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# Anaemia in *Clostridium chauvoei* infection is masked by haemoconcentration

**Nicodemus M. Useh[1,2]*, Andrew J. Nok[3], Najume Ibrahim[1], and King A. N. Esievo[1]**

[1]*Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA*

[3]*Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria*

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**ABSTRACT**

Haemoconcentration has been reported in blackleg, but the exact mechanisms by which it occurs are poorly understood. In the present study, we investigated the role of *Clostridium chauvoei* neuraminidase (sialidase) in the pathogenesis of blackleg in Zebu cattle. Fourteen (14) Zebu bull-calves were either infected with *C. chauvoei* (n = 4), or administered toxins (n = 3) or neuraminidase (n = 4) produced by the bacteria, and comprised a control group (n = 3) respectively. It was observed that anaemia developed in the *C. chauvoei*-infected and neuraminidase-administered groups ($P<0.05$) 7 days after experimental infection with *C. chauvoei* or administration of neuraminidase. The anaemia was attributed to desialylation of erythrocytes by neuraminidase, as there was higher plasma neuraminidase activity ($P<0.05$) and decreased erythrocyte surface sialic acid concentration ($P<0.05$) in the *C. chauvoei* and neuraminidase-administered, compared to the toxin-administered and control groups respectively. Other results revealed that the natural course of blackleg, which does not exceed 3-5 days, is characterized by an anaemia that is masked by haemoconcentration. It was concluded that neuraminidase played a significant role in the development of anaemia, which was masked by haemoconcentration in the early stage of *C. chauvoei* infection, but became evident 7 days post-infection in an experimental model.

**Key words:** *Clostridium chauvoei*, neuraminidase, toxins, anaemia, Zebu cattle

## 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

*Corresponding author:

Nicodemus M. Useh, DVM, PhD, FSB, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, E-mail: nicodemus.useh@fulbrightmail.org; nickuseh@yahoo.com

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reported in 1929 and has remained a major problem of cattle in the country (OSIYEMI, 1975; USEH et al., 2010). It is endemic in both developed and developing countries of the world (the United States of America, India and many countries in Asia and Europe, Latin American countries and Africa) and a well-known cause of financial loss to cattle farmers in these countries (ADAMS, 1998). 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 (USEH et al., 2006b), while in most other countries, the economic losses to the disease have yet to be quantified. The disease is known to cause major economic losses in cattle and minor losses in sheep (ADAMS, 1998). The nomadic Fulani pastoralists of rural Nigeria, who own about 70-80% of livestock resources 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 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 (HEUERMANN et al., 1991; USEH et al., 2004). 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; NOK and BALOGUN, 2003). The enzyme is of great importance in medicine and the pharmaceutical industry for the analysis of oligosaccharides and development of neuraminidase inhibitors (TRAVING and SCHAUER, 1998). 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). Haemoconcentration has been reported in blackleg. In this study, we report for the first time that anaemia occurs in blackleg, but is masked by the haemoconcentration that characterizes the disease.

## Materials and methods

*Animal acquisition, acclimatization and grouping.* Fourteen (14) Zebu bull-calves were purchased, acclimatized, grazed, aged and grouped into 4 groups namely: A (n = 4), B ( = 3) and C (n = 4) and infected with *C. chauvoei* (Jakari strain), toxins and neuraminidase from the bacteria respectively, while group D (n = 3) served as the control. During the period of acclimatization, the animals grazed free range, because of the abundant pasture that characterizes 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, Hyprrhenia rufens, Pennisetum pedicellatum* and *Elionurus probeguinii* 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) and their ages ranged between 19-23 months. There was no statistically significant difference ($P>0.05$) between the mean ages and the mean weights of all the animal groups on day zero of the experiment. Although there is no ethical committee regulating the use of animals for research at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, all the experimental animals were treated as humanely as possible during the experimental period, in accordance with international ethical provisions (BANKOWSKI, 1985).

Packed cell volume (PCV) was determined once weekly (FELDMAN et al., 2006) and used as a basis for grouping the animals, so that there was no statistically significant difference ($P>0.05$) between the mean PCVs of the control, neuraminidase-administered, toxin-administered and *C. chauvoei*-infected groups respectively on day zero of infection.

*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 (experimental group A) was carried out using the method described by SINGH et al. (1993) and the experiment lasted for 21 days. The animals (experimental group A) were administered 40 mL of 10% $CaCl_2$ intramuscularly (thigh) to create muscle damage and simultaneously administered 40 mL ($11.0\times10^9$ cfu/mL) of 36-hour old culture of *C. chauvoei* (Jakari strain) in reinforced clostridial medium (RCM) intramuscularly.

