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ORIGINAL ARTICLE

Effect Of Experimental *Trypanosoma congolense* Infection on Serum Profiles of Lipid and Cholesterol in Pack Donkeys

Samuel, F. U.[1]*; Adamu, S.[2]; Mohammed, B.[2]; Chiezey, P. N.[1]; Mohammed, A. K.[1]; Ereke, S. O.[1] and Ibrahim, M. A.[1]

[1.] National Animal Production Research Institute/Ahmadu Bello University, Zaria, Nigeria. [2.] Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. *Corresponding author: Email: felixsam75@yahoo.com; Tel No: +2348062804899.

SUMMARY

The effect of experimental *Trypanosoma congolense* (*T. congolense*) infection on serum concentrations of lipids in donkeys was investigated. To establish the infection, four apparently healthy pack donkeys were, each, intravenously inoculated with blood (1 ml) from an infected donor donkey containing 1×10^6 *T. congolense* organisms. Following this, 5 ml of blood was collected from each of the experimental animals, starting from day zero and then every other day throughout the experimental period and used for haematological and serum biochemical analyses. Levels of triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol) and low density lipoprotein-cholesterol (LDL-cholesterol) in the serum were measured over a 28-day experimental period, using commercial test kits. The infection with *T. congolense* caused significant (P<0.05) decreases in serum concentrations of total cholesterol and LDL-cholesterol in the experimental animals. Decreases were also observed in the serum concentrations of triglyceride and HDL-cholesterol but were not significant (P>0.05). With the indispensable roles of lipids as integral parts of cell membrane structures and in other metabolic processes in the mammalian hosts, it could be inferred that *T. congolense* infection-induced alterations in serum concentrations of lipids might be contributory pathophysiological mechanisms of some of the reported disorders in trypanosome-infected animals.

Key words: Donkey, Cholesterol, Triglyceride, HDL-cholesterol and LDL-cholesterol *Trypanosoma congolense*.

INTRODUCTION

The donkey or ass *Equus africanus asinus* is a domesticated member of the Family Equidae. Donkeys constitute 70% of the African equine species and are predominantly found in the arid and semi-arid areas providing a reliable, environmentally friendly and renewable source of power to millions of poor communities' worldwide (Fielding and Pearson, 1991). They are docile and easy to manage and provide transport amongst communities in areas with poor terrains or inaccessible areas with poor infrastructure (Blench and Macdonald, 2000) .They have

the ability to withstand harsh conditions in many rural settings in which they are reared but that notwithstanding, the donkey has received least attention especially where they are kept with other livestock. This may be due to the mistaken belief that they are hardy and hardly affected by disease (Svendsen, 1986).

African animal trypanosomosis, caused mainly by *Trypanosoma vivax* (*T. vivax*); *T. brucei* and *T. congolense* constituted a serious setback to improved livestock and mixed farming system in tropical Africa (Kristjanson *et al*., 1999; Irungu *et al*., 2002). Economically, the disease had led to a great negative impact especially in sub-Saharn Africa where the prevalence of the disease is on the increase due to breakdown in surveillance and control measures (Ikede and Losos, 1972; Daniel *et al.*, 1993).

Animal trypanosomosis is transmitted either cyclically through the bite of tsetse fly (*Glossina spp*) or mechanically through the bites of other flies. Trypanosome infections are often protracted illnesses, while the acute forms are less common. In animals, disturbance of equilibrium precipitates or exacerbates the disease. The disturbance takes the form of stress, which may be due to the occurrence of another disease, malnutrition, water deprivation, heat stress, production stress such as pregnancy, lactation or work. Thus, good management may do much to mitigate the effect of trypanosomosis (FAO, 2000). There are no pathognomonic signs and clinical examination, unless coupled with laboratory tests, can only suggest the disease without confirmation and the major clinical signs of the disease are anemia, generalized enlargement of the superficial lymph node, episodic pyrexia, lethargy and progressive loss of body condition (Blood and Radostitis, 2007; Samuel *et al*., 2016). Numerous disorders have been reported to occur in trypanosome-infected animals (Esievo and Saror, 1991; Logan-Hunfrey *et al.,* 1992; Adamu *et al.,* 2008). Conversely,

research efforts made to elucidate the mechanisms of development of these disorders yielded varying and conflicting reports (Nakamura, 1998; Biryomumaisho *et al*., 2003).

This study was therefore carried out to further unravel the possible effect of experimental *T. congolense* infection on the serum levels of triglyceride, high density lipoproteins cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol) and cholesterol in donkeys.

