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Full Length Research Paper

Experimental *Trypanosoma brucei* infection-induced changes in the serum profiles of lipids and cholesterol and the clinical implications in pigs

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The possible effects of *Trypanosoma brucei* (*T. brucei*) infection on serum levels of some lipids and cholesterol were studied in pigs. The infection with *T. brucei* caused significant decline in the serum levels of cholesterol, triglycerides and high density lipoprotein. Mean values of these parameters in the infected group were significantly lower (P < 0.05) than those in the control group, in which the values remained relatively unchanged up to the termination of the experiment. However, the infection appeared not to have significant (P > 0.05) effect on serum low density lipoprotein level. Possible pathophysio-logical mechanisms involved in the *T. brucei* infection-induced lowering of the serum levels of lipids and cholesterol were highlighted just as evidences from literature suggest that the *T. brucei* infection-induced alterations in the serum levels of the lipids and cholesterol could have far-reaching impli-cations on the clinical manifestation of the disease in affected animals and humans. It is imperative, therefore, to conduct more studies to identify biochemical differences, if any exist, between the trypanosomes and animal hosts in the metabolism of the lipids and cholesterol with the view to exploiting the findings in biotechnological development of drugs or other therapeutic approaches.

Key words: Trypanosomosis, pigs, cholesterol, triglycerides, low density lipoprotein, high density lipoprotein.

INTRODUCTION

In spite of the existence of a huge body of research find-ings on African trypanosomosis (trypanosomiasis), the disease has continued to wreak havoc on human and animal lives with consequent effects on the fragile eco-nomy of countries of Tropical Africa (Kristjanson et al., 1999; Bourn et al., 2005). The causative agents of the disease in domestic and wild animals as well as humans are tsetse fly-borne protozoan parasites belonging to the genus *Trypanosoma*. *Trypanosoma vivax* (*T. vivax*), *T. congolense* and *T. brucei* in cattle, sheep and goats, and *T. simiae* in pigs are the most pathogenic species of try-panosomes in animals (Nantulya, 1990). Two major trypanosome species implicated in the pathogenesis of the disease in man are *T. brucei rhodesiense* and *T. brucei gambiense* (Smith et al., 1998; Dumas et al., 1999).

Variable disorders occur sequel to trypanosome infec-tion in animals. An in-depth knowledge of mecha-nisms of their development is pivotal to search for and identi-fication of molecular targets, which could be exploited in evolution of therapeutic approaches, especially, in this cutting edge period of research in molecular medicine and biotechnology. Although several abnormalities in lipid and cholesterol metabolisms have been identified in trypanosome-infected laboratory and domestic animals (Biryomumaisho et al., 2003), attempts to investigate the changes in serum concentration of these molecules occurring sequel to the disease yielded a cocktail of discordant research findings (Adamu et al., 2008), thus, warranting further studies. Moreover, trypanosomosis in pigs has not been extensively studied even as infection, which occurs as single or mixed, with some Nannomonas group of trypanosomes was reported to be common in some African countries (Waiswa, 2005). This work was

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was therefore designed to further investigate the possible effects of trypanosome infection on serum profiles of cholesterol, triglycerides, high and low density lipoprotein cholesterols using different trypanosome and host animal species from our previous study (Adamu et al., 2008); *T. brucei* and pig, respectively, in this case.

MATERIALS AND METHODS

Animals for the experiment

Eleven normal, apparently healthy pigs were purchased from a private pig breeder in Ahmadu Bello University, Zaria, Kaduna State, Nigeria and used in this experiment. The pigs were accommodated in a fly- proof animal house of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. They were adequately fed and water was provided *ad libitum*. The pigs were immediately dewormed on arrival using albendazole (ALBENDAZOLE®, Tuyil Pharm. Ind. Ltd., Nigeria) at 25 mg/kg and treated regularly against external parasites using diazinon (DIAZINTOL®, Animal Care, Nigeria Ltd.). A two-month period was allowed for acclimatization before the commencement of the experiment.

