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AUTOERYTHROPHAGOCYTOSIS BY PERIPHERAL BLOOD MONOCYTES IN RUMINANTS WITH HAEMOPROTOZOAN DISEASES

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Target Audience: Academics/researchers in animal production and health.

ABSTRACT

This study investigated the incidence of autoerythrophagocytosis by peripheral blood monocytes (PBM) in 40 cattle, 30 sheep and 30 goats with or without haemaprotozoan diseases broght for slaughter at the Bodija Cattle Market, Ibadan, between the months of March to June 1997 and October to December, 1998. Standard parasitological and haematological procedures were used to determine haemoparasitic infections and the degree and types of anaemia developed by the animals. A modified plasm/gelatin coated flask method was used to isolate PBM from the blood and their sizes and erythrophagocytic activities determined. Statistical comparisons were made between and within infected and non-infected species of animals. Trypanosomiasis, anaplasmosis and their mixed infections resulted in varying degree of anaemia, increase in mean PBM diameters and erythrophagocytic activities. Between 6 to 28% of PMB of the infected animals were phagocytic, while between 30-85% increases in PBM size were recorded in the infected animals compared with the non-infected ones. PBM isolated from infected animals were found to have engulged between 1 to 5 erythrocytes, most of which were parasitized, especially with *Anaplasma ovis*.

Key words: Erythrophagocytosis, monocytes, ruminants haemoprotozoan diseases.

DESCRIPTION OF PROBLEM

Haemoprotozoan diseases such as trypanosomosis, babesiois and anaplasmosis are responsible for varying levels of morbidites, mortalities and subsequent economic losses in both large and small ruminant stock farming in Africa. In fact, trypanosomosis, caused by free-swimming protozoan haemoflagellates of the genus *Trypanosoma*, has so far remained one of the strongest obstacles to profitable livestock farming in vast areas comprising of at least a third of 10m Km2, covering 37 countries in Africa (1,2,3). Trypanosomosis in man, domestic and wild animals is characterized, among others by extravascular haemolysis (erythrocyte destruction) and variable degrees of anaemia, cachexia and death (4,5). *Babesia spp* and *Anaplasma spp.* are obligate intra-erythrocytic protozoa which are transmitted by various species of ticks in cattle, sheep and goats (6). These

organisms parasitize and multiply in erythrocytes, thus causing both intravascular and extravascular haemolysis, anaemia, jaundice and haemoglobinuria (6).

A common pathogenetic pathway of anaemia in trypanosomosis babesiosis and anaplasmosis is phagocytosis and destruction of effete and/or parasitized erythroytes in the liver, spleen bone marrow and lymph nodes by an activated and expanded mononuclear phagocyte system (MPS) (5,7), of which the tissue macrophage (MQ) is the primary cell (8,9). The peripheral blood monocyte (PBM) is the immediate precursor of the tissue MQ, and itself is derived from the monoblastic precursors in the bone marrow (10). Monocytes appear in small numbers in the peripheral blood consisting of between 1-4%or 20-850 per ul of blood in cattle, sheep and goats (11).

Even though phagocytic, the PBM stay briefly, with a half-life of about 9 hours, in the peripheral blood before being seeded into tissues as MQ. Here they become sessile or inactive before they develop their phagocytic, secretory and immune regulatory capabilities when activated (9,11,12). Once in the tissues, MQ does not re-enter blood circulation (13). Because of the intricacies involved in MQ membrane signal transduction (14), adherence, pseudopodia development and spreading culminating in phogocytosis (10), monocytes in moving peripheral blood may not normally be able to phagocytes effete and/or parasitized erythrocytes as easily and effectively as tissue MQ. However, very few eases of monocyte autoerythrophagocytosis have been reported during *T. brucei* infection in man (15) and *T.congolense* infections in cattle (16,17). No such reports have been made in small ruminant trypanasomosis and in babesiosis and anaplasmosis in cattle, sheep and goats. This study was therefore designed to monitor peripheral blood monocyte activation and erythrophagocytosis in cattle, sheep and goats infected with trypanosomes, babesia and anaplasma organisms.

MATERIALS AND METHODS

Animals: A total of 100 animals consisting of 40 cattle (30 bulls and 10 cows), 30 sheep (18 rams and 12 ewes) and 30 goats (15 bucks and 15 does), all aged above 2 years were used. The animals were amongst those purchased for slaughter at the Bodija cattle market, Ibadan. They were randomly selected for sampling during the ante-mortem inspections carried out a day previous to slaughter, after due consultation with and approval by their owners and the veterinary personnel at the market. The samplings were carried out between the months of March to June 1997 and October to December, 1998.

