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HAEMATOLOGICAL AND SOME NOVEL BIOCHEMICAL CHANGES IN ZEBU CATTLE WITH BLACKLEG

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Summary

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The haematological and biochemical changes in Zebu cattle following a natural outbreak of blackleg were investigated at Kachia, Kaduna state, Nigeria. Blood samples (n=20) from each of two herds involved in the outbreak were collected from both the apparently healthy and sick (infected) cattle for analysis. Mean packed cell volume, haemoglobin and total protein concentrations of the sick animals were higher ($P<0.001$) than those of the apparently healthy ones. There were statistically significant decreases in mean total white blood cell counts ($P<0.001$) but not in differential leukocyte counts ($P>0.001$) of the sick animals. Mean free serum sialic acid levels of the sick animals increased ($P>0.001$), while mean erythrocyte surface sialic acid concentration of the sick animals decreased ($P>0.001$). Also, there was an increase in the mean neuraminidase activity in the plasma of the infected cattle ($P<0.001$) compared to the apparently healthy ones. The implication of these findings in the pathogenesis of blackleg is discussed.

Key words: blackleg, haematological and biochemical changes, natural outbreak, Zebu cattle

INTRODUCTION

Blackleg is a fatal disease of cattle and sheep caused by *Clostridium chauvoei* and was first reported in 1870 (Armstrong & McNamee, 1950). In Nigeria, the disease was first reported in 1929 and is still a major problem for cattle in the country (Osiyemi, 1975; Useh, 2006). The prevalence of blackleg is known to be very high during years of high average annual rainfall (Uzal *et al.,* 2003; Useh *et al.,* 2006a). Vaccination against the disease has been carried out since 1930 in Nigeria, but sporadic outbreaks are recorded annually. Blackleg has been reported in most African countries, Europe, United States of America and Southern America (Useh *et al.,* 2006d). The economic losses of cattle due to the disease in most of the endemic countries have not been quantified, but in Nigeria, they have been estimated at about 4.3 million USD annually (Useh, 2006). The nomadic Fulani pastoralists of rural Nigeria, who own about 70–80% of livestock in the country, rear the Zebu breed of cattle that is highly susceptible to blackleg. They migrate from one place to another in search of pasture for their livestock and many of them request for black-

leg vaccination for their cattle, only if there are outbreaks of the disease in neighbouring herds.

C. chauvoei known to cause blackleg, has been reported to produce neuraminidase (Heuermann *et al.,* 1991; Useh, 2002). Neuraminidases (sialidases, EC 3.2.1.18) are involved in the pathogenesis of some infectious diseases, whose aetiologic agents produce the enzyme (Esievo *et al.,* 1986; Oladele *et al.,* 2002; Nok & Balogun, 2003; Nok & Rivera, 2003; Useh *et al.,* 2004a). The enzyme is of great importance in medicine and the pharmaceutical industry for the analysis of oligosaccharides and development of neuraminidase inhibitors (Von Itzstein *et al.,* 1993; Hayden *et al.,* 1997; Traving & Schauer, 1998; Useh *et al.,* 2006b). There is no consensus on the pathogenesis of blackleg, but toxins and neuraminidase produced by the bacteria are believed to play a significant role in the mechanisms of the disease (Useh *et al.,* 2003; Useh, 2004; Useh *et al.,* 2004b). Most reports on the role of neuraminidase in the pathogenesis of blackleg are based on the speculation that the neuraminidase produced by *C. chauvoei* could be involved in spreading the disease in the tissues of infected animals. We report here for the first time, the haematological and some novel biochemical changes in Zebu cattle with blackleg (reared under native husbandry practices and naturally infected with *C. chauvoei)* in an attempt to explain the possible role of neuraminidase in the pathogenesis of the disease.

