

Some Indices Of Trypanotolerance In Three Breeds Of Nigerian Goats That Differ In Susceptibility To Trypanosomosis

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Citation

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Abstract

A study aimed at identifying potential makers of trypanotolerance was conducted using three breeds of Nigerian goats that differ in susceptibility to animal trypanosomosis. Blood samples of 314 goats adjudged apparently healthy based on freedom from blood and intestinal parasites and a PCV of 25% or higher were used to assess some potential indices of trypanotolerance. The indices were measured using standard procedures, such as Packed cell volume (PCV), haemoglobin concentration (Hb) and complement levels via the classical (CH_{50}) and alternative pathways (AP- CH_{50}). The goats used in the study, consisted of 108 West African Dwarf (WAD), 108 Red Sokoto (RS) and 98 Borno White (BW) breeds of goats of both sexes. There was no significant ($P>0.05$) differences between PCV values in WAD (28.2%), RS (28.3%) and BW (29.2%) goats respectively. Similarly, there was no significant ($P>0.05$), difference between the Hb concentration in WAD (10.1g %), RS (9.7g %), and BW (10.2g %). Complement component C3 levels in WAD (133.2%), RS (107.7%) and BW (92.3%) were significantly ($p<.05$) different. In addition, CH_{50} levels in WAD (59.0 units), RS (57.4 units) and BW (43.1 units) were significantly ($p<.05$) different. No significant ($P>0.05$) difference was observed in the AP- CH_{50} levels in WAD (19.9 units), RS (21.8 units) and BW (20.3 units). The above results suggest that only the levels of C3 and CH_{50} are potential indices of trypanotolerance.

INTRODUCTION

A major constraint to selection for tolerance to trypanosomosis (trypanotolerance) in livestock for both within breed and cross-breeding programmes has been the absence of practical reliable makers of resistance or susceptibility (D'Leteren et al., 1998). In cattle, both natural and experimental studies have shown that certain breeds are more trypanotolerant than others (Murray et al., 1984; FAO, 1991). Many investigators have attributed this phenomenon of trypanotolerance to enhanced humoral immune response, control of anaemia and parasitaemia among other factors (Herbert, 1981; Murray and Urquhart, 1977; Musoke et al., 1981; Otesile and Tabel, 1987; Otesile, et al., 1991). However, there is dearth of information on related comparative studies in small ruminants in Nigeria and elsewhere, particularly on the humoral immune aspects (Haemolytic complement levels, complement C3 level etc). It is conceivable that differences occur among blood components such as PCV, Hb concentration and serum haemolytic complement levels in these animals that show different patterns of resistance or susceptibility to

trypanosomosis.

In order to investigate the phenomenon of trypanotolerance in goats, three apparently healthy breeds of Nigerian goats namely; West African Dwarf (WAD), Red Sokoto (RS) and Borno white (BW) goats were used for the study.

MATERIALS AND METHODS

The WAD goats occur naturally in the southern forest zone of Nigeria, and are known to be trypanotolerant whereas; the RS and BW that occur in the northern part of the country are known to be trypanosusceptible (Mason 1988). The goats were adjudged healthy, based on freedom from gastrointestinal and haemoprotozoan parasites with a packed cell volume (PCV) of 25 percent or greater.

ANIMALS

Out of the 410 goats sampled, 314 were between 1 - 1½ years of age and of both sexes. They comprised of three phenotypically different breeds adjudged apparently healthy. The goats consisted of 108 WAD, 108 RS and 98 BW and were obtained from Ibadan, Oyo State and Maiduguri, Borno

State, Nigeria, from various household units and livestock markets. The ages of all the animals were determined using the rostral teeth development as described by Otesile and Obasaju (1981).

SAMPLE COLLECTION

Blood samples were collected from the external jugular vein. 8mls of blood was collected from which 2mls was immediately dispensed into a Bijou bottle containing 100µl of 100nm of disodium ethylene diamine tetracetic acid (EDTA) solution as anticoagulant. The remaining 6mls of blood was dispensed into plain test-tube to clot. The samples were labeled appropriately and transported to the laboratory on ice pack.