*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. Briefly, meat and liver infusions were mixed in equal parts and suspended in 100 mL distilled water and heated to boiling point. The pH was adjusted to 9.2 while still hot, re-boiled and filtered using a pressure machine. About 7% bacteriological peptone was later added and pH adjusted to 8.0. Sodium chloride (NaCl) (0.5%) and $K_2HPO_4$ (0.4%) were subsequently added. Lyophilized *C. chauvoei* (activated in RCM) was transferred into the above and incubated at 37 °C for 36 h and the culture supernatant containing all toxins produced by *C. chauvoei* was filtered using a pressure machine. Exactly 40 mL of culture supernatant, equivalent to 40 IU of *C. chauvoei* toxin (JAYARAMAN et al., 1962) was administered intramuscularly to animals in experimental group B.

*Culture of C. chauvoei (Jakari strain) for neuraminidase production.* Lyophilized *C. chauvoei* (Jakari strain) was cultivated and neuraminidase isolated as described previously (USEH et al., 2004). The neuraminidase was partially purified using ammonium sulphate saturation, DEAE cellulose chromatography, hydroxyapetite chromatography and phenyl sepharose chromatography respectively, and characterized. The activity of the partially purified and characterized neuraminidase was determined (USEH et al., 2006c) and about 20 IU of it was administered intramuscularly (thigh) to each animal in experimental group C.

*Determination of haematological parameters.* Packed cell volume (PCV), haemoglobin (Hb) and red blood cell counts (RBC) were determined using the procedures described elsewhere (FELDMAN et al., 2006).

*Determination of total protein (TP), erythrocyte surface sialic acid (ESA), serum sialic acid (SSA) and neuraminidase (N-dase) activity in plasma.* Total protein in the plasma was determined using the Biuret method (BUSH, 1991). Haemoglobin-free erythrocyte membranes were prepared from RBCs (DODGE et al., 1963). ESA and SSA were determined using the thiobarbituric acid assay method (AMINOFF, 1961) and plasma neuraminidase (N-dase) activity was also determined (WEBSTER and CAMPBELL, 1972).

*Determination of erythrocyte sedimentation rate (ESR).* This was carried out using the Westergreen method (DAVIDSOHN and HENRY, 1974).

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

**Results**

*Clinical presentation of experimental animals.* Non-specific signs, such as fever and anorexia, were observed in all but the control group. Diarrhoea and dehydration were observed in the bacteria-infected and toxin-administered, but absent in the neuraminidase-administered and control groups respectively. A very severe oedema in the subcutaneous parts of the lower abdomen was observed in the bacteria-infected and neuraminidase-administered groups 18 h post-commencement of the experiment, until day 4 (97 h) when it subsided.

*Haematological changes.* On day zero of infection, the PCVs of all the experimental animals were identical, varying between 27-32%, with mean values of 29.75 ± 2.91%, 28.75 ± 2.65%, 28.57 ± 2.85% and 30.0 ± 2.90% for neuraminidase, bacteria, toxin-administered and control groups respectively (Fig. 1). About 18 h post-administration of the above, mean PCV started appreciating in the neuraminidase, bacteria and toxin-administered groups, compared to the control group, which did not show any increase in mean PCV. The increase continued and the peak values of mean PCVs attained in all the three experimental groups on day 3 (72 h) post -infection were statistically significantly

different (P<0.05) from the control group. Mean PCV values in all the experimental groups declined on day 4, although not statistically significant (P>0.05) and appreciated again on days 5 (120 h) and 6 (in some of the groups) post-infection. On day 7 of the experiment, mean PCV values of neuraminidase and bacteria-administered groups were remarkably decreased, compared to the pre-infection values of day zero, as opposed to those of the control and toxin-administered groups, which did not vary significantly. This finding was noticed until day 11 (245 h) of the experiment. Some animals in the neuraminidase-administered group developed very low PCV values of less than 24% on days 8 (189 h) and 9 (214 and 223 h) post-neuraminidase administration and this reduced the mean PCV values of the group on these days to 26.75 ± 1.10%, 26.5 ± 0.89% and 26.25 ± 1.04% respectively. The mean PCV values of the neuraminidase-administered group on days 8 (189 h) and 9 (214 and 223 h), compared to the pre-infection value on day zero of infection, were statistically significantly different (P<0.05). The same was applicable to the bacteria-administered group, whose mean PCV value decreased on days 8 (189 h), 9 (214 and 223 h), 10 (230 h), 11 (245 h) and 21 (413 h) post -infection to 27± 1.20%, 26.67± 0.94%, 26.67± 1.04, 26.33± 1.01%, 24.67 ± 1.02% and 24.67± 1.04% respectively. There was a statistically significant difference (P<0.05) between the mean PCV values of the bacteria-administered group on days 9, 10, 11 and 21 respectively compared to both the pre-infection values of the same group and the control group.