MATERIALS AND METHODS

Animals

Blood (plasma and sera) samples were collected from 20 apparently healthy and 20 sick Zebu cattle (aged between 8 months and 2 years) belonging to 2 different herds following a reported outbreak of blackleg at Kachia in Kaduna state, Nigeria. At the time of visit to the herds, the apparently healthy animals did not show any sign of disease, even after physical examination. The said outbreak was diagnosed in the field by clinicians at the Ahmadu Bello University Veterinary Teaching Hospital, Zaria, Nigeria and confirmed after a thorough laboratory investigation of the swabs collected from oedematous fluids and infected thigh muscles. Some of the clinical signs observed during physical examination of the sick animals included pyrexia (38.9–41.6 °C), anorexia and dullness. The animals belonged to nomads and had no history of vaccination against blackleg.

Confirmation of diagnosis of blackleg

Blackleg was confirmed by cultural isolation of *C. chauvoei* (Jakari strain) from muscle swabs and oedematous fluid (Dowell & Hawkins, 1981; Useh, 2006) and biochemical tests (Cato *et al.,* 1986).

Determination of haematological and biochemical parameters

Haematological parameters (packed cell volume, PCV; haemoglobin concentration, Hb and differential leukocyte counts) were determined using conventional procedures (Schalm *et al.,* 1975; Hoffbrand & Pettit, 1993). Plasma neuraminidase activity and free serum sialic acid concentrations in both the apparently healthy and sick cattle were determined by measuring the sialic acid cleaved from fetuin by the enzyme in plasma and the concentration of free sialic acid in the serum respectively (Webster & Campbell, 1972). Plasma total protein (TP) concentration was assayed using the hand refractometre

method (Davidson *et al.,* 1998). Haemoglobin-free erythrocyte membranes (ghosts cells) were prepared by lysis of red blood cells using hypotonic solution, followed by thorough washing with isotonic buffer (Dodge *et al.,* 1963) and erythrocyte surface sialic acid concentrations in both the apparently healthy and sick animals were determined as well (Aminoff, 1961).

Statistical analysis

The data obtained was subjected to statistical analysis using Student's t-test and the results were expressed as mean ± standard deviation (SD) (Chartfield, 1983). Values of $P<0.001$ were considered significant.

RESULTS

C. chauvoei (Jakari strain) colonies were isolated from muscle swabs and oedematous fluids of the clinically sick animals. The haematological and biochemical changes observed in the Zebu cattle with blackleg included haemoconcentration (due to increased mean PCV, haemoglobin and total protein concentrations), leukopaenia, lymphopaenia, neutropaenia, eosinopaenia, increases in mean plasma neuraminidase activity and serum sialic acid levels, and decreased mean erythrocyte surface sialic acid concentration. Mean plasma neuraminidase activity in infected cattle was statistically significantly higher than that in the apparently healthy ones ($P<0.001$). Also, mean serum sialic acid concentration of the sick animals was higher than the apparently healthy ones, but the difference was not statistically significant ($P>0.001$). The same was true for mean erythrocyte surface sialic acid concentrations of sick cattle compared to those in apparently healthy ones ($P>0.001$) (Table 1).

DISCUSSION

Leukopaenia in early trypanosomiasis has been linked to the activity of neuraminidase produced *in vivo* by pathogenic trypanosomes (Esievo, 1979; Esievo & Saror, 1983). In Nigeria, blackleg of Zebu cattle is caused by *C. chauvoei* (Jakari strain). All over the world, no study has

Table 1. Haematological and blood biochemical data of apparently healthy Zebu cattle (n=20) and Zebu cattle, naturally infected with blackleg (n=20). Data are presented as mean ± SD (range)

Parameter	Apparently healthy cattle	Blackleg-infected cattle
Packed cell volume (L/L)	0.30±0.08* (0.28–0.38)	0.35±0.02 (0.30–0.38)
Haemoglobin (g/L)	101±6* (93–113)	116±8 (100–127)
Total protein (g/L)	63±6* (55–78)	109±10.6 (87–127)
Total WBC (10^9/L)	12.76±1.10* (12.0–14.70)	11.70±1.34 (9.40–13.80)
Lymphocytes (10^9/L)	8.93±0.77 (7.84–10.29)	8.19±0.96 (6.58–9.66)
Neutrophils (10^9/L)	2.93±0.25 (2.60–3.38)	2.69±0.32 (2.16–3.18)
Eosinophils (10^9/L)	0.76±0.06 (0.66–0.88)	0.70±0.08 (0.56–0.83)
Serum sialic acid (µmol/L)	9.67±1.36 (7.06–11.33)	9.79±1.30 (7.61–11.72)
Erythrocyte sialic acid (µmol/L)	17.1±1.3 (15.2–18.8)	16.4±1.3 (14.1–18.8)
Neuraminidase activity (µmol/min)	43.0±5.6*(37.5–48.5)	68.1±9.1 (56–86.4)