Trypanosoma brucei organism

The parasite used in this experiment was donated by Nigerian Institute of trypanosomiasis research, Kaduna State, Nigeria. Two rats were inoculated with the stabilate, one intraperitoneally and the other subcutaneously, and transported in a cage to fly-proof animal house of the Department of Veterinary Parasitology and Microbiology, where they were closely monitored for development of parasitaemia. Parasitaemia detection was carried out every day on the blood of these rats collected from the tail veins using the method of Woo (Woo, 1969).

Inoculation of donor pig

T. brucei parasites were first detected in one of the two rats 5 days following inoculation with the organisms. At peak parasitaemia, in the rat that was intraperitoneally inoculated, on day 7 post-inoculation (when parasitic load was 1.0×10^5 trypanosomes per ml of blood as estimated using the modified method of Paris et al. (1982) as described by Mutayoba et al. (1994), the rat was put on chloroform anaesthesia. The jugular veins of these rats were then severed in order to collect sufficient blood in heparin-containing vacutainer, which was used to inoculate a donor pig.

Grouping and infection of experimental pigs with *T. brucei*

Following infection of the donor pig with 1 ml of blood containing 1×10^5 *T. brucei* organisms, the blood of the donor pig, 0.5 ml, was collected and tested every day for the appearance of the parasites in peripheral circulation. *T. brucei* organisms were first detected in the blood of the donor pig on day 5 post-infection. On the day 7, when parasitaemia was at its peak level in the donor pig, the ten experimental pigs were ear- tagged and divided into two groups, each comprising 5 animals. Animals in one group, the infected group, were, each, administered 2 ml of blood from the donor pig containing 2.0×10^6 trypanosomes (Sekoni et al., 1990) as estimated using the modified method of Paris et al. (1982) as described by Mutayoba et al. (1994). The other 5 pigs in the second group served as uninfected controls. This day on which animals in the infected group were infected with *T. brucei* organisms was tagged tagged day 0 of infection.

Parasite detection in the blood of the infected animals

Following infection of the pigs, 0.5 ml of blood was collected every day from each of the animals in the infected group and was used for detection of the *T. brucei* organisms so as to ascertain that the disease, trypanosomosis, was really established. The parasites were detected in the blood of two of the infected pigs 4 days post infection. The parasitaemia level in each of these pigs was 1×10^3 trypanosomes/ ml. By the following day, all the pigs in this group were parasitaemic.

Serum lipids and cholesterol analyses

Starting from day 0 and up to day 42 post, 3 ml of blood sample was collected from each animal in the two groups every other day into sterile screw- capped test tube, which contained no anticoagulant. On day 0, the blood sample was collected just before the administration of the infecting blood to the infected group of pigs. This blood was allowed to clot and, after some hours, the serum was harvested and dispensed into properly labeled sterile vials and stored at -20°C until assayed for triglycerides, cholesterol, high and low density lipoprotein-cholesterols. The determination of serum levels of cholesterol was carried out using colorimetric enzymatic end point method. Serum triglycerides were analyzed using colorimetric method after enzymatic hydrolysis with lipases. High density lipoprotein-cholesterol was determined using precipitant method. All these determinations were carried out in the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital, Shika, Zaria using standard commercial test kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom) . The manufacturer's instructions on the assay procedures were strictly followed. Low density lipoprotein cholesterol, measured in mmol/l, was calculated from the values for cholesterol, triglycerides and high density lipoprotein-cholesterol using the formula described by Friedewald et al. (1972) below:

Low density lipoprotein-cholesterol = Cholesterol – Triglycerides/2.2 – High density lipoprotein-cholesterol

Statistical analysis

Data obtained from the animals in the two groups were plotted on graphs using Microsoft Excel Chart Wizard. The post infection mean values in the two groups were also compared statistically using Student's t-test (Steel and Torie, 1980).