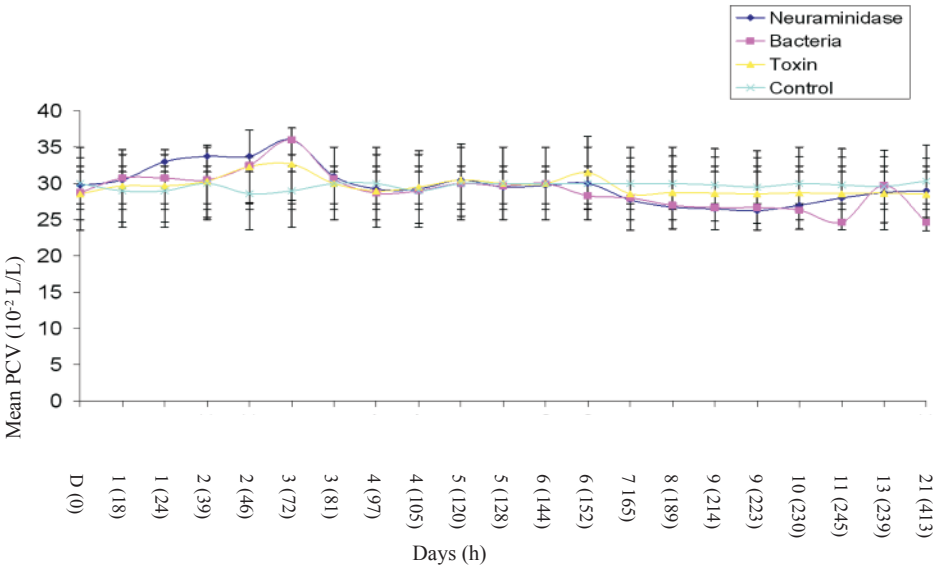


Fig. 1. Variation in mean packed cell volume (PCV) concentration of Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

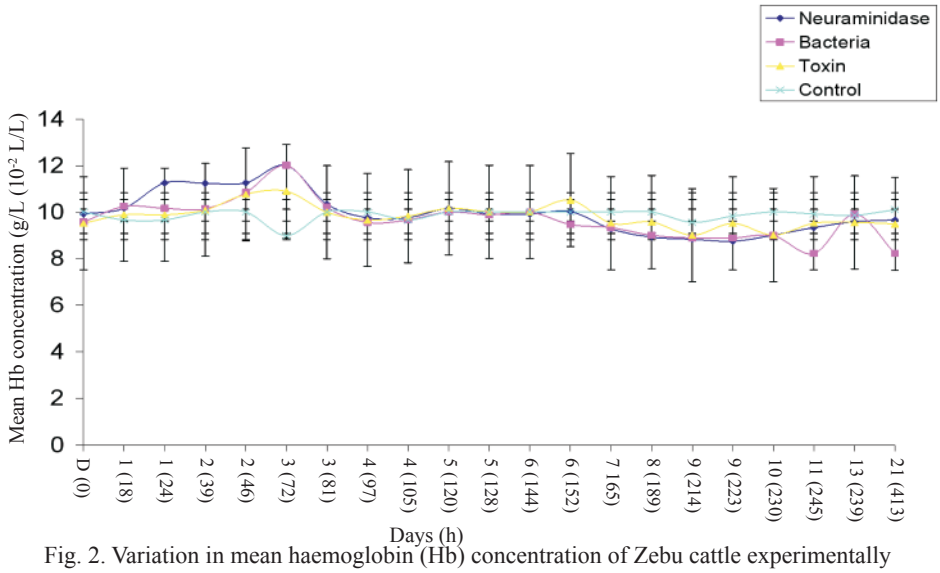


Fig. 2. Variation in mean haemoglobin (Hb) concentration of Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

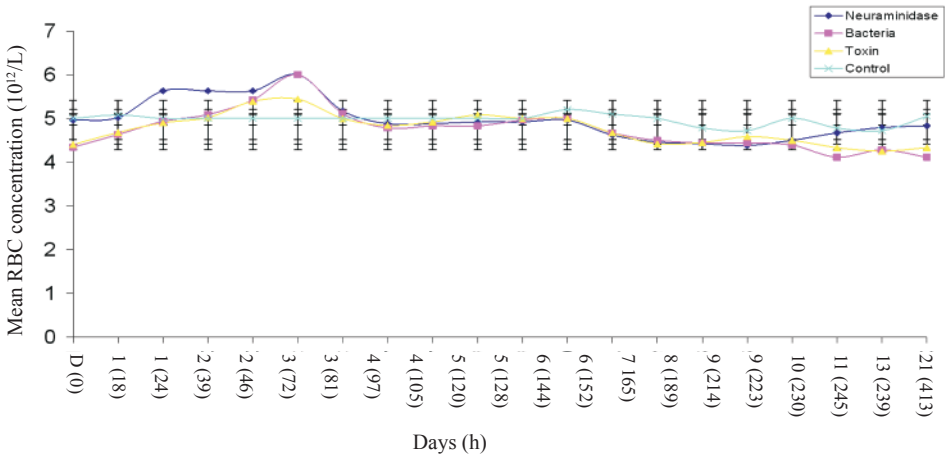


Fig. 3. Variation in mean red blood cell (RBC) counts in Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

