostits et al., 2000). Onchocerciasis and thelaziasis were the most important filarial infection in cattle. Infestation with Onchcerca species adult worms is symptomless except for the presence of subcutaneous nodules. Microfilariae induced hypersensitivity reactions include alopecia, pruritis and dermatitis. Thelazia species infection cause excessive lacrimation, photophobia, conjunctivitis, keratitis, corneal ulceration and abscess formation in the eyelids (Radostits et al., 2000). Many studies were conducted to study the impact of each of these factors on cattle productivity such as milk yield and weight gains (Pholpark et al., 1991; Micheal et al., 1989; Gitau et al., 2001; Scholtz et al., 1991; Muragura et al., 2005). Perez et al. (1994) studied the relationship between the occurrence of Babesia bovis and B. bigemina and some selected factors; he stated that there was an effect of age, breed and season on the occurrence of Babesia. El-Metenawy (2000) studied the effect of season on theileriosis prevalence; he found that season effect on theileriosis prevalence. Bakheit (1998) studied the susceptibility of local cattle to tropical theileriosis in Sudan; he recorded the ability of local cattle to limit the multiplication of Theileria annulata. Bock et al. (1999) studied the effect of cattle breed on innate resistance to inoculations of Babesia bigemina; he stated that pure bred Bos indicus cattle have a high degree of resistance to babesiosis compared to Bos taurus breed.

Flach et al. (1993, 1995) studied the effect of age and sex of cattle on susceptibility to *Theileria* and *Babesia* species infection; he found that infection with *Theileria* annulata increased significantly with age of cattle and that there was no relationship established between infection of engorged nymphs (with Theileria and Babesia) of ticks and sex of host animal. Many workers conducted research in Sudan on the scope of epidemiological aspects of blood parasites in cattle (Malik, 1958; Uilenberg, 1960; Karib, 1961; El-Bihari et al., 1974; Osman, 1992; Rahman et al., 1994; Hassan, 2003).

MATERIALS AND METHODS

Area of study: Omdurman district was chosen as the area of this study. It is located in central Sudan in Khartoum State on the west bank of the White Nile and River Nile, around 12 km of a radius latitude 15°38'N and longitude 32°26'E. Al-Rodwan project in Omdurman was chosen to screen diary cattle for blood parasites. It is located on the North Western site of the locality on an area of 100 acres. It is the main dairy cattle aggregation site in the area with approximately 5,000 heads according to the record of Ministry of Agriculture, Animal resources and Irrigation, Khartoum State.

Study population: Selected cattle from dairy farms in Al-Rodwan project were investigated during dry cool (February-March), dry hot (May-June) and wet hot (August-September) seasons. About 100 animals from the chosen herds of animals were studied during the above seasons. The majority was of cross breeds (89%) and the rest of the chosen population was of local breeds (11%). The population structure consisted of 81% females and

<u>Table 1: Description of study population in Al-Rodwan dairy project</u>
<u>Season</u>

TT. 94	D1	Dry hot	TTT-4 14	
Unit	Dry cool	Frequency (%)	Wet hot	
Total animal examined	100 (100)	95 (100.00)	95 (100.00)	
Breed				
Local	11 (11)	11 (11.58)	11 (11.58)	
Cross	89 (89)	84 (88.42)	84 (88.42)	
Sex				
Male	19 (19)	14 (14.74)	14 (14.74)	
Female	81 (81)	81 (85.26)	81 (85.26)	
Age				
<1 year	33 (33)	28 (29.47)	28 (29.47)	
1-3 years	4 (4)	4 (4.21)	4 (4.21)	
>3 years	63 (63)	63 (66.32)	63 (66.32)	
Milk yield				
<4 kg	5 (5)	5 (5.26)	5 (5.26)	
4-8 kg	39 (39)	39 (41.05)	39 (41.05)	
>8 kg	22 (22)	22 (23.16)	22 (23.16)	

19% males. They were grouped into three age groups namely: <1 year (calves), 1-3 years (heifers) and >3 years (producing animals) giving a percentage of 33, 4 and 63%, respectively (Table 1).

Sampling and Sample collection: The sampling was done according to cluster sampling method (two stage sampling) as described by Thrusfield (1995). About 20% of the clusters (farms) were selected randomly and within each farm only 10% of the herd was sampled randomly to give a total of 100 animals out of 5,000 in 50 farms. The ear tag number of each animal included was recorded together with its age, sex and breed. A total of 290 blood samples were collected during the three different seasons from the same animals identified. The blood was collected in the morning from the jugular veins using vacutainers with EDTA.