MATERIALS AND METHODS
Experimental animals
Four apparently healthy donkeys aged between 6 and 7 years were purchased from Sheme, in Katsina State, Nigeria, which was known to be tsetse-free. On arrival, the donkeys were housed in a fly-proof stable. The animals were adequately fed corn bran, cowpea haulms and fresh pasture and water was provided, *adlibitum*. Following faecal and blood screening for parasitic infections using standard parasitological techniques, the animals were dewormed with Albendazole at 25 mg/kg body weight administered, *per Os*, and treated against external parasites by topical application of Diazintol, while Oxytetracycline long acting at 20 mg per kg body weight, administered intramuscularly, was used to treat against possible haemoparasitic and bacterial infections. The animals were allowed to acclimatize for a month during which they were subjected to routine handlings such as collection of blood samples and determination of rectal temperatures. Each of the donkeys was ear tagged for proper identification.

The trypanosome stock
The *T. congolense* stock was obtained from Nigerian Veterinary Research Institute (NVRI), Vom in Plateau State, Nigeria. The parasite was inoculated into two mice, intraperitoneally, and transported to the Faculty of Veterinary Medicine, Ahmadu

Bello University, Zaria in Kaduna State, Nigeria, where the experiment was carried out.

Inoculation of donor donkey

On arrival, the infected mice were kept in a laboratory and fed adequately. Blood samples were collected daily to monitor parasitaemia using the method described by Woo (1969) and estimated as described by Sannusi (1997). Parasitaemia was detected in the mice 7 days post-infection. When parasitaemia was 1.0×10^6 trypanosomes/ml on day 9 post-infection, one of the mice was euthanized by severing its jugular vein, following chloroform anaesthesia, to obtain blood that was used to intravenously infect a donor donkey. Blood sample was collected every day from the donor animal and used to monitor and estimate parasitaemia as previously described.

Animal allocation and infection

Parasitaemia was at peak level on day 15 post-inoculation of the donor donkey, blood sample was collected and used to infect each of the experimental animals (via intravenous injection of 1 ml of blood containing 1×10^6 trypanosomes). This day was tagged as day 1 of infection.

Blood sampling for parasitological, haematological and biochemical analyses

Beginning from day 14 pre-infection and day 14 post-infection, blood sample (5 ml) was collected at 2-day intervals by jugular venipuncture from each of the experimental animals. Exactly 1 ml of the blood was dispensed into screw-capped test tube containing ethylene diamine tetraacetic acid and used for trypanosome detection and haematological analysis according to methods described by Woo (1969) and Coles (1974), respectively. The other 4 ml of blood was allowed to clot and the serum expressed was removed and dispensed into properly labeled sterile vials. The serum was stored at -20 ^{O}C until needed for serum triglycerides, HDL-cholesterol, LDL-cholesterol and cholesterol assays.

Determination of serum levels of total cholesterol, LDL- and HDL-cholesterols and triglyceride.

Serum concentrations of total cholesterol, LDL- and HDL-cholesterol and triglyceride were determined using standard commercial test kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom) according to the manufacturers' instructions. LDL-cholesterol was calculated from the values of total cholesterol, triglyceride and HDL-cholresterol as follows:

LDL-chol= total Chol- Triglyceride/2.2 – HDL-chol

Statistical analysis

Data on parameters measured were designated as pre- and post-infection values, which were expressed as mean ±standard error of mean (SEM). The levels of significance in differences between the pre- and post-infection means were determined using Student's t-test with Statistical Package for Social Sciences Version 17.0 (SPSS). Values of $P<0.05$ were considered significant.

RESULTS

Clinical observations

Parasitemia (*T. congolense*), were first detected in one donkey in the infected group on day 6 post-infection and by day 8, all the infected donkeys were showing parasitaemia. Peak parasitaemia level of 5 x 105 trypanosomes per millilitre of blood was recorded on day 10 PI. Following the development of parasitaemia, all the infected donkeys came down with clinical trypanosomosis. The first clinically observed manifestations of the disease in these animals were fluctuating pyrexia (104 - 108^{0}F) followed by weakness, dullness, reduced feed intake and rough hair coat. As the infection progressed, mucous

membranes became pale and superficial lymph nodes were enlarged. There was a decrease in the Pack Cell Volume (PCV) value of the infected donkeys from pre-infection to post-infection indicating anaemia.