Blood Sample Collection: Ten millitre (ml) of blood was collected from the each animal by aseptic jugular venapuncture into vacutainer tubes containing ethylene-diamine-tetraacetric acid (EDTA) anticoagulant. The samples were properly mixed, and placed in ice packs for immediate transportation to the laboratory.

Haematology and Parasitology: For each of the sample, packed cell volume (PCV), haemoglobin (HB) concentration, erythrocyte (RBC) and totol leucocyte (TWBC) counts were determined (18). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were caculated (18). Thin smears of each blood sample were made on clean grease-free glass slides and stained by a modified Giemsa techique (18) for examination and identification of anaplasma and babesia organisms (6). In addition, blood samples were examined for the presence trypanosomes by the dark-ground phase contrast buffy coat techique (19).

Isolation of Peripheral Blood Monocytes (PBM): Peripheral blood monocytes (PMB) from each sample were isolated using a modified method (20). The modification consisted of the harvesting of ficoll-paque (Histopaque^{R}, Sigma Chemical Company, USA) separated peripheral blood mononuclear cells (PBMNC) on to autologous plasma and 2% gelatin treated 20mm cover slips placed inside the wells of 22mm diameter polystyrene flat bottom tissue culture plates (Corning, new and non-adherent cells (lymphocytes) removed by gentle washing with cold normal saline. The adherent cells (PMD) were fixed with absoluted methanol and stained by the Giemsa techique (18). Each cover slip was then mounted face down on clean grease free glass slides for examination on the light microscope (Olympus, Japan) fitted with an eye-piece micrometer vernier scale.

Determination of Phagocytosis and PBM Measurement: A total of 50 PBM were randomly selected from each stained slide at x 40 oil immersion objective and the following were determined:

(a) % PBM with engulfed erythrocytes;
(b) total number of erythrocytes phagocytized;
(c) mean diameter of the PBM.

Statistiscal Analysis: Data collected from each animal species and identified protozoan parasite group were pooled and subjected to 2-way analysis of variance (21) and Duncan's Multiple Range Test (22) for determining significant differences, if any, at $P<0.05$ level.

RESULT AND DISCUSSION

Table 1 shows the prevalence of haemoprotozoan diseases, haematology and peripheral blood monocyte (PBM) sizes and their erythrophagocytic activities in cattle, sheep and goats used in this study. The results show that *Trypanosoma congolense, T. vivax, Anaplasma marginale* (cattle) and *A. ovis* (sheep and goats) and mixed infections of these organisms were the most frequently encountered haemoprotozoan diseases of ruminants in the area studied. 57.5%, 50% and 60% of cattle, sheep and goats, respectively were parasite-negative, while between 5 and 20% infections rates were recorded for single infections with these parasites and between 5 and 6.7%

Table 1: Incidence of haemoprotozoan diseases, haematology and PMB characteristics in infected cattle, sheep and goats