The haemoglobin concentrations (Hb) (g/L) of all the experimental animals ranged between 90.1 and 106.7 prior to infection on day zero of infection and were essentially similar, with mean values of 99.2 ± 9.8, 95.8 ± 7.9, 95.2 ± 8.9 and 100.1 ± 9.6 g/L for neuraminidase, bacteria, toxin-administered and control groups respectively (Fig. 2). Mean Hb began to increase in the neuraminidase, bacteria and toxin-administered groups, exactly 18 h from day zero of the experiment, compared to the control group, whose mean Hb values did not vary significantly. The increase in mean Hb continued and peaked on day 3 (72 h) of the experiment. There was a statistically significant difference ($P<0.05$) between the mean Hb of the neuraminidase, bacteria and toxin-administered groups on day 3 (72 h) of the experiment, compared to both the control group and the corresponding pre-infection values on day zero of the experiment. Mean Hb in all the experimental animals decreased on day 4, although the decrease was not statistically significant ($P>0.05$) and increased again on days 5 (120 h) and 6 (in some groups). On day 7 of the experiment, mean Hb of the neuraminidase and bacteria-administered groups decreased, compared to the pre-infection values, as opposed to the control group, whose mean Hb was similar to that of the pre-infection value on day zero of the experiment. The decrease in the former continued unabated, up to day 11 (245 h) of the experiment. The lowest mean Hb of the neuraminidase-administered group was recorded on day 9 (214 and 223 h) of the experiment, compared to days 11 (245 h) and 21 (413 h) of the experiment for the bacteria-administered group.

Red blood cell (RBC) counts varied between 4.33 and 5.33 x $10^{12}$/L in all the experimental groups on day zero of infection, with comparable mean values of 4.96 ± 0.48, 4.34 ± 0.41, 4.40 ± 0.36 and 5.0 ± 0.40 x $10^{12}$/L for neuraminidase, bacteria, toxin-administered and control groups respectively (Fig. 3). Soon after infection (18 h), mean RBC counts in all the experimental groups began to increase and peak values were attained on day 3 (72 h) of the experiment, when the mean values of the experimental groups were statistically significantly different ($P< 0.05$) from the control group. Thereafter, RBC counts started declining, such that on day 21 (413 h), when the experiment was terminated, there was no statistically significant difference ($P>0.05$) between the RBC counts of the neuraminidase, bacteria and toxin- administered groups, compared to both the control group on the same day and the pre-infection mean RBC counts.

*Plasma total protein (TP) concentration.* Plasma total protein concentrations (TP) of the experimental animals varied between 70.5 and 100.30 g/L before infection and on day zero of the experiment, with mean values of 80.65 ± 8.9, 83.33 ± 7.9, 80.00 ± 8.0 and 80.01 ± 7.7 g/L for the neuraminidase, bacteria, toxin-administered and control groups respectively (Fig. 4). An initial increase in mean TP was noticed on day 1 (18 h) of the experiment and there was a statistically significant difference ($P<0.05$) between the mean TP of neuraminidase, bacteria and toxin-administered groups, compared to the

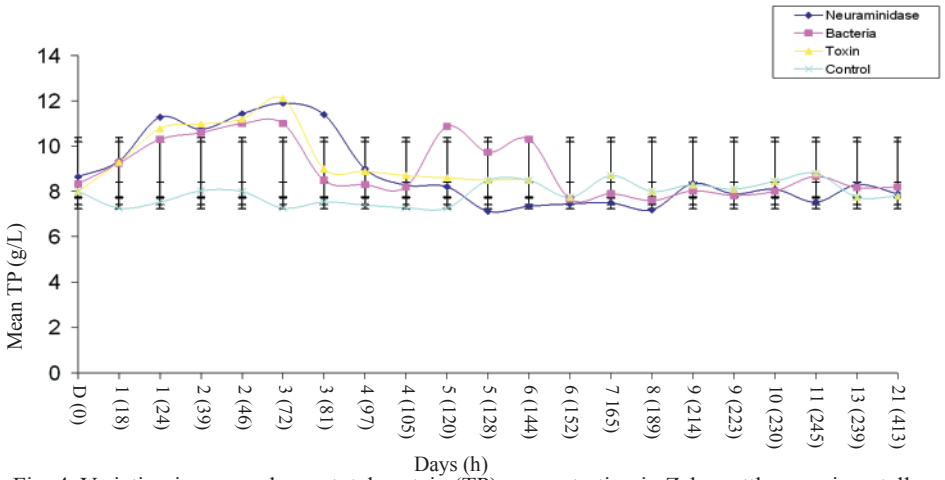


Fig. 4. Variation in mean plasma total protein (TP) concentration in Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

