

NEURONAL DEGENERATION AND MENINGEAL SIALIC ACID
COMPLEMENT OF ZEBU CATTLE EXPERIMENTALLY
INFECTED WITH *CLOSTRIDIUM CHAUVOEI*

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Summary

Useh, N. M., A. J. Nok, N. D. G. Ibrahim & K. A. N. Esievo, 2012. Neuronal degeneration and meningeal sialic acid complement of Zebu cattle experimentally infected with *Clostridium chauvoei*. *Bulg. J. Vet. Med.*, 15, No 1, 51–56.

For a long time, blackleg was mistakenly assumed to be an exclusively animal disease, because of poor diagnostic tools in the past. Current report of the disease in human beings has made research on *Clostridium chauvoei* a hot topic. Pathology of the nervous system in blackleg has yet to be exhaustively investigated. In this study, we report for the first time, neuronal degeneration in the brain and the meningeal sialic acid complement of Zebu cattle experimentally infected with *Clostridium chauvoei*. Mean meningeal sialic acid complement of the infected cattle was much lower than that of controls ($P < 0.05$), while mean plasma neuraminidase activity of the infected bull-calves that died on day 3 (72 hours) post-infection was five times higher than that of the controls, possibly suggesting that the low sialic acid complement in infected animals resulted from enhanced cleavage of sialic acid in the meninges by the neuraminidase produced *in vivo* by *C. chauvoei*. This study suggests that sialic acid cleavage from meninges may possibly assist the spread of *C. chauvoei* and opportunistic pyogenic bacterial organisms to the brain to cause meningitis, accompanied by pus.

Key words: blackleg, *Clostridium chauvoei*, meningeal sialic acid, neuronal degeneration, Zebu cattle

INTRODUCTION

Blackleg is a disease of cattle, sheep and other ruminants, caused by *C. chauvoei* (Kijima-Tanaka *et al.*, 1998). The economic losses of Nigeria's Zebu cattle from the disease have been estimated at 4.3 million USD annually (Useh *et al.*, 2010). The prevalence of the disease is known to increase parallelly to average annual rainfall (Uzal *et al.*, 2003; Useh *et al.*, 2006a). Vaccination has been carried out since 1930, but sporadic outbreaks are reported

annually (Osiyemi, 1975). *C. chauvoei* is known to produce neuraminidase, an enzyme that is believed to assist in spreading blackleg in the tissues of infected ruminants (Useh *et al.*, 2004a). The use of neuraminidase inhibitors to clinically manage blackleg is being investigated (Useh *et al.*, 2006b) and it is believed that natural products may possibly provide a better therapeutic advantage over penicillin (Useh *et al.*, 2004b).

Neuraminidase and toxins produced by *C. chauvoei* are known to play significant complementary roles in the pathogenesis of blackleg (Useh *et al.*, 2003). Very little is known about the pathological changes that occur in the nervous system of cattle with blackleg (Singh *et al.*, 1993). This study is the first to report neuronal degeneration in the brain and changes in meningeal sialic acid in Zebu cattle experimentally infected with *C. chauvoei*.

MATERIALS AND METHODS

Animals

Zebu bull calves were allocated to 2 experimental groups for this study. Four animals were infected with *C. chauvoei*, while three served as controls. During the period of acclimatization, the animals were grazed on free range, because of the abundant pasture that characterized the rainy season in Zaria, Nigeria, but when the experiment commenced they were confined in the appropriate experimental pens and fed a combination of groundnut hay and hay prepared from *Andropogon gayanus*, *Hypprrhenia rufens*, *Pennisetum pedicellatum* and *Elionurus probeguunii* until the experiment was terminated. They were supplied feed at 4% of their individual body weights daily and water *ad libitum*. The weights of the animals were estimated using 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 was carried out using the method described by Singh *et al.* (1993) and the experiment lasted for 21 days. Although there is no ethical committee for animal experimentation at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, the experimental animals were treated most humanely.

Determination of plasma neuraminidase activity

Plasma neuraminidase activity in both infected and control animals was measured by the method of Webster & Campbell (1972). Samples were collected until animals died or were sacrificed. The method determines free sialic acid cleaved by neuraminidase produced by *C. chauvoei*.

Postmortem examination

One bull calf died in the *C. chauvoei* infected group and its brain and meninges were collected for pathological studies and determination of meningeal sialic acid complement. At the end of the experiment, one animal from each infected and control groups were also sacrificed. Postmortem examination was carried out using the procedure described by Igbokwe (1989). Brain sections for histopathology were obtained, preserved and fixed in 10% buffered neutral formalin for at least 48 h. The tissues were sectioned at 5–6 μm and stained with haematoxylin/eosin.

Determination of sialic acid complement of the meninges

One (1) gramme of the meninges of both infected and control animals were collected from animals that died or were sacrificed on termination of the experiment

and thoroughly homogenized. The resultant slurry was transferred into a 2 mL tube and 1 mL of distilled water was added. Sialic acid in the homogenized meninges was determined using 50 different aliquots of the homogenates as described previously (Aminoff, 1961). The principle of this method is that of quantification of free sialic acid cleaved from the meninges of the brain by sialidase produced by *C. chauvoei* *in vivo*.