The samples were labeled with animal number, placed in an ice box at 4°C and transported as soon as possible to the laboratory before processing for parasitological examinations. Body temperature of the animal was taken directly from the rectum using a thermometer. The milk yield of producing animals was taken from the farm records.

Parasitological examinations: Parasitological examinations included wet blood examination, buffy coat and thin blood films examination.

Wet mount: One drop of fresh blood was placed on a slide covered with a cover slip and examined microscopically for detection of motile parasites at 10×40 magnification.

Buffy coat examination (Woo, 1970): A capillary tube was taken the end of capillary tube was put on a drop of the

blood sample filled to about three-quarters and sealed by plastoseal at one end. It was placed in the haematocrit centrifuge which was run for 5 min. After centrifugation the Packed Cell Volume was read and then the capillary tube was placed onto a clean slide and covered with one drop of distilled water and examined microscopically at 10×40 magnification to detect trypanosomes and microfilariae.

Thin blood film: A small drop of fresh blood was put in the middle of one end of the slide and spread right across the slide and then air dried. The slide was labeled using a pencil. Blood films were fixed in absolute methyl alcohol for 2 min stained in 5% diluted Giemsa's stain for 45 min and washed in distilled water and then dried. Immersion oil was put on the blood film and examined microscopically for the detection of blood parasites at 10×100 magnification.

Data analysis: Microsoft Excel (Windows 2003) and Stata 6.0 for Windows 98/95/NT were used for data analysis. Chi-square (χ^2) was used for as a statistical analysis to assess the effect of various factors (body temperature, age, sex, breed and PCV). The student t-test was also employed to find out the effect of blood parasites on milk yield.

RESULTS AND DISCUSSION

The presence of blood parasites in cattle in Al-Rodwan dairy project was investigated during different seasons. The results showed that 8 (8%), 5 (5.25%) and 6 (6.32%) for dry cold, dry hot and wet hot, respectively were recorded as positive for blood parasites using thin blood film.

All results are shown in Table 2. No motile organisms Trypanosoma and Microfilaria were detected. The prevalence of Theileria sp. infection was 7, 5.26 and 6.32% in dry cold, dry hot and wet hot seasons, respectively. Prevalence of Babesia species infection was only recorded in dry cool season as 1% (Table 3). There was no effect of season ($\chi^2 = 3.145$, p>0.05) on the presence of blood parasites (Fig. 1). There was no relationship ($\chi^2 = 3.145$, p>0.05) between Packed Cell Volume (PCV) and occurrence of blood parasites (Table 4). A positive correlation (p<0.01) was found between presence of blood parasites and milk yield of cows resulting in reduction in milk production (Fig. 2). A strong correlation was recorded between temperature and occurrence of blood parasites ($\chi^2 = 111.2$, p<0.01) (Table 4). No association was found to occur between presence of blood parasites and breed or sex ($\chi^2 = 0.005$, p>0.05 and $\chi^2 = 0.483$, p>0.05, respectively) (Table 4).

Table 2: Summary of the result of blood parasites survey in dairy cattle in Al-Rodwan dairy project

	Seasons Dry hot			
Unit	Dry cool	Frequency (%)	Wet hot	
Total of animal examined PCV	100 (100)	95 (100.00)	95 (100.00)	
Normal	32 (32)	36 (37.89)	32 (33.68)	
Abnormal	68 (68)	59 (62.11)	63 (66.32)	
Buffy coat	, ,	, ,	, ,	
Positive	0 (0)	0 (0.00)	0 (0.00)	
Negative	100 (100)	95 (100.00)	95 (100.00)	
Wet mount	, ,	, ,	,	
Positive	0 (0)	0 (0.00)	0 (0.00)	
Negative	100 (100)	95 (100.00)	95 (100.00)	
Thin blood stain				
Positive	8 (8)	5 (5.26)	6 (6.32)	
Negative	92 (92)	90 (94.74)	89 (93.68)	

Packed Cell Volume (PCV): Adults: normal 28.4-38.8. Calves: normal 32.0-39.7

Table 3: The prevalence of blood parasites during seasons in dairy cattle in
Al-Rodwan dairy project

	No. of	Prevalence (%)		
	animal			Over all
Seasons	examined	<i>Theileria</i> sp.	Babesia sp.	prevalence (%)
Dry cool	100	7.00	1	8.00
Dry hot	95	5.26	0	5.26
Wet hot	95	6.32	0	6.32

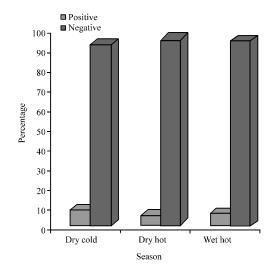


Fig. 1: The effect of season on presence of blood parasites in dairy cattle in Al-Rodwan dairy project. Chi-square (χ^2) = 0.609, p-value = 0.738 (Not significant, p>0.05)

Age was also not found related with occurrence of blood parasites ($\chi^2 = 0.222$, p>0.05) (Table 4).