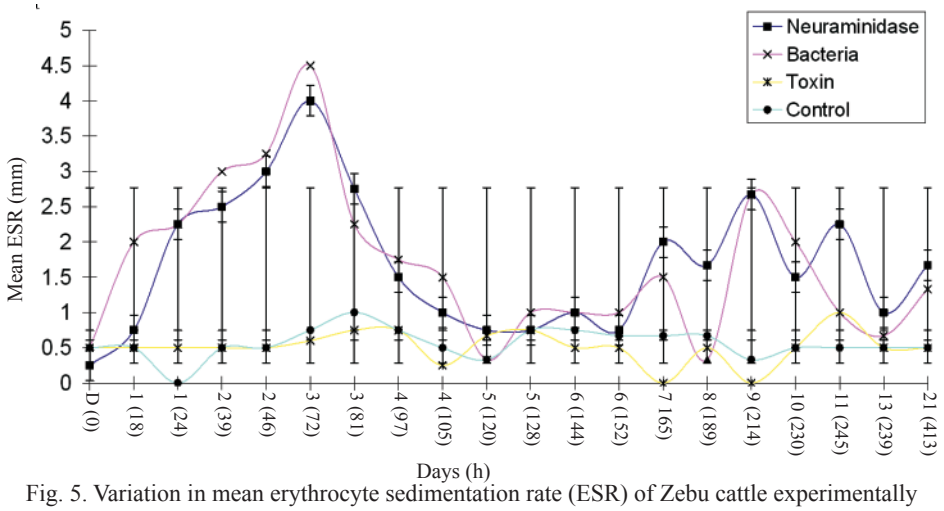


Fig. 5. Variation in mean erythrocyte sedimentation rate (ESR) of Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

control group, both on day 1, and the pre-infection mean values of each group. This pattern was sustained up to day 3 (72 h) of the experiment. A peak increase in TP was noticed in all, except the control group, on day 3 (72 h) of the experiment, with mean values of 119.0 ± 6.4, 110.0 ± 5.8, 121.0 ± 10.4 and 72.7 ±9.0 g/L for the neuraminidase, bacteria, toxin-administered and control groups respectively. Subsequently, a decline in TP commenced from day 3 (81 h) of the experiment onwards, but the decrease at 81 h was not statistically significant ($P>0.05$). On days 5 (120 h) and 6 (144 h) of the experiment, there was a statistically significant difference ($P<0.05$) between the mean TP of the bacteria-administered, compared to the neuraminidase, toxin-administered and control groups. Mean TP on day 21 (413 h) of the experiment for the neuraminidase and bacteria-administered groups were less than the respective mean pre-infection values on day zero, although the differences were not statistically significant ($P>0.05$).

*Erythrocyte sedimentation rate (ESR).* On day zero of infection, the mean ESR was 0.25 ± 0.25, 0.50 ± 0.29, and 0.50 ± 0.29 mm for the neuraminidase, bacteria, and toxin-administered groups, which did not vary from the control group (0.50 ± 0.29 mm) (Fig. 5). Mean ESR began to increase in the neuraminidase and bacteria-administered groups 18 h after the experiment commenced, compared to the toxin-administered and control groups, whose mean ESR remained relatively stable (between 0.50 ± 0.25 and 0.75 ± 0.48 mm) throughout the experimental period. Mean ESR in the neuraminidase and bacteria-administered groups reached their peaks on day 3 (72 h) of the experiment (4.0 ± 1.83 and 4.5. ± 1.29 mm for neuraminidase and bacteria-administered groups respectively). Shortly after this period, mean ESR in the neuraminidase and bacteria-administered groups began to fluctuate, until mean values of 1.50 ± 0.58 and 2.0 ± 1.0 mm were attained on day 21 (413 h) for the neuraminidase and bacteria-administered groups respectively.