Serum biochemical changes

T. congolense caused decrease in the serum values of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol in all the infected animals.

The mean value of total serum cholesterol was observed to reach minimum at day 2 PI, (2.0±0.05 mmol/l) and the maximum day 11 PI, (2.80±0.05) mmol/l. The mean PI value (2.38±0.05mmol/l) of the total cholesterol was significantly (P<0.05) lower than that (2.59 ± 0.03mmol/l) at pre-infection period. (Figure 1)
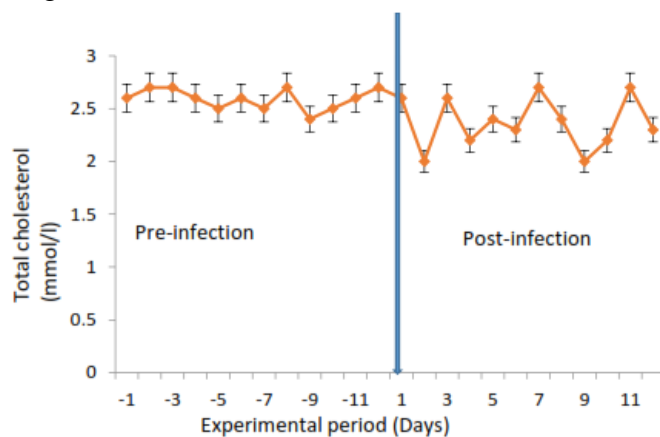

Figure 1: Showing the effect of *T. congolense* on total cholesterol
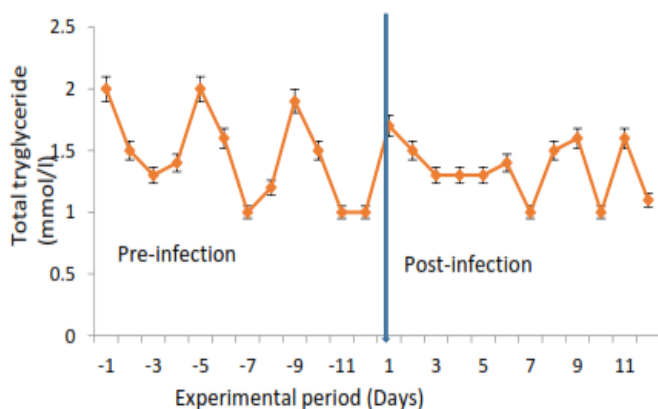

Figure 2: Showing effect of *T. congolense* on total triglyceride

Decrease in the mean value of serum triglyceride was discernable on the day 1 PI, the lowest value of the serum triglyceride was recorded on the day 7 and 10 PI, this was followed by gradual increase to reach peak value of 2.0±0.06mmol/l on days 11 PI. The mean PI value of the serum triglyceride (1.53±0.06mmol/l) at post infection was not significantly (P>0.05) lower when compare with that (1.45±0.11mmol/l) at pre infection period. (Figure 2)

The mean value of HDL-cholesterol was observed to rise to sharp peak on the days 5 PI, (9.0±0.39mmol/l) and fall abruptly on day 7 PI, to a mean value of 1.0±0.39mmol/l, and then rises gradually and high again on the day 9 PI, to a mean value of 8.0 ± 0.39mmol/l.

The overall mean value PI (1.82 ± 0.39mmol/l) was not significantly (P>0.05) different from pre infection value (1.23 ± 0.05 mmol/l), (as shown in Figure 3). The mean value of LDL-cholesterol was observed to decrease on day 2 PI and then rises slightly after which its falls to a minimum value of 0.3 ± 0.05mmol/l on day 4 PI. The maximum value was observed on the day 5 PI, of 1.0 ± 0.05mmol/l. The difference between the mean serum values (Post infection, 0.45±0.05mmol/l; pre infection, 0.76±0.07mmol/l) of LDL-cholesterol of the experimental animals were statistically significant (P<0.05). (Figure 4).

