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In vitro activity of selected ruminant Sera on T*rypanosoma brucei brucei* Plimmer & Bradford, 1899

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Abstract

This study aimed at evaluating the *in vitro Trypanosoma brucei brucei* activity of sera from some ruminants viz: West African dwarf goat, Red Sokoto goat, Sahel goat, Yankassa sheep, Uda sheep, Balami sheep, N'Dama cattle, Sokoto Gudali cattle, Friesian cross/Bunaji cattle, White Fulani cattle. Blood samples from donor ruminants were screened using thick film and haematocrit centrifugation technique (HCT). to ensure that they were free from trypanosomes. *Trypanosoma brucei brucei* (Federe strain) was suspended in RPMI supplemented with FCS. After addition of each test serum, the motility of the parasite was used as index of viability. Among the sera from selected small ruminants, only the West African Dwarf goat serum exhibited some anti-trypanosomal activities *in vitro*, with a slight inhibition index/Anti-trypanosomal Activity Index (ATI) of 0.29, and a significant reduction ($P<0.05$) in survival time of 21.33±1.15 hours compared with that of all the sera from other small ruminants including the control (30.00±0.00 hours). On the other hand, among the sera from selected large ruminants, only the N'Dama cattle serum showed some anti-trypanosomal activities *in vitro*, with an inhibition index/ATI of 0.38, and a significant reduction ($P<0.05$) in survival time of 18.67±1.15 hours compared with that of all the sera from other large ruminants including the control (30.00±0.00 hours). The *in vitro* anti-trypanosomal activity of sera from West African Dwarf goat and the N'Dama cattle was cidal in nature. The *in vitro* anti-trypanosomal activity of West African Dwarf goat serum appeared to be related to the xanthine oxidase (XO) activity; the highest ATI of this serum coincided with the highest XO content. This study shows that sera from West African Dwarf goat and N'Dama cattle have innate immunity against *T. brucei brucei*.

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Introduction

Animal trypanosomiasis is a major constraint to livestock productivity in the tropics and has a significant impact on the life of millions of people globally (mainly in Africa, South America and south-east Asia) (Gutierrez *et al.*, 2006). Chamond *et al.*, (2010) reported about 3 million deaths in cattle annually, causing annual production losses of about US$ 1.2 billion. The complex nature of the trypanosome parasite renders the control methods of trypanosomiasis very problematic. Attempts to control trypanosomiasis are based mainly on the use of trypanocidal drugs and on vector control.

Researches have highlighted some trypanoresistant mammals that present a high incidence of trypanosome infections but with low parasitemia (Reduth *et al.*, 1994; Thomson *et al.*, 2009, 2014), and the mechanisms that allow those animals to control blood trypanosomes are being investigated. Reduth *et al.* (1994) and Muranjan *et al.* (1997) isolated a trypanocidal protein from Eland and Cape buffalo sera; the trypanocidal protein from the Cape buffalo was identified as xanthine oxidase – an enzyme that cathalyses the conversion of hypoxanthine to xanthine, and xanthine to uric acid.

In addition to a specific antibody, there are some non-specific factors that kill trypanosomes or limit their proliferation, contributing to host resistance (Black *et al.,* 1999). Ng'ayo *et al.* (2005) detected trypanosomes in small ruminants and pigs, while Trail and collaborators previously evaluated Trypanotolerance and the value of conserving livestock genetic resources (Trail *et al.*, 1989). In this regards, this study evaluated the activity of sera of some ruminants on *T. brucei brucei in vitro*, with the aim of determining their levels of trypanotolerance for better conservation of livestock genetic resources.

Materials and methods

Ethical statement

This research had ethical approval from the Committee on Animal Use and Care, Directorate of Academic Planning & Monitoring, Ahmadu Bello University, Zaria, with the Approval No: ABUCAUC/2017/007. Local approval was obtained from the Assistant Director of Research, NAPRI, Ahmadu Bello University, Zaria. In the course of this study, recommendations on guidelines for the accommodation and care of animals used, based on Directive 86/609/EEC (EU Directive 2010/63/EU for animal experiments), were taken into consideration.

Sampling site

Zaria is located on the high plains of Northern Nigeria, 652.6 meters above the sea level, some 950 km away from the coast (112 031N 7Q 42E). Zaria is a city of Kaduna state which extends from the tropical grassland known as the Guinea Savannah to Sudan Savannah. Vegetation is thick and grasses about 3.6 meters tall with big trees, which grow shorter as one approaches the Sudan Savannah (https://abu.edu.ng/zaria-at-a-glance.html).