RESULTS

Parasitaemia and clinical observations

T. brucei organisms were first detected in the blood of two of the infected pigs on day 4, post-infection, probably, due to variation in individual susceptibility to the organism. By day 5 post infection, all animals in the infected group were parasitaemic. Parasitaemia was low, with mean level fluctuating between 1.0×10^3 and 1.0×10^4 trypanosomes per ml of blood up to day 14 post infection. A sudden surge in the parasitaemia level to a peak value (1.0×10^5 trypanosomes per ml of blood) was observed on day 15 post infection. Thereafter, the mean parasitaemia fluctuated at very low levels up to the termination of

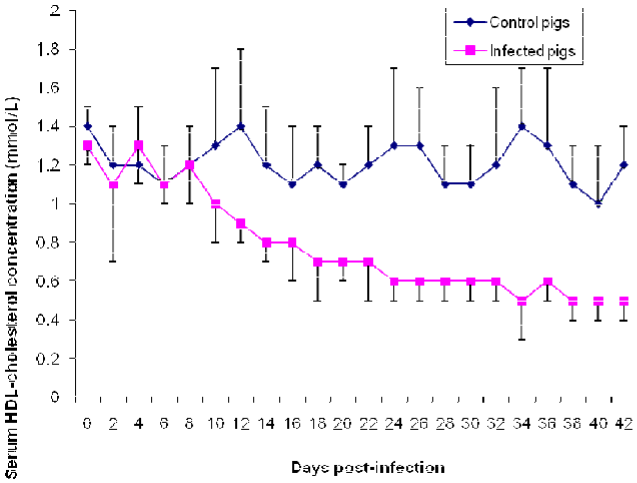

Figure 1. Mean serum High density lipoprotein concentration in *T. brucei*-infected and control pigs.

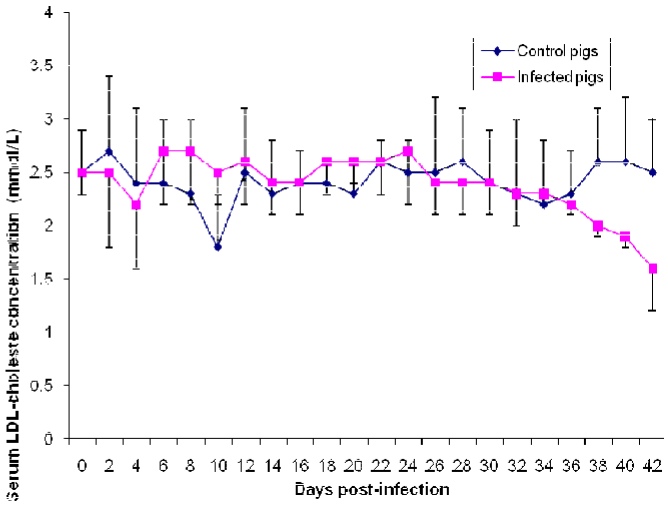

Figure 2. Mean serum low density lipoprotein concentration in *T. brucei*-infected and control pigs.

the experiment on day 42 post infection, a period sufficient for acute infections. Parasitaemia was intermittent in individual pigs of the infected group. Following the development of parasitaemia, pigs in the infected group became febrile with recta temperatures ranging between 105 and 109°F. With time in the infection lethargy, reduced feed intake (as measured by the quantity of the feed left uneaten by the infected pigs when compared with that of the controlled pigs each day) and roughness of hair coat were observed.

High density lipoprotein

Mean serum values for high-density lipoprotein in the infected and control groups on day 0 of infection were 1.3 ± 0.1 mmol/L and 1.4 ± 0.1 mmol/L, respectively. Following infection, levels of high density lipoprotein were comparable in the two groups of animals up to day 8 post-infection after which a rapid decline in the serum level of the high-density lipoproteins was observed in the infected pigs (Figure 1). This decline in the concentration of high density lipoprotein progressed to reach a minimum level of 0.6 ± 0.2 mmol/L on day 34 post-infection. Thereafter, the level stabilized, with only some minor fluctuations, up to the termination of the experiment (Figure 1). Serum levels high density lipoprotein in the control pigs remained fairly at the same levels relative to the pre-infection value on day 0 of infection, although some fluctuations were observable (Figure 1). The mean post infection value (0.8 ± 0.2 mmol/L) of this parameter in the infected was significantly lower ($p < 0.05$) than that (1.3 ± 0.1 mmol/L) in the control group.