DISCUSSION

Findings made in the present study clearly indicate that *T. congolense* infection of donkey results in lowered values of Packed Cell Volume, serum HDL- cholesterol , LDL-cholesterol and toatal cholesterol, thus, corroborating the earlier findings made by Samuel *et al.* (2016), who showed

that *T.*

congolense infection of donkey resulted in (anaemia) lowered PCV; Biryomumaisho *et al.* (2003) who reported a decreased serum HDL-cholesterol , LDL-cholesterol and total cholesterol in *T.congolense* and *T. bruice* infected Small East African goats as well as the findings by Adamu *et al.* (2008) who also reported a gradual decline in PCV, serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in *T. congolense* infection of sheep. The present reports are also in conformity with those of Wellde *et al.* (1989) and Katunguka-Rwakishaya *et al.* (1992) who also reported a reduced PCV, total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in *T. Rhodensiesi* infection of cattle and *T. congolense* infection of sheep respectively. The present findings however contradicted those made by Nakamura, (1998) who showed in increased serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and inflammatory cytokines in *T. brucei* infection of rabbits. The present observations are also in complete deviance from those made much earlier by the Diehl and Risby (1974); Rouzer and Cerami, (1980) who reported increased levels of serum lipids (*T. gambiense*) and Hypertriglyceridaemia (*T. brucei*) in infected rabbits, respectively.

There are several pathophysiological mechanisms involved in the lowering of serum levels triglycerides, HDl-cholesterol, LDL-cholesterol and cholesterol in trypanosome-infected animals (Adamu *et al.,* 2008) as have been suggested in the present study. For example, it has been shown that lipoprotein requirement of trypanosomes is dependent upon serum HDL- cholesterol, LDL-cholesterol of the host, in order for the parasite to multiply
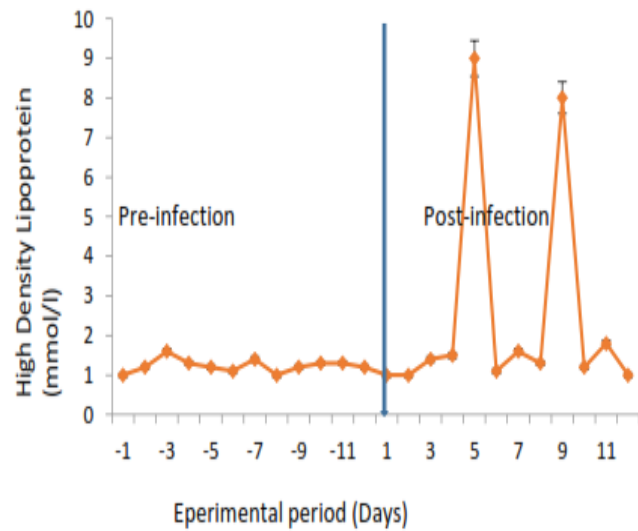

Figure 3: Showing effect of *T. congolense* on high density lipoprotein
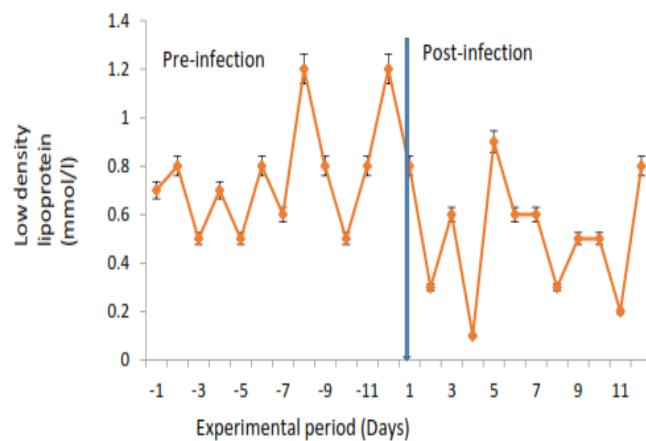

Figure 4: Showing effect of *T. congolense* on Low density lipoprotein

under axenic culture (Black and Vanderweed, 1989). This could partly explain the decrease in serum HDL-cholesterol, LDL-cholesterol, in *T. congolense*-infected animal.

Also, blood-stream forms of trypanosome, which are unable to synthesis cholesterol are known to require it along with phospholipid and total lipids for synthesis of their membrane and growth there by depleting the host reserve of the lipids (Black and Vandetrweed, 1989; Hue *et al*., 1990; Katunguka-Rwakishaya, *et al.*, 1991; Green

et al., 2003; Nok *et al.*, 2003; Adamu *et al.*, 2008). The continuous removal and utilization from the blood stream, of these molecules could partly be responsible for the lowered serum level of lipids and total cholesterol observed in the present studies. Impaired synthesis and subsequent release of cholesterol from the liver could also be a contributory factor to the decrease in serum levels of total cholesterol observed in the trypanosome infected animals in the present study. This is because pathologic changes occurring as a consequence of trypanosomes infection have been reported in the liver (Logan –Henfrey *et al.*, 1992). Impaired synthesis of cholesterol in the liver could also be the result of insufficient hepatocellullar respiration due to hypoxia caused by aneemia in the *T. congolense* as reported by Adamu *et al.* (2008) in infected sheep. Also, lowered LDL-cholesterol serum level such as observed in the present study could retard cholesterol transport from the liver and contribute to its lower serum level.

