FIELD TRIAL OF SODIUM ALGINATE-ADSORBED *CLOSTRIDIUM PERFRINGENS* TYPES C AND D TOXOID AGAINST CLOSTRIDIAL ENTEROTOXEMIA IN SHEEP

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ABSTRACT

A field-trial evaluation confirmed the efficacy of Clostridium perfringens types C and D vaccine prepared from local strains of C. perfringens as suitable for preventing sheep enterotoxaemia in Nigeria. The vaccine comprised beta and epsilon toxins (C. perfringens types C and D) inactivated with 0.6 % formalin and adsorbed to sodium alginate. In three private farms, known to experience the disease in the Plateau State, a total of 2240 Yankasa sheep were selected to investigate whether immunization of feedlot sheep with sodium alginate C. perfringens toxoid would reduce subsequent clinical symptoms and mortality due to natural enterotoxaemia. A total of 22 (1.9 %) deaths were recorded among vaccinated animals while 271 (25.3 %) animals died in unvaccinated flocks through the 10 months study period. The mean disease-specific mortality for vaccinates was 1.9 % while that for controls (unvaccinated) was 25.3 %. Vaccine efficacy was defined as lower death rates of the vaccinated sheep compared with the unvaccinated sheep. The vaccine efficacy was 98.6 %. Unvaccinated sheep were 13.3 times more likely to become affected than vaccinated sheep. The association between the survival rates among sheep from 3 different farms vaccinated with sodium alginate toxoid, and Unvaccinated (control) to enterotoxaemia was significant ($P<0.05$) by chi-square test {X^2 (cal) =28.03, X^2 (tab) =5.99, df=2}.

Keywords: sodium alginate, C. perfringens toxoid, enterotoxaemia, sheep.

INTRODUCTION

Enterotoxaemia is a disease of economic importance among sheep in Nigeria, but immunization against the disease is not yet a regular practice (8, 13, 14). The disease affects both sexes without bias, and has a prevalence rate as high as 51.1 % with a peak incidence during the months of July to September in the area of this study (8). The most practical way to handle *C. perfringens* related illnesses is to prevent them. The widely used vaccines are the alum precipitated and oil adjuvant vaccines (16). The duration of immunity conferred by oil adjuvant vaccine, which is generally accepted as the better of the two, was found to vary considerably, and its range was shown to be between 8 and 14 months (5, 15, 16,). Although vaccines incorporating adjuvants such as sodium alginate have been used experimentally, they are not yet in regular use.

The husbandry patterns and management practices prevailing in most parts of Nigeria where enterotoxaemia is enzootic do not permit frequent access to the animals for the purpose of vaccination. Furthermore, under the conditions prevailing in these areas the use of a thick emulsion such as an oil adjuvant vaccine presents practical difficulties. Thus, there is need for a vaccine which requires simple storage and transport facilities, which is easy to administer to animals and which will give adequate immunity of long duration. The sodium alginate-adsorbed toxoid used in this study is immunogenic, easier to prepare and administer and its colloidal nature tends to make it more stable than other preparations. This field test was performed to confirm the efficacy of the sodium alginate adsorbed *C. perfringens* types C and D toxoid as a potential vaccine for preventing enterotoxaemia in sheep in Nigeria.

MATERIALS AND METHODS

Vaccine

The vaccine was prepared from local toxigenic strains of *C. perfringens* type C (KNC381) and type D (PD881) as described (11), and was tested in enterotoxaemic, enzootic areas of Plateau State, Nigeria (8, 10, 14). Basically, pure cultures of *C. perfringens* type C (12 hours) and type D (20 hours) growth in reinforced clostridia medium of Hirsch and Grinsted (6) were filtered, and the pH of the filtrates adjusted. Type D filtrate was treated with trypsin (Merck) to a final concentration of 0.25% to activate epsilon toxin. Each filtrate was treated with formalin to a final concentration of 0.6%. The formalin-treated filtrates were pooled together in equal volumes, thoroughly mixed and allowed to stand at 37°C for 14 days till the product became non-toxic for mice. The toxoid was then mixed in equal volumes with 2% sodium alginate (BDH Chemicals Limited, London) in distilled water. The product was tested and certified sterile, safe and potent, and then dispensed into amber- colored bottles and stored at 4°C until used.

Study area and population

The trials were carried out at three private farms located in enterotoxaemia enzootic areas (Chaha, Barkin Ladi and Vom) of Plateau State. The three farms practiced the same management and husbandry methods. The farms were managed under extensive conditions with practically all the flocks being grazed on unimproved pasture with occasional supplementation with potato, groundnut and acha (*Digitalis exilis*) wastes, with periods of plenty (during the rain) alternating with times of scarcity during the dry season. The farms had ready access to veterinary services. Enterotoxemia had neither been vaccinated against nor treated successfully in the area. Efforts to treat the condition using general management methods had not yielded positive results (7).