Low density lipoprotein

The values of pre-infection mean serum low density lipoprotein respectively. Mean values of this parameter in the two groups were at comparable levels up to day 36, except that on day 10 (Figure 2) post-infection, a sudden drop in the mean serum lipoprotein concentration was observed in the control group. After day 36 post-infection, a sharp and progressive drop in the mean serum level of the parameter in the infected group was observed. This decline continued up to the termination of the experiment (Figure 2). The difference between the post-infection mean serum low density lipoprotein concentrations in the *T. brucei*-infected and control groups, which were 2.3 ± 0.3 and 2.4 ± 0.2 mmol/L, respectively, was not significant ($P > 0.05$). Except for the minor fluctuations, mean serum concentration of this parameter in the control pigs remained fairly the same throughout the experimental period (Figure 2).

Cholesterol

The pre-infection mean serum values for cholesterol in the infected and control pigs were 4.6 ± 0.4 mmol/L and 4.5 ± 0.5 mmol/L respectively. Following infection with *T.* brucei, there was a gradual decrease in the serum concentration of the cholesterol in the infected group which started from day 12 post-infection (Figure 3). This drop in the mean serum level of cholesterol in the *T. brucei*-infected group continued without abating up to the end of the experiment (Figure 3). On the contrary, no appreciable change was observed in the mean serum cholesterol concentration in the control group during the experiment. The difference between the post-infection mean serum cholesterol concentration (3.8 ± 0.6 mmol/L) in the infected group and that (4.5 ± 0.2 mmol/L) in the control pigs was significant ($P < 0.05$).

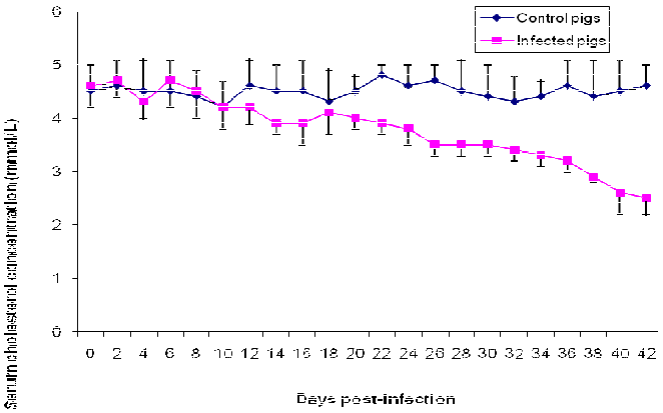

Figure 3. Mean serum cholesterol concentration in *T. brucei* infected and control pigs.

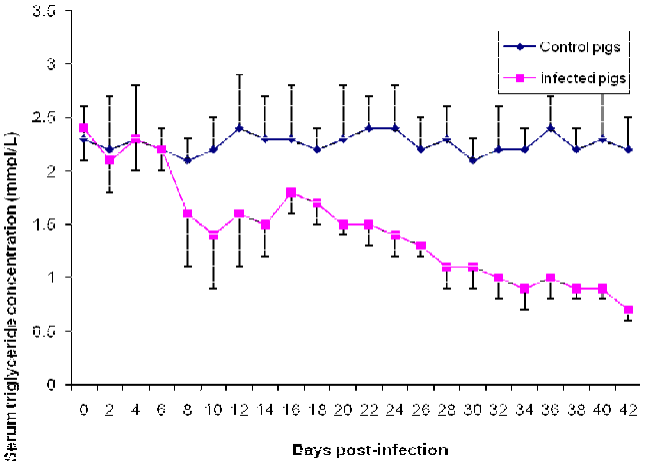

Figure 4. Mean serum triglyceride concentration in *T. brucei*-infected and control pigs.