Statistical analysis

Data obtained from sialic acid complement of meninges was expressed as mean±SD and analyzed using Student's *t* test (Chatfield, 1983).

RESULTS

Histopathological studies showed degeneration of brain neurons (Fig. 1). Menin-

gitis and meningeal haemorrhages were also observed (results not shown). Mean sialic acid level in the meninges of infected animals was lower than that of controls and the difference was statistically significant ($P<0.05$).

Mean plasma neuraminidase activity on day 3 (72 h) of infection when one animal died was about 5 times higher than that in the controls and the difference was statistically significant ($P<0.05$) (Table 1).

DISCUSSION

For many decades, blackleg was considered to be an exclusively animal disease (Radostits *et al.*, 2000) because of the non availability of precise diagnostic tools. The report by Nagano *et al.* (2008) that *C. chauvoei* also caused human disease with fatal outcome has placed blackleg in the list of hot topics for research today. These

Table 1. Meningeal sialic acid complement and plasma neuraminidase activity of *C. chauvoei*-infected and control Zebu cattle (mean±SD).

	Infected cattle	Control
Meningeal sialic acid (mg/mL)	1.31±0.27 ^a	2.41±0.31 ^b
Plasma neuraminidase activity (mg/mL/min)	5.88±1.17 ^a	1.30±0.27 ^b

^{a,b} values in a row with different superscripts are statistically significant ($P<0.05$).

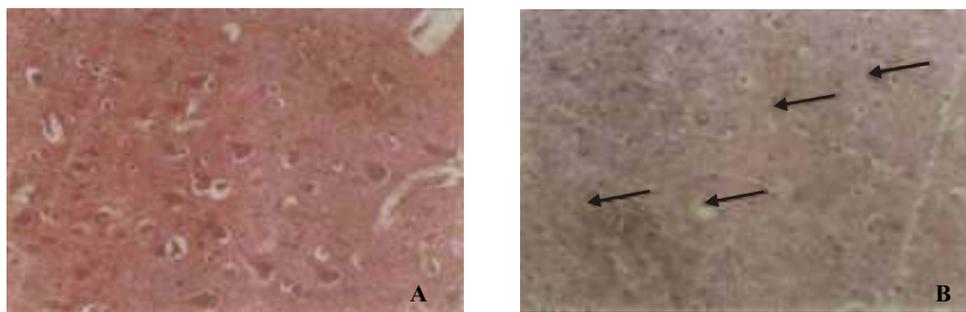


Fig. 1. Photomicrograph of the brain. A. Healthy Zebu bull-calf showing normal neurons; B. Zebu bull-calf infected with *Clostridium chauvoei*, showing degenerated neurons (arrows); H/E, ×200.

authors reported the death of a 58-year old Japanese 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. The report stimulated research on novel molecular approaches in the diagnosis of blackleg and gas oedema in man and animals (Halm *et al.*, 2010; Lange *et al.*, 2010; Garofolo *et al.*, 2011).

Pathology of the nervous system has not been extensively investigated in blackleg (Singh *et al.*, 1993). In the present study, neuronal degeneration was observed in the brain of Zebu bull calves experimentally infected with *C. chauvoei*, as opposed to the control animals, whose brain neurons were normal. *C. chauvoei* is reported to produce neuraminidase and the enzyme is known to cleave the terminal sialic acids from sugar residues and glycoproteins (Useh *et al.*, 2006c). The mean sialic acid complement of the degenerated neurons, compared to that of normal neurons in the control animals was not investigated in this study, but it is our guess that the neuronal degeneration observed may in part be possibly attributed to sialic acid cleavage from the brain neurons by neuraminidase produced *in vivo* by *C. chauvoei* during the experimental infection. Mean plasma neuraminidase activity in the infected cattle was about 5 folds higher than the control, thus giving more support to our suspicion.

The meninges of the brain are made of sialic acids, bound to glycoproteins (Schauer *et al.*, 1995). Meningitis accompanied by pus was reported in cattle with blackleg (Malone *et al.*, 1986) but *C.*

chauvoei, which causes blackleg is not a pyogenic bacterium. In the present study, meningitis was also observed in Zebu bull calves experimentally infected with *C. chauvoei*. The mean sialic acid complement of the meninges of the infected calves was lower than that of the control and the difference was statistically significant ($P < 0.05$). In a previous study (Useh *et al.*, 2006c), meningitis in blackleg was speculated to occur following the cleavage of sialic acid from the meninges by neuraminidase produced by *C. chauvoei*. The study suggested that sialic acid cleavage from the meninges by neuraminidase may assist the spread of *C. chauvoei* and opportunistic bacterial organisms to the meninges to cause inflammation (meningitis) and pus formation. The observation of low sialic acid complement in the meninges of infected unlike non-infected control cattle in the present study suggest that meningitis in the former may actually result from sialic acid cleavage accompanied by superimposed secondary (pyogenic) bacterial infection as reported previously. The identification of *C. chauvoei* in the meninges on smear further supported the above report. During the spread, *C. chauvoei* may have been accompanied by opportunistic pyogenic bacteria, hence the pus observed by Malone *et al.* (1986) and also confirmed in the present study.

It is concluded that further studies should be carried out to ascertain other possible mechanisms of the neuronal degeneration observed in blackleg.

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