*Neuraminidase activity (N-dase).* Neuraminidase activity (N-dase) varied between 0.80 and 1.60 µmol/min on day zero of the experiment, with mean values of 1.15 ± 0.38, 1.25 ± 0.21, 1.30 ± 0.36 and 1.30 ± 0.27 µmol/min for the neuraminidase, bacteria, toxin-administered and control groups respectively (Fig. 6). After about 18 h of the study, mean N-dase began to increase in the neuraminidase (5.05 ±1.99 µmol/min) and bacteria-administered (4.55±1.88 µmol/min) groups, compared to the toxin-administered (1.27±0.36 µmol/min) and control groups (1.13± 0.31 µmol/min) respectively, representing a statistically significant difference ($P<0.05$) between the former and the later. The increase in mean N-dase in the neuraminidase-administered group continued, with the neuraminidase group attaining peak N-dase (6.08 ± 2.00 µmol/min) on day 1 (24 h) post-administration, while the bacteria-administered group attained peak mean N-dase (5.88 ± 1.17 µmol/min) on day 3 (72 h) post-infection. These values were statistically significant ($P<0.05$), compared to the toxin-administered and control groups. The mean N-dase decreased sharply thereafter, and stabilized near moderate levels, in both the

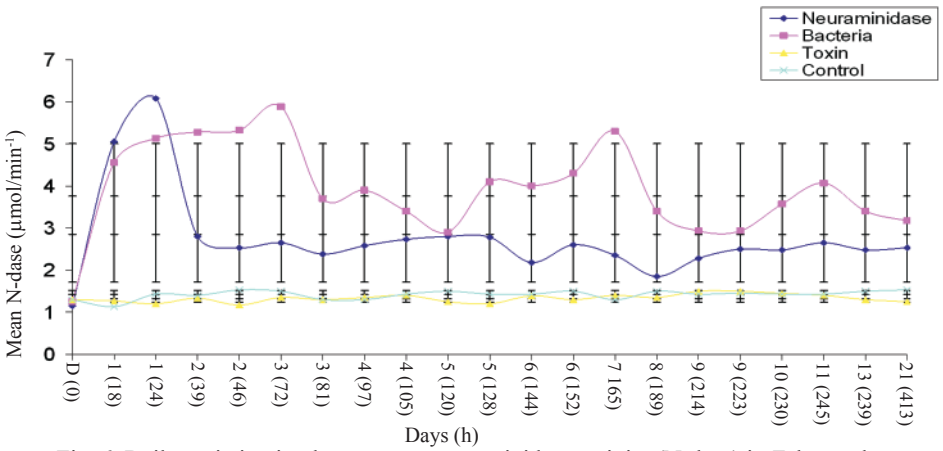


Fig. 6. Daily variation in plasma mean neuraminidase activity (N-dase) in Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

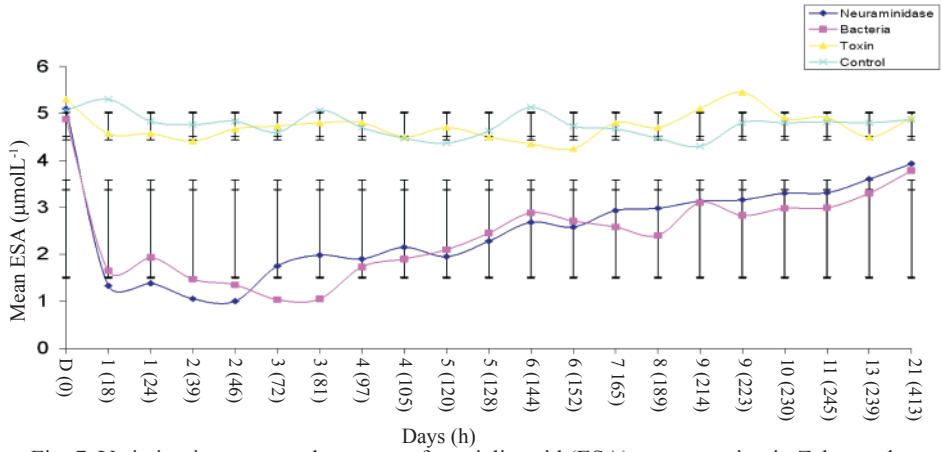


Fig. 7. Variation in mean erythrocyte surface sialic acid (ESA) concentration in Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

neuraminidase and bacteria-administered groups, with occasional significant increases in the bacteria-infected group (4.10 ± 0.30 µmol/min on day 5 or 128 h post-infection; 4.30 ± 0.60 µmol/min on day 6 or 152 h post-infection and 5.30 ± 1.30 µmol/min on day 7 or 165 h post-infection respectively). Mean N-dase values in all the experimental groups on day 21 (413 h) of the experiment were above the pre-infection values, although those of toxin and control groups were not statistically significantly different (P>0.05).