Triglycerides

Values of the pre-infection mean serum triglyceride concentrations in the infected and control groups were 2.4 ± 0.3 mmol/L and 2.3 ± 0.3 mmol/L, respectively. *T. brucei* infection induced a significant decrease in the mean serum triglyceride level in the infected group (Figure 4). The sharp decrease was discernible from the value on day 6 of 2.2 ± 0.2 mmol/L to reach a low value of 1.6 ± 0.5 mmol/L on day 8 post-infection. It remained fairly at this level up to day 14 post-infection before rising, slightly, on days 16 and 18 post-infection after which the decrease continued up to the end of the experiment (Figure 4). The mean serum value of this parameter remained fairly unchanged during the course of the experiment (Figure 4) . The mean post infection values for this parameter in the infected and control groups, which were 1.5 ± 0.4 mmol/L and 2.3 ± 0.1 mmol/L, respectively, differed significantly ($p < 0.05$) from each other.

DISCUSSION

From the findings in this study, it is reasonable to infer that *T. brucei* infection of pigs causes significant decrease in the serum levels of cholesterol, high density lipoprotein and triglyceride. Reasons for the sudden drop in serum low-density lipoprotein on day 10 could not be ascertained at the time of the experiment but remains to the best of our knowledge subject of investigation.

Sudden and unexplained changes in lipid catabolism in the control sheep could be a possible explanation on this finding. The infection apparently has minimal or no effect on serum low density lipoprotein concentration. The findings in this study are in conformity with those in the reports of Biryomumaisho et al. (2003), in *T. congolense* and *T. brucei* infection of goats, and Adamu et al. (2008), in *T. congolense* infection of sheep, respectively. Other findings that tally with the ones in the present study are those in the reports of Welde et al. (1989) and Katunguka-Rwakishaya et al. (1992) in *T. rhodesiense* and *T. congolense* infections of cattle and sheep, respectively. The findings in the present study, like in the previous one (Adamu et al., 2008), are in complete deviance to those made in *T. brucei* infection of rabbits (Nakamura, 1998).

T. brucei infection induced a more drastic and progressive drop in serum triglyceride concentration in pigs than was observed with *T. congolense* infection of sheep (Adamu et al, 2008). Unlike *T. congolense* infection of sheep (Adamu et al., 2008), *T. brucei* infection caused progressive decline in serum level of cholesterol in pigs. Although the cause of disparities in the serum levels of lipids and cholesterol in some of the previous reports was not investigated, differences in species or strain of trypanosomes or of the host animal breed and species used in the different studies might have contributed to the variable results obtained. Irrespective of the host and parasite species involved, the clinical disorders inflicted by trypanosomes are generally the same and most of the times are consistent (Logan-Henfrey et al., 1992).

The alterations observed in the serum concentrations of the triglyceride, high density lipoprotein and cholesterol could involve many pathophysiological mechanisms (Adamu et al., 2008). It has been reported that trypanosomes require lipoproteins for them to multiply under axenic culture (Black and Vanderweed, 1989). Thus, the lowering of the serum the lipids and cholesterol as observed in the present and previous studies (Welde et al., 1989; Katunguka-Rwakishaya et al., 1992; Biryomumaisho et al., 2003; Adamu et al., 2008) could, partly, be the result of trypanosomal utilization of the molecules. Also, blood-stream forms of trypanosomes are unable to synthesize cholesterol and so require it along with phospholipids and total lipids for synthesis of their membranes and growth (Hue et al., 1990; Katunguka-Rwakishaya et al., 1991; Green et al., 2003; Nok et al., 2003). Thus, the continuous utilization, from the blood stream, of these

molecules could therefore be a contributory factor to lowering of the serum levels of lipids and cholesterol.