The study of blood parasites in dairy cattle during different seasons in Omdurman area revealed a higher prevalence of *Theileria* species infection compared to *Babesia* species infection. Similarly, different workers recorded the presence of blood parasites in both intensive

Table 4: The relationship between breed, sex, age, PCV and body temperature and occurrence of blood parasites in dairy cattle in Al-Rodwan dairy project

Factor	Chi-square (χ²)	p-value
Breed	0.005	0.942
Sex	0.483	0.480
Age	0.222	0.090
PCV	3.145	0.076
Body temperature	111.2	0.000**

p>0.05 Not significant; **p<0.01: High significant

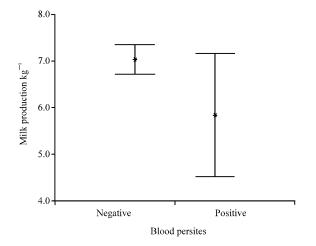


Fig. 2: The relationship between blood parasites and milk production in Al-Radwan dairy project; High significance (p<0.01)

and pastoral production systems of Sudan (Abdalla, 1984; Hassan, 2003). The presence of blood parasites infection in dairy cattle in Al-Rodwan project was attributed to the fact that most of the farms in this area were infested with ticks particularly, all the farms built of mud and block stones which constitute a suitable environment for that ticks. The study revealed that there was no effect of season $\chi^2 = 3.145$, p>0.05) on the prevalence of blood parasites. This finding disagreed with the results of different researchers. Perez et al. (1994) found that season was a risk factor of presence of Babesia bovis infection. El-Metenawy (2000) found during a study aimed at investigating the parasites infecting cattle blood at Al-Qassim region in Saudi Arabia, that theileriosis prevalence reached a maximum in (84.3%) in both autumn and summer seasons while it dropped to 59.4% in spring. The disagreement of this study could be attributed to application of acaricides and administration of antipiroplasmal drugs by farm owners at intervals which could have affected the prevalence of blood parasites during different seasons. It could also be due to the mismanagement practiced at Al-Rodwan while allows for continuous tick challenge throughout the year. Negative relationship was obtained between the Packed Cell Volume (PCV) and occurrences of blood parasites $\chi^2 = 3.145$, p>0.05). In contrast, many researchers explained that there was a positive relationship between occurrence of blood parasite and PCV. Gill *et al.* (1977) stated that *Theileria annuata* infection caused anemia. Also, Fukasama *et al.* (2002) recorded that *Theileria orientalis sergenti* infection is one of the most harmful anemic disease. Bovine babesiosis showed decrease in PCV and caused sever anemia (Callow and Pepper, 1974; De Vico *et al.*, 1999; Radostits *et al.*, 2000). The disagreement could be attributed to the fact that most of cattle population in Al-Rodwan project (68%) were found to have a low PCV which could be nutritional or due to other infections.

That means there were other diseases and factors causing anemia such as internal parasites and nutritional insufficiency. This finding is very important as the health care in dairy farms need to have a defined protocol in Sudan which can be followed by farmers. This study revealed that there was an association (p<0.01) between presence of blood parasites and milk yield of producing animals. Similar results were reported by different researches. Michael et al. (1989) studied the effect of theileriosis on milk yield and suggested that it caused decrease in milk yield. Patarroyo et al. (1995) stated that bovine babesiosis caused by Babesia bigemina remains a significant constraint to milk cattle production. Although, PCV could not be linked with blood parasites, yet this could be one of the major factors that affect milk yield. As shown from the results, there was a strong correlation $\chi^2 = 111.2$, p<0.01) between occurrence of blood parasites and body temperature. Similarly, many researchers recoded this result such as De Vico et al. (1999) who stated that bovine babesiosis showed fever; also Smith and Kilborne (1893) recorded Babesia bovis infection characterized by high fever.

