

A report on biochemical changes in the cerebrospinal fluid of Zebu cattle experimentally infected with *Clostridium chauvoei*

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ABSTRACT

The mechanisms by which blackleg occur need to be thoroughly investigated for effective treatment, prevention and control. A study was conducted to investigate the biochemical changes in the cerebrospinal fluid (CSF) of Zebu cattle experimentally infected with *Clostridium chauvoei*, in an attempt to determine the role of neuraminidase in the pathogenesis of blackleg. The experimental design specified the use of a total of 14 Zebu bull-calves. These were allocated into 4 experimental groups namely: *C. chauvoei*-infected (n = 4), toxin-administered (n = 3), neuraminidase-administered (n = 4) and control (n = 3) groups respectively. Mean neuraminidase activity was very high in the *C. chauvoei*-infected and neuraminidase-administered groups, compared to the toxin-administered and control groups (P<0.05). Mean free sialic acid concentrations in the CSF also followed a similar pattern to the neuraminidase activity. The variation in mean total protein concentration in the CSF and mean pyruvic acid levels and the significance of these findings in the pathogenesis of blackleg and the effect of the disease on food security are discussed.

Key words: biochemical changes, cerebrospinal fluid, Zebu cattle; *Clostridium chauvoei*

Introduction

Blackleg is a fatal disease of cattle and sheep caused by *C. chauvoei*, and was first reported in 1870 (ARMSTRONG and McNAMEE, 1950). In Nigeria, the disease was first reported in 1929 and has remained a major problem of cattle in the country (OSIYEMI

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1975). 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, but sporadic outbreaks are recorded annually. The economic losses of cattle to blackleg in Nigeria have been estimated at about 4.3 million United States dollars annually. 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 blackleg vaccination for their cattle, but only if there are outbreaks of the disease in neighboring herds.

C. chauvoei, which is the known cause of blackleg, has been reported to produce neuraminidase (HEUERMAN et al., 1991; USEH et al., 2004). Neuraminidases (sialidases, EC 3.2.1.18) are involved in the pathogenesis of some infectious diseases, whose aetiological agents produce the enzyme (NOK and RIVERA, 2003). The enzyme is of great importance in medicine and the pharmaceutical industry for analysis of oligosaccharides and the development of neuraminidase inhibitors (TRAVING and SCHAUER, 1998). There is no consensus on the pathogenesis of blackleg, but the toxins and neuraminidase produced by the bacteria are believed to play a significant role in the mechanisms of the disease (USEH et al., 2003). No studies have been carried out so far, on the biochemical changes in the cerebrospinal fluid of cattle infected with *C. chauvoei*. In this report we present, for the first time, the biochemical changes in the CSF of Zebu" cattle experimentally infected with *C. chauvoei*, and the possible role of neuraminidase in the derangements observed.

Materials and methods

Animal acquisition, acclimatization and grouping. Fourteen (14) Zebu bull-calves were purchased, acclimatized, grazed, aged and grouped into 4 groups. Groups A (n = 4), B (n = 3), and C (n = 4) were infected with *C. chauvoei* (Jakari strain), toxins and neuraminidase from the bacteria respectively, while group D (n = 3) served as control. During the period of acclimatization, the animals grazed in a free range, because of the abundant pasture that characterizes the rainy season in Zaria, Nigeria, but when the experiment commenced they were confined in appropriate experimental pens and fed a combination of groundnut hay and hay prepared from *Andropogon gayanus*, *Hyppurhenia rufens*, *Pennisetum pedicellatum* and *Elionurus probeguunii*, until the experiment was terminated. They were supplied feed commensurate with 4% of their individual body weights daily and water *ad libitum*. The weights of the animals were estimated using a waist band and ranged between 80-140 kg. The animals were aged using dental eruption (WOSU, 2002).

Cultivation of C. chauvoei for infection. Lyophilized *C. chauvoei* (Jakari strain) donated by the National Veterinary Research Institute (NVRI), Vom, Plateau state,

Nigeria, was used for the experiment. The organism was first isolated from Zebu cattle with blackleg and its pathogenicity indices have been fully determined (PRINCEWILL, 1965). The preparation of the bacteria and infection of Zebu bull-calves (group A) was carried out using the method described by SINGH et al. (1993), and the experiment lasted for 21 days.

Culture of C. chauvoei (Jakari strain) for neuraminidase production. The bacteria were cultivated and neuraminidase was isolated, as described previously (USEH et al., 2004). The neuraminidase was partially purified, as described earlier (USEH et al., 2006b) and administered to experimental group C.

Cultivation of C. chauvoei (Jakari strain) for toxin production. The method of JAYARAMAN et al. (1962) was used to cultivate the bacteria and produce the toxins which were administered to experimental group B.