Yet still, blood-stream trypanosomes scavenge blood glucose as a source of energy (Chaudhuri et al., 2006), which could ultimately lead to hypoglycemia in the trypanosome- infected animal. Although blood glucose level was not determined in this study, hypoglycemia which could be caused by the trypanosome infection could undoubtedly result in increased catabolism of lipids and cholesterol in order to meet some strategic energy needs in the body of the host animal. Consequently, this would lead to decrease in serum levels of these molecules. Gluconeogenesis from lipids for some essential physiological processes in the body could deplete serum lipids and cholesterol. Generally, depressed feed intake of animals typically associated with trypanosomosis (Reynolds and Ekwuruke, 1988; Adamu et al., 2008) and such as observed in the *T. brucei*-infected pigs in the present experiment could ultimately reflect on the levels of the triglyceride, high density lipoprotein and cholesterol. Moreover, there is a suggestion that lipolysis is a major mechanism of supply of the high energy needed in the body during fever induced by trypanosomes (Akinbamijo et al., 1992) . Also, Faye et al. (2005) reported that the high energy demands of trypanosome infection in animals may lead to severe energy shortage and this could affect energy, which could include the serum lipids, cholesterol among others, and protein metabolisms.

It is pertinent to note that impaired synthesis and subsequent release of cholesterol from the liver, due to pathologic changes occurring consequent to trypanosome infection of animals (Logan-Henfrey et al., 1992), could also be partly responsible for the lowered serum level of cholesterol. Impaired synthesis of cholesterol in the liver could also be the result of insufficient hepatocellular respiration due to hypoxia caused by anaemia in the *T. congolense*–infected sheep in the present study. Also, lowered high density lipoprotein such as observed in the present study could retard cholesterol transport in the body and contribute to its lowered serum level.

The consequent effects of significantly lowered lipid and cholesterol serum levels in the pathophysiology of some of the disorders reported in trypanosome-infected animals could only be appreciated with good understanding of the functions of such lipids and cholesterol in the mammalian physiology. The liver is the site of synthesis of cholesterol. At the same time, cholesterol is being supplied in human and animal diets. Cholesterol is the building block for cell membranes and it is essential in the formation of bile (which subsequently 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. Low density lipoprotein is the major cholesterol carrier in the blood and is responsible for transporting cholesterol from the liver to organs and tissues of the body. High density lipoprotein on the other hand is responsible for carrying cholesterol from various organs and tissues to the liver for recycling or degradation.

It is glaring from the foregoing that any significant change in the serum concentration of lipids and cholesterol could have detrimental effects in affected animals. Some clinical disorders have been reported, which could occur as consequences of trypanosome infection in animals. Included in this category are reproductive endocrine disorders that could result in infertility or sterility, in extreme cases of trypanosome infection of animals (Mutayoba et al., 1994; Sekoni, 1994; Adamu et al., 2004, 2006, 2007). Haemolytic crisis partly caused by free fatty acids released by autolysing trypanosomes (Assoku et al., 1977; Biryomumaisho et al., 2003), though not investigated in the present study, could contribute to anaemia, which majorly exacerbates tissue degenerative changes (Logan-Henfrey et al., 1992). The pathophysiological mechanisms involved in the development of nervous manifestations in human, for example, are not currently understood, but autoimmune mechanisms as in Chagas' disease are thought to be involved (Rhind et al., 1997) . It is worth noting that significant decline in serum lipids and cholesterol levels could aggravate the neurological disorders since cholesterol is vital in cell signaling in neuronal synapses formation.

Conclusion

In conclusion, *T. brucei* infection resulted in a drop in serum concentrations of lipids and cholesterol in pigs just like *T. congolense* did in infected sheep in our previous study (Adamu et al., 2008). More investigations are needed to elucidate the mechanisms involved in the decline in serum levels of the lipids and cholesterol. It is also imperative to conduct studies that would help identify biochemical differences, if any exist, between the trypanosomes and animal hosts in the metabolism of lipids and cholesterol with the view to exploiting the findings in biotechnological development of drugs or other therapeutic approaches.

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