	PVC (%)	HB (g/dl)	RBC (x10^{6}u)	MCV (fl)	MCHC (%)	%PBM with engulfed	Mean number of erythrocytes	Mean PBM Diameter (u)
Cattle								
-ve (23)*	34.1± 2.9^{ab}	11.0 ±1.4^{a}	8.9± 2.4^{a}	38.3 ± 2.8^{a}	32.3 ± 4.1	0^{d}	0^{c}	14.4 ±0.8^{b}
TC (5)	30.6 ± 0.5 ^{ab}	8.6 ± 0.7^{b}	8.7 ± 0.8 ^{b}	35.7 ± 3.7^{a}	28.1 ± 2.8	6.4 ± 1.4^{c}	9.8 ± 2.6^{d}	18.3 ± 1.3^{a}
TV (2)	24.5 ± 1.3 ^{b}	7.3 ± 1.7^{b}	6.3 ± 1.0^{b}	38.9 ± 1.8^{a}	29.8 ± 3.1	7.3 ± 2.4^{c}	13.0 ± 1.5^{c}	19.4 ± 1.7^{a}
AM (4)	37.5 ± 2.8 ^{a}	10.8 ± 1.6^{a}	9.4 ± 1.2^{a}	39.9 ± 3.3^{a}	28.8 ± 2.6	6.0 ± 2.7^{c}	9.0 ± 2.8^{d}	15.8 ± 2.2 ^{ab}
TC/TV (2)	20.0 ± 1.0 ^{c}	5.6 ± 1.2^{c}	6.3 ± 1.2^{b}	31.7 ± 2.7^{a}	28.0 ± 3.3	15.5 ± 1.3^{a}	28.0 ± 1.0^{a}	20.1 ± 1.7^{a}
TC/AM	21.3 ± 1.3^{c}	6.1 ± 1.8^{c}	6.2 ± 2.0^{b}	34.3 ± 1.8^{a}	28.7 ± 2.8	12.5 ± 1.8^{b}	18.5 ± 1.5^{b}	17.0 ± 1.3 ^{ab}
TV/AM (2)	19.8 ±1.8^{c}	6.6 ± 1.0^{c}	6.9 ± 1.3^{ab}	28.7 ± 1.5^{b}	33.3 + 1.3	13.5 ± 10^{b}	12.8 ± 10^{b}	19.8 ± 0.1^{a}
Sheep								
-ve (15)*	29.7 ± 3.0^{a}	9.5 ± 1.5^{a}	12.3 ± 1.7^{a}	25.1 ± 1.8^{a}	32.0 ± 2.4^{a}	0^{d}	0^{c}	12.8 ± 0.8^{d}
TC (6)	21.3 ± 1.8^{b}	6.5 ± 1.5^{b}	8.9 ± 1.2^{b}	23.9 ± 2.6^{a}	30.5 ± 2.6^{a}	13.1 ± 3.2c	16.3 ± 5.8^{b}	16.7 ± 2.5^{c}
TC/TV (2)	14.0 ± 1.0^{c}	3.6 ± 0.5^{c}	6.9 ± 0.8^{c}	20.3 ± 2.0^{a}	25.7 ± 1.8^{b}	28.0 ± 1.3^{a}	32.0 ± 1.3^{a}	22.2 ± 2.8^{a}
AO (5)	21.4 ± 2.2^{b}	6.3 ± 0.9^{b}	10.2 ± 3.8^{a}	21.0 ± 3.3^{a}	29.4 ± 2.3^{a}	14.4 ± 4.1^{c}	19.0 ± 6.3^{b}	18.0 ± 2.3^{b}
TC/AO (2)	17.0 ± 3.0^{c}	1.5 ± 0.7^{b}	9.2 ± 1.8^{b}	18.4 ± 1.3^{b}	26.5 ± 1.0^{b}	20.0 ± 1.0^{b}	21.0 ± 1.0^{ab}	20.8 ± 0.8 ^{ab}
Goats								
-ve (18)	32.5 ± 2.8 ^{a}	9.8 ± 1.6^{a}	13.4 ± 2.4	24.3 ± 1.6^{a}	30.2 ± 2.5^{a}	0^{c}	0^{c}	13.2 ± 0.6^{c}
TC (5)	28.2 ± 3.6^{a}	8.6 ± 2.2^{a}	14.4 ± 3.2	19.6 ± 0.8^{ab}	30.5 ± 1.6^{a}	7.7 ± 2.6^{b}	10.6 ± 5.2^{b}	18.0 ± 1.2^{a}
AO (5)	30.8 ±2.89^{a}	9.1 ± 2.6^{a}	15.3 ± 3.2	20.1 ± 1.2^{ab}	30.3 ± 2.4^{a}	6.2 ± 2.1^{b}	8.4 ± 1.7^{b}	15.5 ± 1.5^{b}
TC/AO (2)	21.0 ± 4.2^{b}	6.8 ± 0.0^{b}	13.2 ± 2.9	15.9 ± 2.6^{b}	32.4 ± 1.5^{a}	20.8 ± 3.7^{a}	27.0 ± 2.5^{a}	20.0 ± 1.9^{a}

a,b,c = Means on the same vertical line without a common superscript differ significantly (P<0.05)
*-ve = parasite negative; TC = *Trypanasoma congolense* alone = *T vivax* alone; AM = *Anaplasma marginale* alone
AO = *A. ovis* alone; TC/TV, TC/AM, TV/AM, TC/AO = Mixed infections (number of animals).

occurred as mixed infections either with *T. congolense* and T. *vivas* as in cattle and sheep (Table 1), or with *T. congolense* and *Anaplasma spp* (in all the animals).