Preparation of goat anti-rabbit haemolysin

The procedure of Mayer (1971) and Lanchman et al., (1973) were used with slight modifications, to raise haemolysins against goat red cells. Briefly, two adult chinchilla rabbits weighing between 1.8 to 2 kg were inoculated intravenously with 1ml of goat RBC membranes (containing 2 mg suspension) in isotonic saline solution as described by Dodge et al. (1963). Intravenous injection of RBC membranes was repeated at a 24 hourly interval with 11 injections administered. Seventy-two hours after the last injection, blood collected from the rabbits was allowed to clot for one hour at room temperature. Serum containing haemolysin was collected by centrifugation at 5,000-x g for 10 minutes at 4°C. Haemolysins from the two rabbits were pooled together, dispensed in 0.5ml aliquots into sterilized Bijou bottles, and immediately stored at -20°C until used.

PREPARATION OF ANTI-C3 SERA

Anti-C3 serum was prepared as described by Hudson and Hay, (1980). Briefly, goat serum was activated with zymoson by mixing 13.5 mg/ ml serum and incubated at 37°C for 30 minutes. The zymoson C3 complex was washed six times in barbitone buffered saline (pH 8.6). Freund's incomplete adjuvant (FIA) was added and contents thoroughly mixed. Appropriately, 0.2ml of zymoson – C3/FIA mixture was injected subcutaneously to each of the four sites of an adult chinchilla rabbit namely; the right and left shoulder regions and right and left rump regions. Seven days after the last immunization with zymoson C3/ FIA emulsion, blood from the rabbits were collected through the marginal ear veins and Anti- C3 serum was collected and dispensed in 0.5ml aliquots and stored at -20°C until use.

ESTIMATION OF TOTAL AND ALTERNATIVE HAEMOLYTIC COMPLEMENT (THC AND AHC) LEVELS.

A micro method of the standard haemolytic assay was used for the determination of total serum complement (THC) level as described by Ogundele (1988). Briefly, 50µl of 1:50 initially titrated dilution complement was pipetted into 84 wells of U-shaped bottom microtitre plates and 50µl of 5×10^8 cells/ml of washed sheep red cell was added into each well. The plates were incubated at 37°C for one hour with constant shaking at 10 minutes interval after which, the sensitized rabbit red cell suspension was washed three times with Tris-Saline buffer and supernatant solution decanted. The volume of sensitized RBC was adjusted to 50µl with Tris-saline buffer and 50µl of 40 percent fresh goat serum (FGS) added into each test well and gently shaken to ensure even suspension of erythrocytes with FGS (giving a final reaction concentration of 20 percent FGS). Positive and negative control wells were set up. The plates were incubated for one hour at 37°C with constant shaking at 10 minutes intervals and later spun at 200 x g for 10 minutes. The supernatant was pipetted and transferred into flat bottom microtitre plates. The optical density (OD) reading of the released haemoglobin was read at 541nm. The degree of lysis (which reflects complement activity) was calculated and converted into CH_{50} as described by Mayer (1971).

The method of assaying the alternative haemolytic complement level (AHC) is the same as described for THC. The only exception was that, rabbit erythrocyte replaced sheep erythrocyte (without sensitization with haemolysin) while, Ethylene- Glycol bis- amino tetra-acetic acid (EGTA) replaced calcium chloride in Tris- saline buffer (Talwar, 1983).

DETERMINATION OF SERUM LEVELS OF COMPLEMENT C3

Serum levels of complement C3 was determined by single radial immuno diffusion (RID) technique as described by Tabel (1982). The levels were expressed in relation to the level in a standard reference pool of apparently healthy goat serum.

ESTIMATION OF HAEMATOLOGICAL PARAMETERS

Packed cell volume (PCV) and haemoglobin concentration (Hb) concentration were estimated using autohaemolyzer.* (* Autohaematology Analyser (Mindray) Bc 2800 Vet.).

EXAMINATION FOR HAEMOPARASITES

Each blood sample was examined for haemoprotozoans by preparing thin blood smears stained with 10% Giemsa stain and positive blood samples were discarded. In addition, the buffy coat examination technique and ELISA was used to screen for trypanosome infection (Murray et al., 1977, and Luckins 1992).

EXAMINATION OF FAECAL SAMPLES

To screen for gastrointestinal parasites, fresh faecal samples were collected directly from the rectum of the goats. They were subjected to sedimentation and floatation techniques using saturated sodium chloride or zinc sulphate solution as floatation medium (Anon, 1977).

STATISTICAL ANALYSIS

The student's test was used to determine the level of significance between the variables where ($p < 0.05$) were considered significant and subjected to 2 x 2 contingency table for relative risks at 95% confidence limit. Chi-square was also employed to give a measure of strength of association between the variables under study (Graph Pad Instat, 2000).