Aspiration of cerebrospinal fluid from experimental animals. In the first 3 groups, CSF was collected at post-mortem, immediately after the animal died or during sacrifice at the end of the experiment, while CSF was collected from control animals following slaughter, both at the beginning and end of the experiment, using the sub-occipital approach. Each animal was placed on lateral recumbency, with the head fully flexed and firmly held in that position by one or two assistants and the CSF was collected using a 3 to 4 inch, 16 gauge needle and stylet.

Determination of free sialic acid in the cerebrospinal fluid. Free sialic acid in the CSF of both the infected and control groups was determined using the thiobarbituric acid method (AMINOFF, 1961).

Determination of neuraminidase activity in the cerebrospinal fluid. Neuraminidase activity in the CSF of both the infected and control animals was measured by the method of WEBSTER and CAMPBELL (1972).

Determination of total protein concentration in the CSF. Total protein (TP) concentration in the CSF of both the infected and control groups was determined using the Biuret method described by DAVIDSON et al. (1998).

Determination of pyruvic acid concentration in the CSF. Pyruvic acid levels in the CSF of both the infected and control animals were determined using the 3, 5 dinitrosalicylic acid (DNS) method described by PLUMMER (1987).

Determination of specific gravity of CSF. Specific gravity (SG) of CSF from both the infected and control animals was determined using the procedure described by BUSH (1991).

Statistical analysis. Data obtained from the study were computed as mean \pm standard deviation (SD), analysed using the analysis of variance (ANOVA, the Duncan multiple range test) and values of $P < 0.05$ were statistically significant (CHATFIELD, 1983).

Results

The biochemical changes in the cerebrospinal fluid (CSF) of Zebu cattle, experimentally infected with *C. chauvoei*, its toxins and neuraminidase, are presented in Table 1. There was no statistically significant difference ($P > 0.05$) between mean neuraminidase activity in the CSF of Zebu cattle administered *C. chauvoei* or neuraminidase, but there was a statistically significant difference ($P < 0.05$) between these and those administered toxins from *C. chauvoei* and the controls. Mean free sialic acid concentrations in the CSF of *C. chauvoei*-infected and neuraminidase-administered groups were much higher than in the toxin-administered or control groups ($P < 0.05$). Mean total protein concentrations in the toxin-administered and control groups were higher than the *C. chauvoei*-infected and the neuraminidase-administered groups ($P < 0.05$). The mean specific gravity (SG) of the CSF of the toxin-administered and control groups were similar, and both were higher than the SG of the *C. chauvoei*-infected and neuraminidase-administered groups ($P < 0.05$). Mean pyruvic acid concentrations in the CSF of the bacteria-infected and neuraminidase-administered groups were lower ($P < 0.05$) than the toxin-administered and control groups. There was no statistically significant difference ($P > 0.05$) between the mean age and the mean weights of all the animal groups on day zero of the experiment.

Table 1. Biochemical changes in the CSF of Zebu cattle experimentally infected with *Clostridium chauvoei*, or administered its toxins and neuraminidase (mean \pm SD)

Biochemical parameter	Control group	<i>C. chauvoei</i> group	Toxin group	Neuraminidase group
Neuraminidase activity (mg/mL/min)	1.90 \pm 0.26 ^a	3.90 \pm 0.67 ^b	1.85 \pm 0.18 ^a	3.95 \pm 0.88 ^b
Free sialic acid (mg/mL)	0.55 \pm 0.10 ^a	1.10 \pm 0.08 ^b	0.65 \pm 0.14 ^a	2.40 \pm 0.21 ^b
Total protein (mg/mL)	23.88 \pm 2.17 ^a	0.35 \pm 0.19 ^b	23.0 \pm 1.41 ^a	Undetectable ^b
Specific gravity (SG)	1.040 ^a	1.000 ^b	1.038 ^a	1.000 ^b
Pyruvic acid (mg/mL)	33.94 \pm 4.62 ^a	10.85 \pm 5.64 ^b	30.67 \pm 0.28 ^a	Undetectable ^c

^{a, b, c} Values in different columns with different superscripts are statistically significant ($P < 0.05$)

Discussion

Blackleg has a long history of veterinary importance and for many years was mistaken for an exclusively animal disease (WEATHERHEAD and TWEARDY, 2012) because of the non availability of precise diagnostic tools. It is possible that many human deaths, which were erroneously attributed to *C. septicum* infection (because of its close phylogenetic

relationship with *C. chauvoei*), may actually have occurred as a result of *C. chauvoei* infection. DNA-based PCR identification provides an alternative that is more reliable and faster. The report by NAGANO et al. (2008) that *C. chauvoei* also caused human disease and is in fact lethal to man, did not only launch the organism into scientific space, but has placed blackleg on the list of hot topics for research today. These authors reported the death of a 58-year old Japanese national caused by *C. chauvoei* infection. The 16S-23S rRNA gene intergenic spacer region of the pathogen, isolated from the patient, was amplified by polymerase chain reaction (PCR), generating a 522-bp *C. chauvoei* specific product, coinciding with that of *C. chauvoei* ATCC 10092, confirming that the 58-year old man actually died of *C. chauvoei* infection. This stimulated research into new molecular diagnostic techniques against *C. chauvoei* and *C. septicum* infections in man and animals (LANGE et al., 2010; HALM et al., 2010; GAROFOLO et al., 2011).