Radostits et al. (2000) explained that bovine theileriosis and babesiosis caused increase in body temperature. Disturbed normal physiology during body temperature rise could result, among other things in reduced milk production. The study revealed that there was no effect $\chi^2 = 0.005$, p>0.05) of breed on occurrence of blood parasites. This result disagreed with different researchers. For instance, Bakheit (1998), reported that the ability of the Kenana cattle to limit the microschizont multiplication of Theileria annulata, resulting in less sever damage of lymphoid tissues during the acute phase of the disease was the basis of their resistance. Also, Bock et al. (1999) stated that pure breed Bos indicus cattle have a high degree of resistance to babesiosis compared to Bos indicus cross Bos taurus breed and Bos taurus breed. The disagreement of this study could be attributed to the different breeds in the study particularly; most of the cattle populations in diary farms in Al-Rodwan project were cross breeds (89%). The low number of pure local breeds did not allow the finding of a significant difference between the two breeds included. Also no pure foreign breed was encountered in this project. No correlation $\chi^2 = 0.483$, p>0.05) was found in our study between sex and presence of blood parasites. Similar results were reported by Flach *et al.* (1993), who stated that there was no relationship was established between infection of engorged nymphs of ticks and sex of host animal. Thus sex is not a determining factor in susceptibility to tick-borne parasites.

This study revealed no relationship $\chi^2 = 0.222$, p>0.05) between prevalence of blood parasite and age of animals. In contrast, Flach et al. (1995) stated that infection with Theileria annulata increased significantly with age of cattle, although the age effect on new infections may be a result of increased tick numbers on older animals. Perez et al. (1994) revealed that age was a risk factor in the presence of Babesia bovis infection. The disagreement with this study could be attributed to the composition of study cattle. Most of the cattle population in diary farms in Al-Rodwan project was producing cows over 3 years (63%) and the rest of population were heifers (4%) and calves (33%). Also it could be due to some degree of enzootic stability where all age groups are equal in the morbidity of infection. Other blood parasites, particularly Trypanosoma or microfilaria were not encountered during this study, although reported in other parts of the capital Khartoum. Possible explanation is that Al-Rodwan project is found in an area where present conditions are not suitable for insect propagation. This should not be overlooked as micro-climates may be created through negligence and lack of awareness and that used permit the infestation of insect species that are known as mechanical or biological vectors of some parasites. This may come as a result indiscriminate introduction of cattle which may originate from infected herds e.g., with Trypanosoma species or microfilaria.

CONCLUSION

In this study, infection with *Theileria* species and *Babesia* species were prevalent in Omdurman. Infection with blood parasites had economic impact due to reduction in milk production.

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REFERENCES

- Abdalla, H.M., 1984. Studies on *Babesia bigemina* in cattle in Northern Sudan. M.Sc. Thesis, University of Khartoum.
- Bakheit, M.A., 1998. Susceptibility of Kenana cattle to tropical theileriosis in the Sudan. M.Sc. Thesis, University of Khartoum.
- Bock, R.E., T.G. Kingston, N.F. Standfast and A.J. de Vos, 1999. Effect of cattle breed on innate resistance to inoculations of *Babesia bigemina*. Aust. Vet. J., 77: 465-466.
- Callow, L.L. and P.M. Pepper, 1974. Measurement of and correlations between fever, changes in the packed cell volume and parasitaemia in the evaluation of the susceptibility of cattle to infection with *Babesia argentina*. Aust. Vet. J., 50: 1-5.
- De Vico, G., B. Marci, C. Sammartino and G.R. Loria, 1999. Bovine babesiosis in Sicily: Preliminary study on pathology. Parassitologia, 41: 37-38.
- El-Bihari, S., F.A. Gadir and H. Suleiman, 1974. Incidence and behavior of microfilariae in cattle. Sud. J. Vet. Anim. Husb., 15: 82-85.
- El-Metenawy, T.M., 2000. Prevalence of blood parasites among cattle at the central area of Saudi Arabia. Vet. Parasitol., 87: 231-236.
- Flach, E.J., H. Ouhelli, D. Waddington and M. El-Hasnaoui, 1993. Prevalence of *Theileria* in the tick *Hyalomma detritum* in the Doukkala region, Morocco. Med. Vet. Entomol., 7: 343-350.
- Flach, E.J., H. Ouhelli, D. Waddington, M. Oudich and R.L. Spooner, 1995. Factors influencing the transmission and incidence of tropical theileriosis (*Theileria annulata* infection of cattle) in Morocco. Vet. Parasitol., 59: 177-188.
- Fukasama, M., T. Kikushi, H. Shinohara, A. Nishida and T. Yamgishi, 2002. Genetic parameters for packed red blood cell volume and daily gain during grazing in Japanese Shorthorn calves facing a natural challenge from Japanese theileriosis. Livest. Prod. Sci., 77: 167-174.
- Gill, B.S., Y. Bhattacharyulu and D. Kaur, 1977. Symptoms and pathology of experimental bovine tropical theileriosis (*Theielria annulata* infection). Ann. Parasitol. Hum. Comp., 52: 597-608.