RESULTS

HEMATOLOGY

The mean packed cell volume (PCV %) and haemoglobin (Hb) concentration of apparently healthy three breeds of indigenous goats is presented in Table 1. There was no significant ($P > 0.05$) difference between the PCV and Hb concentration of the three breeds of goats studied. In addition, there was no significant ($P > 0.05$) difference between the PCV and haemoglobin (Hb) concentration of the males and females of the three breeds.

Figure 1

Table 1: Mean packed cell volume and haemoglobin concentration (\pm SE %) of apparently healthy 3 breeds of indigenous goats

| Goat Breed | Males | | | Females | | | Both sexes | | |
|------------|-------|----------------|----------------|---------|----------------|----------------|------------|----------------|----------------|
| | No. | Hb Conc | PCV | No. | Hb Conc | PCV | No. | Hb Conc | PCV |
| WAD | 56 | 10.3 \pm 1.4 | 29.5 \pm 2.6 | 52 | 9.9 \pm 1.4 | 28.4 \pm 2.5 | 108 | 10.1 \pm 1.4 | 28.9 \pm 2.6 |
| RS | 56 | 10.1 \pm 0.8 | 28.0 \pm 2.0 | 52 | 9.4 \pm 1.3 | 28.7 \pm 2.6 | 108 | 9.8 \pm 1.1 | 28.3 \pm 2.3 |
| BW | 50 | 10.2 \pm 1.4 | 28.9 \pm 2.0 | 48 | 10.5 \pm 1.7 | 29.5 \pm 2.2 | 98 | 10.4 \pm 1.6 | 29.2 \pm 2.2 |
| All breeds | 162 | 10.2 \pm 1.1 | 28.8 \pm 2.3 | 152 | 9.9 \pm 1.5 | 28.7 \pm 2.4 | 314 | 10.1 \pm 1.4 | 28.8 \pm 2.4 |

Keys: WAD = West African Dwarf, RS = Red Sokoto, BW = Borno White

SERUM COMPLEMENT

The mean relative complement C3 concentration of apparently healthy three breeds of indigenous goats is presented in Table 2. C3 levels of WAD goats (133.2 \pm 3 units) was significantly ($P < 0.05$) higher than the corresponding values in BW goats (92.7 \pm 18.7 units) and RS goats (107.7 \pm 12.6 units). Similarly, the C3 levels of RS goats was significantly ($P < 0.05$) higher than the corresponding value in BW goats. There was no significant ($P > 0.05$) difference between the C3 concentration of males and females of the three breeds (Table 2).

The mean total and alternative hemolytic complement levels (CH_{50} and AP- CH_{50}) of apparently healthy three breeds of indigenous goats are presented in Table 3. CH_{50} of WAD goats (59.0 \pm 6.2 units) was significantly ($P < 0.001$) higher than the corresponding value in BW goats (43.1 \pm 3.8 units) but was not significantly ($P > 0.05$) different from the value in RS goats (57.4 \pm 9.8 units). Mean CH_{50} of RS goats was significantly ($P < 0.05$) higher than the corresponding values in BW goats (Table 3). There was no significant ($P < 0.05$) difference between the CH_{50} of the males and females of the three breeds (Table 3). In addition, there was no significant ($P > 0.05$) variation in the AP- CH_{50} of WAD goats (19.9 units), RS goats (21.8 units) and BW goats (20.3 units).

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Figure 2

Table 2: Mean relative complement (C3) concentration of apparently healthy breeds of indigenous goats (\pm SE %)

| Goat breeds | Males | | Females | | Both sexes | |
|-------------|-------|------------------|---------|------------------|------------|------------------|
| | No. | Mean C3 conc. | No. | Mean C3 conc. | No. | Mean C3 conc. |
| WAD | 56 | 131.1 \pm 13.0 | 52 | 133.2 \pm 13 | 108 | 132.9 \pm 12.9 |
| RS | 56 | 108.3 \pm 11.5 | 52 | 107.7 \pm 12.6 | 108 | 106.0 \pm 13.1 |
| BW | 50 | 94.1 \pm 20.4 | 48 | 92.7 \pm 8.7 | 98 | 93.5 \pm 18.1 |
| All breeds | 162 | 111.4 \pm 14.9 | 152 | 111.2 \pm 11.4 | 314 | 111.2 \pm 14.7 |