Donors ruminants

Blood was collected, with the assistance of veterinary assistants, from some ruminants reared in areas free of trypanosomes (Zaria in Nigeria) and were also certified trypanosome free by the thick film and haematocrit technique (Woo, 1989). West African dwarf goat (*Capra aegagrus hircus*), Red Sokoto goat (*Capra aegagrus hircus*), Sahel goat (*Capra aegagrus hircus*), Yankassa sheep (*Ovis aries*), Uda sheep (*Ovis aries*), Balami sheep (*Ovis aries*), N'Dama cattle (*Bos taurus*), Sokoto Gudali cattle (*Bos taurus*), Friesian cross/Bunaji cattle (*Bos taurus*), White Fulani cattle (*Bos taurus*) were all obtained from the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Blood Sample collection and processing

Ten millilitres (10mL) of blood sample was aseptically collected from each donor ruminant through the jugular vein; 2mL of this was dispensed into EDTA (ethyl-diamine-tetra-acetic acid) coated tube placed in a beaker containing iceblocks while the remaining (8mL) was dispensed into sterile plain glass test tube and allowed to clot for 2-3 hours at room temperature from which serum was collected after centrifugation at 4,000 g for 5min (Black *et al.*, 1999).

Sera samples obtained were stored in a freezer at -20°C until required for further analysis. Blood samples in the EDTA tubes were used to screen for the presence of trypanosomes on the basis of which the serum of trypanosome free samples were used.

Screening of blood for trypanosomes

Each blood sample in the EDTA coated tubes was screened for trypanosomes by the thick films and haematocrit centrifugation technique (HCT) (Woo, 1989).

Parasite

Trypanosoma brucei brucei (Federe strain), was obtained from the Nigerian Institute of Trypanosomiasis Research (NITR), Kaduna, Nigeria. The parasite was maintained through serial passaging in Wistar rats. Blood was collected from the tail of infected rats by capillary action into heparinized microhaematocrit tubes up to three-quarter their length, one end of each capillary tube was sealed with plastiseal. The sealed capillary tubes were placed in a microhaematocrit centrifuge and spun at 9000 g for 5 min. The parasites were extracted from the buffy-coat and used for the experiment.

In vitro detection of anti-trypanosomal activity of the test sera

This was done using microtitre plates as follows:

Trypanosomes from the buffy coat were suspended in RPMI 1640 (Rossvelt Park Memorial Institute 1640) medium supplemented with 2% glucose, 2mM sodium pyruvate, 10% complement - inactivated (56°C for 30 min) fetal bovine serum (Black and Van-deweerd, 1989), sodium bicarbonate and sodium pyruvate, and antibiotics (streptomycin 100µg/ml, penicillin 100U/ml). A total of 50µl of each ruminant serum sample was introduced into one of the 96 wells of a microtitre plate and 50µl of *T. brucei brucei* suspension 8 per field (31.62×10^6/mL of blood) was added to it, rocked gently to mix and incubated at room temperature. Thereafter, a drop of about 5µl of each mixture was examined microscopically on hourly basis using wet film method. Cessation in motility of parasites was taken as indication of inhibitory/cidal serum activity against the parasites (Bulus *et al*., 2008). The motility of *T. brucei brucei* in each well was compared with the motility of the same parasites in the control well without test serum. A formula was derived (Djieyep *et al*., 2017) to determine the Anti-Trypanosomal Activity Index (ATI) of each serum sample.

$$ATI = \frac{T_c - Ts}{T_c}$$

Where, T_c is the survival time of the parasites in the control medium

T_s is the survival time of the parasites in the sample

Infectivity assessment

80µl of the mixture of the *in vitro* affected parasite with effective serum was inoculated into mice and monitored by microscopy for trypanosomes on daily basis for 10 days to determine if the observed anti-trypanosomal activity was inhibitory or cidal.

Detection of Anti-Trypanosomal Antibodies in Serum Samples

This was done using a slightly modified Ouchterlony Double Diffusion test (Ouchterlony and Nilsson, 1978).

Detection of the xanthine oxidase content of the selected sera

The technique of Black *et al*. (1999) was adopted to detect the xanthine oxidase content of the test sera. Accordingly, aliquots (100 µl) of each serum was added to 900 µl of H_2O_2-assay buffer (0.5mm xanthine and 1mm 2, 4, 6 tribromo-3-hydroxybenzoic acid in 0.1mm 4-amino-antipyrine with a final concentration of 8 units horse-radish peroxidase permL). The mixture was incubated at 25°C for 30 min, immediately chilled in an ice bath, and absorbance read at 512 nm wave length was recorded after zeroing the spectrophotometer with an equivalent mixture lacking horseradish peroxidase. A serial dilution of the commercial cow's milk xanthine oxidase was done; absorbance was also recorded at 512 nm wave length and used to plot a standard curve. It has been established that detection of H_2O_2 produced in serum by this assay is not affected by other enzymes in serum, including catalase (Le Tissier *et al*., 1994). The xanthine oxidase content of serum was determined by reading the serum value against the cow's milk xanthine oxidase standard curve.