- Gitau, G.K., J.J. McDermott, B. McDermott and B.D. Perry, 2001. The impact of *Theileria parva* infections and other factors on calf mean daily weight gains in smallholder diary farms in Muraga District, Kenya. Prev. Vet. Med., 51: 149-160.
- Hassan, D.A.S., 2003. Epidemiological studies on tropical theileriosis (*Theileria annulata* infection of cattle) in the Sudan. M.Sc. Thesis, University of Khartoum.
- Karib, A., 1961. Animal trypanosomiasis in the Sudan. Sudan J. Vet. Sci. Anim. Husb., 2: 39-46.
- Malik, E.A., 1958. Occurrence of *Onchocerca armillata* in Sudanese cattle, *Bos indicus*. J. Parasitol., 44: 30-31.
- Michael, S.A., A.H. El-Refaii, N. McHardy and D.G. Rae, 1989. Effect of treatment of chronic theileriosis with buparvaquone on milk yields. Trop. Anim. Health Prod., 21: 218-222.
- Muragura, G.R., A. McLeod, J.J. McDermott and N. Taylor, 2005. The incidence of calf morbidity and mortality due to vector-borne infection in smallholder dairy farms in Kwale District, Kenya. Vet. Parasitol., 130: 305-315.
- Osman, O.M., 1992. *The ileria annulata* in the Sudan. In: Recent Development in Research and Control of *The ileria annulata*. Dolan, T.T. (Ed.). The International Laboratory For Research On Animal Diseases, Nairobi, Kenya, pp: 125.
- Patarroyo, J.H., A.A., Prates, C.A. Tavares, C.L. Mafra and M.I. Varga, 1995. Exoantigenes of an attenuated strain of *Babesia bovis* used as a vaccine against bovine babesiosis. Vet. Parasitol., 59: 189-199.
- Perez, E., M.V. Herrero, C. Jimenez, D. Herd and G.B. Buening, 1994. Effect of management and host factors on seroprevalence of bovine anaplasmosis and babesiosis in Costa Rica. Prev. Vet. Med., 20: 33-46.

- Pholpark, S., M. Pholpark, C. Polsar, A. Charoenchai, Y. Paengpassa and Y. Kashiwazaki, 1991. Influence of *Trypanosoma evansi* on milk yield of diary cattle in Northern Thailand. Prev. Vet. Med., 42: 39-44.
- Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary Medicine. 9th Edn., Harcourt Publisher Ltd., London, UK., pp. 1289-1338.
- Rahman, M.B.A., A.M. Zakia, H.A. Bakheit, M.O. Halima, A.O. Fayza and A.Y. Osman, 1994. Epidemiology of bovine tropical theileriosis in the Sudan. Proceeding of the Workshop Sudan Veterinary Association Residence, May 4-5, Khartoum, Sudan, pp. 6-17.
- Scholtz, M.M., A.M. Spickett, P.E. Lombard and C.P. Enslin, 1991. The effect of tick infestation on the productivity of cows of three breeds of cattle. Onderstepoort J. Vet. Res., 58: 71-74.
- Seifert, H.H., 1995. Tropical Animal Health. 1st Edn., Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 152-169.
- Smith, T. and F.L. Kilborne, 1893. Investigation into the nature causation and prevention of texas or Southern cattle fever. Bur. Anim. Ind. Bull., 1: 151-152.
- Soulsby, E.J.L., 1982. Helminthes, Arthropod and Protozoa of Domesticated Animals. 7th Edn., Bailliere, London, pp. 306-327.
- Thrusfield, M., 1995. Veterinary Epidemiology. 2nd Edn., Blackwell Science, Oxford, UK, pp. 183.
- Uilenberg, G., 1960. Trypanosomiasis in Kosti and El-duiem districts tour. Annual Report. Vet. Dept. Sudan, pp: 40.
- Woo, P.T., 1970. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. Acta Trop., 27: 384-387.