* statistically significant difference between both categories of cattle ($P<0.001$).

been carried out to investigate the role of neuraminidase in the pathogenesis of blackleg in cattle with experimental or natural infection. In the present study, we investigated the derangements associated with blackleg in Zebu cattle reared under native husbandry practices and naturally infected with *C. chauvoei* (Jakari strain). There was a decrease in the mean total white blood cell counts (leukopaenia) and mean differential leukocyte count (lymphopaenia, neutropaenia, eosinopaenia and monocytopaenia) in the infected Zebu cattle compared to their apparently healthy counterparts and the leucopaenia was statistically significant ($P<0.001$). These reductions in total and differential leukocyte counts may be attributed to the action of neuraminidase, whose activity was significantly higher ($P<0.001$) in the plasma of sick vs apparently healthy cattle. Neuraminidase is known to desialylate white blood cells, leading to their decrease in peripheral blood (Woodruff & Woodruff, 1976). Mean packed cell volume (PCV) and TP concentrations were also higher in the sick than in the apparently healthy animals and the increase was statistically significant ($P<0.001$). This finding corresponds with reports of other authors (Pemberton *et al.,* 1974; Radostits *et al.,* 2000) who found haemoconcentration and hypovolaemia in cattle infected with blackleg. Recently, the pathogenesis of haemoconcentration and hypovolaemia in blackleg was also attributed to the activity of neuraminidase, whose low optimum pH of 4.5 was thought to stimulate anaerobiosis and vascular permeability, leading to oedema, hypovolaemia and haemoconcentration (Useh, 2002; Useh *et al.,* 2006c).

Mean erythrocyte surface sialic acid concentration in the apparently healthy cattle was higher than that of the sick ones, although the difference was not statistically significant ($P>0.001$). The low sialic acid concentration in the later could be as a result of enhanced cleavage of sialic acids from the erythrocyte surfaces of sick animals which had higher plasma neuraminidase activity than the apparently healthy ones, since neuraminidase is known to cleave the terminal sialic acids from sugar residues and glycoproteins (Esievo *et al.,* 1982; Nok *et al.,* 2003; Useh *et al.,* 2003). Although the sick cattle were treated after sample collection and diagnosis of blackleg, it was not possible to collect blood samples (serum and plasma) thereafter to assess the response of plasma neuraminidase to therapy, because the Fulani's of rural Nigeria being nomads, live in remote areas which can not be reached on a daily basis. It was therefore impossible to collect samples to assess the therapy clinically and to monitor impaired haematological and biochemical changes. Much later, reports from the nomads notified the researchers that the animals recovered after the treatment. The mean erythrocyte surface sialic acid concentrations in both the apparently healthy and sick cattle need to be investigated in experimental infection to explain why there was no statistically significant difference in their amounts. The mean serum sialic acid concentration in the sick animals was higher than that in the apparently healthy ones, although the difference was not statistically significant ($P>0.001$). This finding may be attributed to the action of the enzyme sialyltransferase, which is produced by the thyroid gland to regenerate sialic acids on the surfaces of erythrocytes, both in health and disease (Kraemer, 1966), although we did not assay sialyltransferase activity in the present study.

We conclude that the daily changes in sialyltransferase activity in the plasma of cattle infected with blackleg should be investigated *in vivo* in both experimental and natural infection, to ascertain any possible regeneration of sialic acids on the erythrocyte surfaces of both apparently healthy and blackleg-infected cattle. Further work is on the way to determine the possibility of developing an antiserum against blackleg using neuraminidase from *C. chauvoei*.

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