The biochemical changes in the CSF of cattle, with either natural or experimental infection with *C. chauvoei*, have yet to be investigated, although a comprehensive study on the role of sialidase in the pathogenesis of blackleg was reported recently (USEH et al., 2012). In this study, there was higher mean neuraminidase activity in the CSF of Zebu cattle either experimentally infected with *C. chauvoei* or administered exogenous neuraminidase, compared to the toxin-administered and control groups ($P < 0.05$). Also, mean free sialic acid in the CSF followed a similar pattern. It is logical to state, based on these findings, that the high mean neuraminidase activity in the CSF of the neuraminidase-administered and bacteria-infected groups may have cleaved sialic acids from glycoconjugates in the CSF, to elaborate high amounts of free sialic acid in the CSF, as observed in this study. High neuraminidase activity is known to cause increased vascular permeability in cattle infected with *C. chauvoei*, leading to extravasation of plasma protein to the tissues and the development of oedema (SINGH et al., 1993). Mean total protein concentrations in the CSF of the toxin-administered and control groups were much higher than the neuraminidase and bacteria-administered groups ($P < 0.05$), indicating that the increased vascular permeability, produced by neuraminidase, induced protein trafficking from the CSF into the plasma, leading to reduced mean total protein concentrations in the former. Mean SG of the toxin-administered and control groups were also higher than the neuraminidase-administered and bacteria-infected groups ($P < 0.05$), understandably so, because of the decreased amount of solutes (mean total protein) in the later. Mean pyruvic acid levels also followed the same pattern as total protein and SG, and this may also be possibly due to increased vascular permeability and, hence, the trafficking of pyruvate into the plasma and peripheral circulation. In another study (USEH et al., 2007), pyruvic acid levels of the plasma were very high due to anaerobiosis, which caused the inability to catabolise pyruvate, leading to its accumulation in the plasma (peripheral circulation). The present study shows that neuraminidase plays a key role in determining the biochemical composition of the CSF of cattle infected with *C. chauvoei*,

and it is suggested that the use of neuraminidase inhibitors, some of which are natural products, to manage blackleg clinically should be thoroughly investigated. In this era of global warming, when new animal diseases are predicted to emerge, because of changing environmental factors, knowledge of the changing dynamics of disease is important in instituting control measures. Livestock production is a major source of household revenue in northern Nigeria and should disease overwhelm farmers, who most often employ native practices, it will not just increase the poverty rate in the region, but will also constitute a major threat to protein supply and hence be detrimental to food security. An increase in livestock diseases will lead to increased deaths of animals, a decreased protein supply for human nutrition and hence a threat to food security in Nigeria in particular and Africa in general. The nomadic lifestyle of the Fulani pastoralists, who are essentially transhumance, makes livestock disease control a difficult task to achieve, not just in Nigeria, but throughout Africa, since they encounter more diseases as they migrate from one place to another in search of pasture. New knowledge of the mechanisms of animal diseases that are prevalent in Nigeria is therefore important to predict changes in the occurrence of the diseases, predicated by global warming. This study has identified the pathogenic roles of neuraminidase and toxins produced by *C. chauvoei* in the cerebrospinal fluid of Zebu cattle, experimentally infected with *C. chauvoei*, and it is recommended that future chemotherapeutic protocols and vaccination strategies against the disease should exploit the findings in this study.

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SAŽETAK

Istražene su biokemijske promjene u cerebrospinalnoj tekućini Zebu goveda pokusno zaraženih bakterijom *Clostridium chauvoei* radi određivanja uloge neuraminidaze u patogenezi šuštavca. Pokus je bio proveden na 14 muške teladi, koja je bila podijeljena u četiri skupine. Prva skupina (n = 4) bila je zaražena bakterijom *C. chauvoei*, teladi druge skupine (n = 3) primijenjen je toksin, treće skupine (n = 4) neuraminidaza, a četvrta (n = 3) je bila kontrolna skupina. Srednja aktivnost neuraminidaze bila je vrlo jaka u zaražene teladi i teladi kojoj je bila primijenjena neuraminidaza u usporedbi sa skupinom kojoj je bio primijenjen toksin i kontrolnom skupinom ($P < 0,05$). Srednja koncentracija slobodne sijalinske kiseline u cerebrospinalnoj tekućini bila je slična neuraminidaznoj aktivnosti. Razmatrane su promjene srednje koncentracije ukupnih proteina u cerebrospinalnoj tekućini, srednje razine pirogroždane kiseline, kao i značenje njihovih nalaza u patogenezi šuštavca i učinak bolesti na sigurnost hrane.

Ključne riječi: biokemijske promjene, cerebrospinalna tekućina, Zebu govedo, *Clostridium chauvoei*