Keys: WAD = West African Dwarf, RS = Red Sokoto, BW = Borno White

Figure 3

Table 3: Mean total haemolytic complement and alternative haemolytic complement levels (CH and AP \pm SE %) of apparently healthy breeds of indigenous goats

| Goat breeds | Males | | | Females | | | Both sexes | | |
|-------------|-------|------------------|------------------|---------|------------------|------------------|------------|------------------|------------------|
| | Mean | | | Mean | | | Mean | | |
| | No. | AP ₅₀ | CH ₅₀ | No. | AP ₅₀ | CH ₅₀ | No. | AP ₅₀ | CH ₅₀ |
| WAD | 56 | 20.6 \pm 3.4 | 57.7 \pm 6.1 | 52 | 18.5 \pm 4.8 | 59.8 \pm 6.6 | 108 | 19.5 \pm 4.1 | 58.7 \pm 6.2 |
| RS | 56 | 20.3 \pm 4.8 | 51.1 \pm 8.0 | 52 | 23.4 \pm 5.2 | 58.8 \pm 8.4 | 108 | 21.8 \pm 5.0 | 57.4 \pm 8.8 |
| BW | 50 | 20.8 \pm 3.4 | 61.4 \pm 5.9 | 48 | 19.9 \pm 3.4 | 42.6 \pm 6.0 | 98 | 20.3 \pm 4.5 | 43.1 \pm 3.8 |
| All breeds | 162 | 20.5 \pm 4.3 | 53.4 \pm 4.7 | 152 | 20.6 \pm 4.5 | 53.7 \pm 7.0 | 314 | 20.5 \pm 4.5 | 53.0 \pm 6.5 |

Keys: WAD = West African Dwarf, RS = Red Sokoto, BW = Borno White

DISCUSSION

This study was aimed at investigating some indices of tolerance to trypanosomosis using breeds of apparently healthy Nigerian goats. This objective was achieved by collecting blood samples to determine some haematological parameters, haemolytic complement and C3 levels. This investigation was necessitated by the fact that most work on haematolytic complement, complement component C3 and other potential indices of trypanotolerance were studied in large ruminants (cattle and domestic buffaloes) with little attention to small ruminants, particularly goats. There was no significant ($P > 0.05$) difference in the packed cell volume (PCV) and haemoglobin concentration (Hb) between the three breeds of goats studied. Similarly, sex was not associated with any significant ($P > 0.05$) difference between the means of the PCV and Hb values. This observation is in agreement with earlier works by Oduye and Okunaiya (1971), and Adulugba and Joshua (1990).

The WAD goats however, had significantly ($P < 0.05$) higher (CH₅₀) total haemolytic complement and C3 levels compared to RS and BW goats. This observation may suggest possible roles of THC and C3 levels as indices of trypanotolerance

since this breed has been reported to be trypanotolerant (Mason 1988). This has been attributed to innate immunity, a genotypic trait of resistance, mostly responsible for the survival of wild or trypanotolerant breed in tsetse-infested areas (Selfert, 1992). It has been reported that a direct relationship exist between humoral immunity, PCV, Hb and serum haemolytic complement (C3) in trypanosomosis (Musoke et al., 1981; Otesile and Tabel, 1987; Otesile, et al., 1991). Most of the investigators have attributed this phenomenon of trypanotolerance to enhanced humoral immune response, control of anaemia (PCV, Hb) and parasitaemia among other factors (Herbert, 1981; Murray and Urquhart, 1977; Musoke et al., 1981; Otesile and Tabel, 1987; Otesile, et al., 1991). Ecologically, WAD goats are mostly restricted to the tsetse infested rainforest belt of southern Nigeria, and are constantly threatened by trypanosomosis, which may have resulted to the phenomenon of parammunity (D'letern, 1998). It is therefore, conceivable that continuous and sustained stimulation of the host's immune system by these parasites can lead to a higher level of humoral immune components in these animals compared to their counterparts in the northern dry part of the country where vector borne diseases are very minimal.

In all the three breeds studied, there was no significant ($P > 0.05$) difference in the alternative haemolytic complement level (AP₅₀). It is therefore, possible that trypano-susceptibility or trypanotolerance in goats will not be associated with the above parameter. However, further experimental infection of individual breeds may shed more light on this observation.

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