The diseases were characterized by varying degrees of normocytic, normochromic anaemia in single infections and with severe microcytic hypochromic anaemia in mixed trypanosome/anaplasma infections, especially in sheep and goats. While PBM of uninfected animals did not phagocytose any erythrocyte, haemoprotozoan infections in all the animal species were characterized by significant increases in PBM size and erythrophagocytic activity. Between 6.4+1.4 - 15.5+ 1.3%, 13.1+ 3.2 - 28.0+ 2.0% and 6.2+ 2.1 - 20.8 + 3.7% of the PBM in cattle, sheep and goats respectively were enlarged, foamy and phagocytic, with each PBM engulfing between 1-5 erythrocytes, some of which were paratized (Fig. 1). The intensity of phagocytosis correlated positevely with the degree of the anaemia developed by the animals. PBM from infected animals showed variable increased in size ranging from 30 - 85% above the mean PBM size in uninfected animals (Table 1). Animals with mixed infections, especially with *congolense/vivax,* trypanosome/anaplasma organisms recorded the highest erythrophagocytosis and PBM diameter increase with rusultant severe anaemia. Sheep appeared to have suffered the most severe anaemia, increased PBM diameter and erythrophagocytic activity than cattle and goats (Table 1).

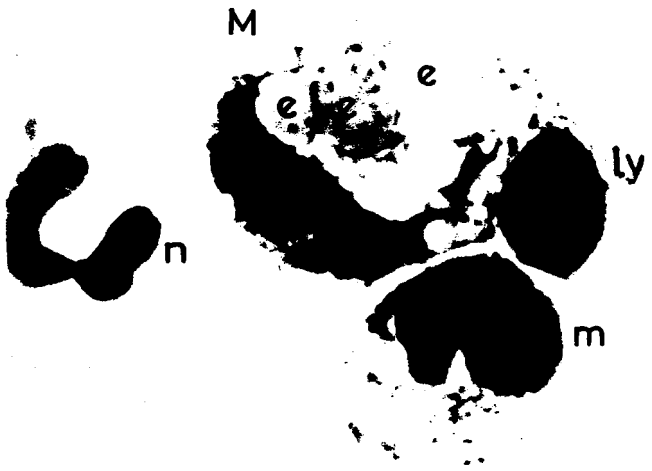

Fig. 1 Giemsa stained ovine PBM showing an enlarged monocyte (M) with engulfed *anaplasma ovis*-parasitized erythrocytes (e). Note another foamy monocyte (m) and occasional contaminating lymphocyte (ly) and neutrophil (n). x750.

The results from this study have shown that peripheral blood monocytes (PBM) take a very active part in erythrocyte phagocytosis and destruction during haemoprotozoan disease of cattle, sheep and goats. This was characterized by PBM enlargement, intensely foamy cytoplasm and erythrophagocytosis. It has been shown that structural, biochemical and functional changes accompany the differentiation of PBM into macrophages (MQ) both *in vivi* and *in vitro* (9,13). These changes include increase in cell size and number of cytoplasmic organelles such as Golgi body, mictochondria and rough endoplasmic reticulum, increases in cellular protein content and glucose utilization. Other changes include increased secretory activities such as increased lactate production, enzyme synthesis and activity of lysosomal enzymes, increased respiratory burst, and increased receptor expression (11,13). As they became seeded into various tissues as macrophages (MQ), they differed in their cytochemical, biochemical and function activities. These characteristics are dependent on their location, state of maturation, the degree, type and persistence of antigenic stimulation (14,23).

MQs were reported to become activated during trypanosome infection (24), and have been shown to avidly engulf both mature and immature erythrocytes, granulocytes and platelets in the spleen, bone marrow, liver and lymph nodes of infected animals (7,25) leading to anaemia, which is one of the most important clinical features of human and animal trypanosomiasis (4,5). The development and severity of the anaemia have been reported to follow the development and level of parasitaemia in infected hosts (26).

Even though the population of PBM is comparatively lower than their tissure MQ counterparts (18), the fact that PBM could be so activated to phagocytes and destroy erythrocytes while still in circulation attests to the versatility of the members of the monocytic phagocytic series (MPS) (27). There has been no report of erythrophagocytosis by PMB during anaplasmosis or babesiosis in ruminants, hence this study has shown that PBM also plays an active role in the pathogenesis of anaemia in these conditions in ruminants.

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