*Erythrocyte surface sialic acid (ESA) concentration.* Erythrocyte surface sialic acid concentrations (ESA) varied between 4.20 and 5.50 µmol/L and were similar in all groups on day zero of the experiment, with mean values of 5.10 ± 0.58, 4.88 ± 0.35, 5.30 ±0.41 and 5.07 ± 0.22 µmol/L for neuraminidase, bacteria, toxin-administered and control groups, respectively (Fig. 7). About 18 h post-commencement of the experiment, there was a statistically significant decrease in the mean ESA of the neuraminidase (1.33 ± 0.65 µmol/L) and bacteria-administered (1.65 ± 0.66 µmol/L) groups, compared to the toxin-administered (4.57 ± 0.44 µmol/L) and the control groups (5.30 ± 0.35 µmol/L) ($P<0.05$). The maximum decrease in ESA in the neuraminidase group (1.00 ± 0.21 µmol/L) was attained on day 2 (46 h) of the experiment. The decrease continued until day 4 (97 h) of the experiment, when mean ESA in the neuraminidase and bacteria-administered groups began to appreciate again. From days 1 to 9 of the experiment, there was a statistically significant difference ($P<0.05$) between mean ESA in the neuraminidase and bacteria-administered groups, compared to those of the toxin-administered and control groups. On day 21 (413 h) of the experiment, mean ESA appreciated greatly in the neuraminidase and bacteria-administered groups, so that there was no statistically significant difference between the mean ESA of all the experimental groups under study.

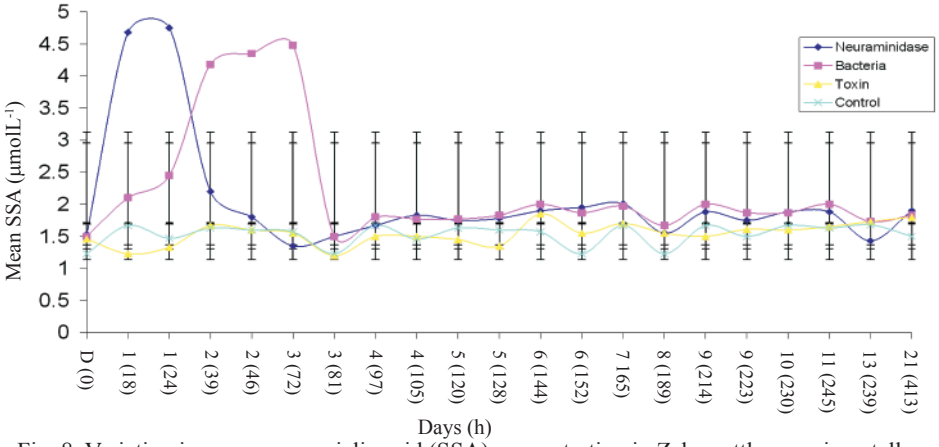


Fig. 8. Variation in mean serum sialic acid (SSA) concentration in Zebu cattle experimentally administered *C. chauvoei*, its toxins and neuraminidase

*Serum sialic acid (SSA) concentration.* Serum sialic acid concentrations (SSA) were similar, varying between 1.10 and 1.80 µmol/L on day zero of the experiment, with mean values of 1.53 ± 0.58, 1.50 ± 0.49, 1.46 ± 0.56 and 1.23 ± 0.48 µmol/L for the neuraminidase, bacteria, toxin- administered and control groups respectively (Fig. 8). Mean SSA increased significantly ($P<0.05$) in the neuraminidase and bacteria-administered groups 18 h post-commencement of the experiment, with mean values of

4.68 ± 2.11, and 2.10 ± 0.98 µmol/L respectively, compared to 1.23 ± 0.21 and 1.67 ± 0.25 µmol/L for the toxin-administered and control groups respectively. The same finding was observed 24, 39 and 46 h into the experiment. On day 3 (72 h) of the experiment, the mean values of SSA were 1.35 ± 0.01, 4.48 ± 0.96, 1.55 ± 0.54 and 1.57 ± 0.49 µmol/L for the neuraminidase, bacteria, toxin-administered and control groups respectively. There was therefore a statistically significant difference ($P<0.05$) between the mean SSA of the bacteria-administered group and that of all the other experimental groups. Thereafter, mean SSA regressed in all the experimental groups, but the mean SSA in the neuraminidase and bacteria-administered groups (in most cases) were consistently higher than those of the toxin-administered and control groups. After day 3 (72 h) of the experiment, there seemed to be no statistically significant difference ($P>0.05$) between the mean SSA in all the experimental groups. Mean SSA in all the experimental groups decreased on day 21 (413 h) of the experiment, although there was no statistically significant difference between the mean SSA of all the experimental groups on that day.