Furthermore, blood-stream forms of some trypanosomes scavenge blood glucose as a source of energy (Chaudhuri *et al.*, 2006). This may partly contribute to the development of hypoglycemia observed in some trypanosome infected animals. Although blood glucose was not determined in the present study, hypoglycemia induced by trypanosome infection could undoubtedly result in the increased catabolism of lipids in order to meet some strategic energy needs in the body of the host animal. Gluconeogenesis from lipid for some essential physiological processes in the body could also cause lowered lipid and cholesterol serum levels. Decrease feed intake of animals, typically associated with trypanosomosis (Blood and Radostits, 2007) and such as the one observed in the trypanosome infected animals in the present experiment could ultimately reflect on the nutritional status of the affected animals and hence, lower serum concentrations of lipids and cholesterol . Increase serum non-esterified fatty acid in trypanosome-infected

animals has led to the suggestion that lipolysis is the major mechanism for supplying the high energy demanded by the fever following trypanosome infection (Akinbamijo *et al.*, 1992). Indeed, Faye *et al.* (2005) reported that the high energy demands of trypanosome infection may lead to severe energy shortage and this might be reflected in the changes to energy and protein metabolism.

The role of decrease in the serum concentrations of lipids and cholesterol in the pathophysiology of some of the disorders reported in trypanosomes-infected animals can better be appreciated when the function of such lipids and cholesterol in the mammalian physiology are taken into cognizance. Cholesterol is produced in the liver as well as being supplied to the body in human and animal diets. Cholesterol is the building block for cell membranes and it is essential in the formation of bile (which aids in the digestion of fats), vitamin D, other steroids and hormones such as progesterone, testosterone and oestrogen. Cholesterol helps secure proteins involved in cell signaling, allowing for example, neurons to find each other when forming synapses, the formation of which is a basic part of learning and the formation of memories. LDL-Cholesterol is the major cholesterol carrier in the blood and is responsible for transporting cholesterol from the liver to organs and tissues of the body. HDL-Cholesterol on the other hand is responsible for carrying cholesterol from various organs and tissues to the liver for recycling or degradation. Consequently, any significant alteration in the serum levels of these molecules could result in disorders in affected animals. Although free fatty acids have not been determined in the present experiment, it has been shown that autolysing trypanosomes release such compounds mostly, stearic, linoleic, palmitic and oleic acids were reported to be generated (Assoku *et al.*, 1977). These free fatty acids are potentially cytotoxic and haemolytic in vitro. Their increase in

trypanosome-infected animals may confirm their cytotoxic properties on erythrocytes of the host animals and hence their contribution to anaemia and other tissue pathological changes (Biryomumaisho *et al*., 2003).

The pathogenesis of neurological involvement in human African trypanosomosis is not currently understood, but it is thought to involve autoimmune mechanisms as in Chagas' disease (Blood and Rodostitis, 2007). Significant decrease in serum lipids and cholesterol levels, such as observed in the present study, may contribute to the development of the reported neurological disorders since cholesterol is vital in cell signaling, a phenomenon that allows neurons to find each other when forming synapses.

In conclusion, *T. congolense* infection resulted in a drop in serum concentrations of lipid and cholesterol in donkey. The significance of the reduction in serum levels of these molecules on the pathogenesis of African trypanosomosis can be viewed from two perspectives; first that the rapid depletion of the serum lipid and cholesterol re-affirms earlier suggestions that these molecules are, probably, essential in some of the parasite's metabolic processes for it to thrive and cause disease in the animal host. Secondly, the decrease in serum levels of lipids and cholesterol could have deleterious effects on some body systems, which could result in derangement of their functions. Thus fall in serum levels of lipids and cholesterol may be one of the pathophysiological mechanisms in the development of some of the reported disorders observed in trypanosame-infected animals. Further investigation is undoubtedly needed to elucidate the biochemical differences that exist in the lipid and cholesterol metabolism between the animal host ad trypanosome parasite so that metabolic target could be identified in the parasite with the view to incorporating the knowledge in the design and development of chemotherapeutic agents.

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