Data analysis

The software Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis. One-way Analysis of variance (ANOVA) was used to analyze the variations in the survival time of trypanosomes exposed to test sera as well as the xanthine oxidase content of the test sera. The significant difference was at the level of probability 0.5. All data were expressed as means ± Standard Error.

Results

Trypanosome Infection Status of Sera from Selected Ruminants

All the blood samples from the selected ruminants were negative for trypanosomes by thick blood film and haematocrit centrifugation technique (HCT).

Effect of the Selected Ruminant Sera on Trypanosoma brucei brucei

In vitro activity of sera from selected small ruminants on Trypanosoma brucei brucei

Sera from the selected small ruminants, except the West African Dwarf goat, did not exhibit any anti-trypanosomal activity but significantly increased (P<0.05) the survival time of the parasites. The survival times of *T. brucei brucei* in the sera of the selected small ruminants were significantly higher (P<0.05) than the control (30.00±0.00 hrs), except the West African Dwarf goat with a survival time of 21.33±1.15 hrs which was significantly lower (P<0.05) than that of the parasites in the control medium, indicating a slight ATI of 0.29 (Table 1). Sera from all selected breeds of sheep had similar supportive activity. Serum from Balami sheep had the highest supportive activity as the ATI was the highest on the negative side, - 0.5, with a mean survival time of 45.00±0.00 hrs.

In vitro activity of sera from selected large ruminants on Trypanosoma brucei brucei

The results in Table 2 show that there were significant differences (P<0.05) in the interaction of sera from the selected large ruminants with *T. brucei brucei in vitro*, and the control, except of N'Dama serum which recorded a survival time of 18.67±1.15 hrs, with an anti-trypanosomal activity index of 0.38. *Trypanosoma brucei brucei* survival times in sera from White Fulani, Sokoto Gudali and Friesian cross/Bunaji were significantly higher (P<0.05) than the control, with negative ATI values, indicating a supportive activity on *T. brucei brucei*. The highest survival time, 59.67±6.81 hrs, was recorded from the serum of Friesian cross/Bunaji.

Table 1. *In vitro* activity of sera of some small ruminants on *Trypanosoma brucei brucei*.

Source of serum	Mean survival time (hr)	Mean ATI
Capra aegagrus hircus (West African dwarf goat)	21.33±1.15^{a}	0.29
Capra aegagrus hircus (Red Sokoto goat)	41.33±1.15^{cd}	-0.38
Capra aegagrus hircus (Sahel goat)	37.33±6.43^{c}	-0.24
Ovis aries (Yankassa sheep)	43.00±1.73^{d}	-0.43
Ovis aries (Uda sheep)	44.00±1.73^{d}	-0.47
Ovis aries (Balami sheep)	45.00±0.00^{d}	-0.50
RPMI 1640*	30.00±0.00^{b}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different (P<0.05).

ATI: Anti-trypanosomal activity Index.

*Culture medium without serum (control).

Table 2. *In vitro* activity of sera of some large ruminants on *Trypanosoma brucei brucei*.

Source of serum	Mean survival time (hr)	Mean ATI
Bos taurus (N'Dama cattle)	18.67±1.15^{a}	0.38
Bos taurus (Sokoto Gudali cattle)	51.33±11.01^{b}	-0.71
Bos taurus (Friesian cross/Bunaji cattle)	59.67±6.81^{b}	-0.99
Bos taurus (White Fulani cattle)	47.33±4.62^{b}	-0.58
RPMI 1640*	30.00±0.00^{a}	0

Values are means ± standard deviation. Values with different superscripts down the column are significantly different (P<0.05).

ATI: Anti-trypanosomal activity Index.

*Culture medium without serum (control).

Xanthine Oxidase Content of Sera from Selected Ruminants

Xanthine oxidase content of sera of selected small ruminants

It was observed as shown in Fig. 1, that West African dwarf goat serum had the highest concentration of

xanthine oxidase (2.06±0.09 µg/ml), followed by that of Uda sheep, then Balami sheep, Sahel goat, Yankassa sheep and Red Sokoto goat which had the lowest concentration (0.70±0.00 µg/ml).