**Discussion**

Neuraminidase is known to cleave sialic acids from erythrocyte surfaces to cause anaemia in disease conditions, whose aetiologic agents produce the enzyme (ESIEVO et al., 1982). Although haemoconcentration was observed 18 h from day zero of the experiment, with peak values occurring 72 h into the experiment in the neuraminidase, toxin and *C. chauvoei* infected groups (evident by increased PCV, Hb, RBC and TP), it is clear that the concomitant increase in plasma neuraminidase activity, increased serum sialic acid levels, decreased erythrocyte surface sialic acid levels and increased erythrocyte sedimentation rate during the period suggest that neuraminidase may have cleaved sialic acids from the erythrocyte surfaces at a faster rate, to cause anaemia, which was masked by the severe haemoconcentration observed as a result of diarrhoea and anorexia. Increased ESR occurs in anaemia (DAVIDSOHN and HENRY, 1974) and the increased ESR levels, which began 18 h after the experiment commenced, suggest that anaemia occurred, beginning in the early stage of the infection, but was not observed, because dehydration (caused by diarrhoea and anorexia) indeed masked the anaemia. It is known that calves have higher haematological parameters, compared to adult cattle (SCHALMS et al., 1975) and since the haematological values of the experimental animals as from day 7 post-infection were statistically significantly lower ($P<0.05$) than the pre-infection values, it is safe to report that anaemia may have occurred, during this period, including the early stage of the disease when it was masked by haemoconcentration. The elevated PCV, HB, RBC and TP decreased only after diarrhoea and oedema had subsided, giving more support to our hypothesis that haemoconcentration was actually responsible for the false elevation of these parameters to mask anaemia.

Mean plasma neuraminidase activity increased in the neuraminidase-administered and bacteria-infected groups compared to the toxin-administered and control groups ($P<0.05$) (Fig. 6) confirming the *in vivo* production of neuraminidase by *C. chauvoei.* Neuraminidase administered exogenously or produced *in vivo* may have cleaved sialic acid from erythrocytes of the neuraminidase-administered and bacteria-infected groups, leading to decreased erythrocyte sialic acid complement and increased serum sialic acid concentration in these groups, compared to the toxin-administered and control groups, whose erythrocyte sialic acid levels were significantly higher ($P<0.05$) (Fig. 7). Mean serum sialic acid concentration initially increased in the neuraminidase-administered and bacteria-infected groups compared to the toxin-administered and control groups ($P<0.05$) (Fig. 8) and thereafter, there was no statistically significant difference ($P>0.05$) between the mean serum sialic acid levels of all the experimental groups. This finding agrees with KRÄMER (1966), who reported that calf thyroid glands produce sialyl transferase, which regenerates sialic acid on the surfaces of desialylated erythrocytes, although sialyl transferase activity was not investigated in this study. The present study is in agreement with previous report on trypanosomal anaemia, which is known to be due to sialic acid cleavage from erythrocytes by neuraminidase produced by trypanosomes *in vivo* and subsequent erythrophagocytosis (ESIEVO et al., 1982). It is concluded that anaemia occurs in *C. chauvoei* infection, but is masked by haemoconcentration, observed in the early stage of the disease as a result of dehydration caused by anorexia and diarrhoea.

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**SAŽETAK**

Točan mehanizam nastanka hemokoncentracije kod pojave šuštavca slabo je istražen. U ovom je radu istražena uloga neuraminidaze (sijalidaze) koju tvori *Clostridium chauvoei* u patogenezi šuštavca u zebu goveda. U pokus je bilo uzeto 14 muške teladi zebu goveda od kojih je jedna skupina (n = 4) bila zaražena bakterijom *C. chauvoei*, druga skupina (n = 3) dobivala je bakterijske toksine, treća (n = 4) neuraminidazu, a četvrta (n = 3) je bila kontrolna skupina. Anemija se razvila u telića zaraženih bakterijom *C. chauvoei* i telića kojima je bila primijenjena neuraminidaza ($P<0,05$) i to sedam dana nakon pokusne zaraze ili primjene neuraminidaze. Pripisana je desijalilaciji eritrocita neuraminidazom, jer je ustanovljena jača aktivnost neuraminidaze u plazmi ($P<0,05$) te smanjena koncentracija sijalinske kiseline na površini eritrocita ($P<0,05$) u zaraženoj skupini i skupini koja je dobila neuraminidazu u usporedbi sa skupinom koja je dobila toksin i kontrolnom skupinom. Drugi rezultati pokazuju da se prirodni tijek šuštavca, koji ne traje duže od tri do pet dana, očituje anemijom prikrivenom hemokoncentracijom. Zaključuju se da neuraminidaza ima značajnu ulogu u razvitku anemije, koja je bila prikrivena hemokoncentracijom u ranom stupnju zaraze, ali je u pokusnom modelu postala očita sedam dana nakon zaraze.

**Ključne riječi:** *Clostridium chauvoei*, šuštavac, neuraminidaza, toksini, anemija, zebu govedo