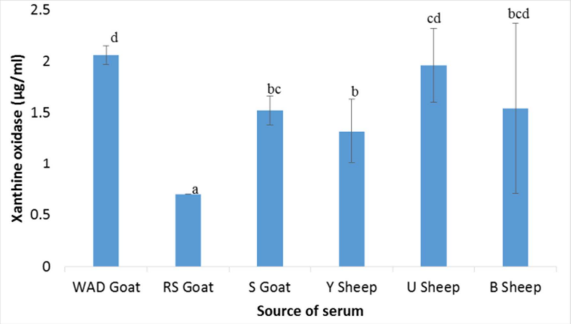

Fig. 1. Xanthine oxidase content of sera from selected small ruminants.

Values are mean concentrations. Values with different superscripts are significantly different (P<0.05).
Key: WAD: West African Dwarf, RS: Red Sokoto, S: Sahel, Y: Yankassa, U: Uda, B: Balami.

Xanthine oxidase content of sera of selected large ruminants

Result on xanthine oxidase content of sera of the selected large ruminants is illustrated in Fig. 2. It shows that sera from all the selected large ruminants had the same range of xanthine oxidase activity, as their concentrations were not significantly different (P>0.05).

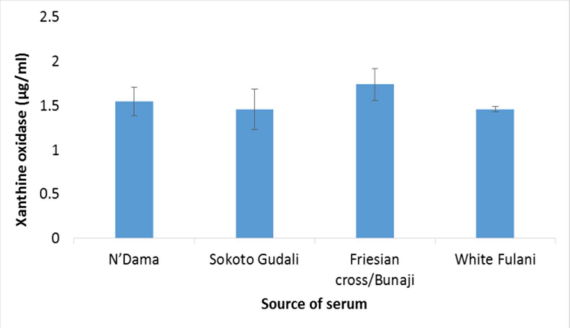

Fig. 2. Xanthine oxidase content of sera of selected large ruminants. Values are mean concentrations.

Discussion

This study has revealed that sera from the selected small ruminants are mostly supportive of the survival of *T. brucei brucei in vitro*; sera from sheep were found to be more supportive since the highest survival times was recorded for *T. brucei brucei* in Balami sheep serum. This result suggests that sheep, most especially the Balami breed are more susceptible to *Trypanosoma brucei brucei* than goat. This repport is in line with that of Ng'ayo *et al.* (2005) who reported that sheep were more succeptible to infection by trypanosomes than goats. The fact that the least supportive activity was recorded in the serum of West African Dwarf goat in this study could be attributed to the trypanotolerance of these goats. This seems to suggest some relative genetic purity of this goat, which needs to be preserved in order to maintain their trypanotolerance. Geerts *et al.* (2009) reported that the introgression of genes of trypanosusceptible breeds into the West African Dwarf goat populations, which is being practised, could lead to their loss of trypanotolerance.

The supportive activity for *T. brucei brucei* observed with sera from the large ruminants *in vitro,* with the exception of serum from N'Dama cattle, may be associated with the succeptibility of these ruminants to *T. brucei brucei* and the reputable trypanotolerance of the N'Dama cattle that has been demonstrated by many findings (Hornby, 1921; Murray, 1974; Morrison *et al.*, 1981; Morrison and Murray, 1985; ILRAD, 1986; Paling *et al.*, 1991). The highest activity recorded with Friesian cross serum suggests that this breed of cattle is the most susceptible among the large ruminants to *T. brucei brucei.*

In ruminants, the serum with the lowest supportive activity, from the West African dwarf goat, having the highest concentration of xanthine oxidase suggests some association of the trypanosomal activity with the xanthine oxidase activity. The low concentration of the enzyme in Red Sokoto goat serum seems to be in order with the lack of anti-trypanosomal effect observed.

That sera from all the selected large ruminants including the N'Dama serum had similar xanthine oxidase activity suggests that the trypanotolerance ability of N'Dama cattle is genetic and not catabolic. D'leteren *et al.* (1998) also observed that trypanotolerance is not only a breed characteristic but is also a heritable trait within the N'Dama population.

Our findings also corroborate with that of Trail *et al.* (1989) who examined phenotypes that appeared more characteristic of the N'Dama in comparison with those of East African Zebu cattle for associations with trypanotolerance traits.

Conclusion

Among the sera from the selected ruminants, sera from West African Dwarf goat and N'Dama cattle have anti-trypanosomal activity on *T. brucei brucei in vitro*, which is a cidal activity. None of the sera contain antibodies reactive to *T. brucei brucei*. The anti-trypanosomal activity of the West African Dwarf goat serum coincides with its xanthine oxidase content.

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