The sheep were of the. Yankasa breed, 6 weeks to 10 months old and of both sexes. All animals were screened for protective antibodies by mouse protection tests before the starting the vaccination. Those with measurable antibody level (above 0.1 i.u per ml) were excluded from the tests. In three trials, a total of 1170 animals were twice vaccinated with a dose of 5 ml each via the subcutaneous route, at 3 weeks apart with the sodium alginate toxoid (SaT). The 1070 unvaccinated sheep were injected subcutaneously with 5 ml each of sterile normal saline to compare the possible effect of injection. The vaccinated and control animals were identified with indelible ink on the ears and neck region respectively. They were observed together in the feedlots under the same management condition for ten months (March – January).

Immunity

Immunity was assessed by the mouse antibody protection test (4). The test was easy and affordable. Ten randomly selected vaccinated animals and 10 controls were bled occasionally, and the sera were used for the tests. A group of 3 mice was used for each serum sample. Each mouse was inoculated with 0.5 ml of undiluted serum by the subcutaneous route and challenged 30 minutes later with 1 mouse lethal dose of a fresh preparation of *C. perfringens* types C and D toxins via the tail vein. Challenged mice were observed for 3 days. Mouse protective antibody was considered to be present if one or more mice survived challenge provided that all three control mice died.

Mortality Data and Analysis

All experimental animals were monitored throughout the ten months period for signs of disease. Necropsy was performed on dead animals within 24 hours of death. A gross diagnosis was made for each animal. When the diagnosis was tentative, gut contents for toxin test, (mouse antibody - protection) and urine sample for sugar and protein test processed by glucose oxidase and Biurett methods (3) were conducted to confirm diagnosis. Histopathological examinations were also done on kidney samples taken from confirmed cases and some vaccinated (normal) sheep. Tissues were immersion-fixed in 10 % buffered formalin and processed to paraffin wax as described by Buxton *et al*. (2). Sections were cut and stained with hematoxylin eosin and examined. Disease-specific mortality was recorded for each group. The differences in mortality rate between the groups were compared and their association with vaccination was statistically analyzed by chi-square distribution. The vaccine efficacy was calculated by odds-ratio methods as described by Orienstein *et al* (18).

RESULTS

Immunity and protection

Based on the mouse-antibody tests, there was no detectable pre- vaccination immunity in the experimental animals. If the result was doubtful such animals were excluded from the study. Tests carried out 3 weeks post-vaccination showed that all vaccinated animals had measurable antibody titers, 2.5 - 8.0 i.u per ml. No adverse reactions were observed in any of the vaccinated animals.

Out of 2240 animals used for this trial a total of 293 (13.0 %) died of enterotoxaemia during the study period. A total of 22 (1.9 %) deaths were recorded among vaccinated animals while 271 (25.3 %) animals died in unvaccinated flocks. The mean disease-specific mortality rate for vaccinates in the 3 farms was 1.9 % and for the

controls was 25.3%.
Two hundred and eighty four (96.9 %) of the total deaths were due to *C. perfringens* type D while 9 (3.1 %) of the total deaths was due to *C.* perfringens type C. Only 1 (0.09 %) death among vaccinated animals was due to *C. perfringens* type C.
Eight (36%) vaccinated sheep and 81 (30%) controls died in the months of June and July. Most of the deaths, 14 (63%) vaccinates and 190 (70%) controls, occurred in the months of August, September, and October. The number of animals affected and percentage mortality rates are shown in Table1. Chi-square analysis of associations between vaccination and mortality rates as observed in the 3 farms showed a significant difference [$P<0.05, X^2(Cal.)=28.03$, $x^2(tab.)=5.99$, df=2] between the two groups in the rates of survival as a result of vaccination. X^2 test on the association in the survival rates between the two groups of sheep obtained from farms 1 and 2 showed a significant difference. A similar result was obtained for farms 2 and 3. The difference in survival rates between vaccinated and unvaccinated animals from farms 1 and 3 was not significant [$X^2(cal.) =3.34$, $X^2(tab.) =3.84$, df =1, $P >0.05$]. The vaccine efficiency was 98.6% (Appendix 1).

Clinical Findings
The dominating symptoms presented by most affected sheep were spastic movements, dullness, lassitude, and recumbence. Uncontrolled defecation of semi-solid fecal matter by some sheep was noted in all the farms. Retraction of the head and kicking of the hind limbs were signs which preceded death of most affected animals.

Necropsy Finding
The urinary bladders of affected animals were full with brown urine. There was inflammation of the kidneys (pulpy) and livers of affected unvaccinated sheep. The most consistent findings were congestion of the gastro-intestinal tract and excess fluid and fibrin in the pericardial sac.

Biochemical analysis
Urine from enterotoxaemia affected sheep was positive for glucose (++) and protein. These parameters were negative for vaccinated normal sheep.

Histopathology
Postmortem examination of kidney tissues from enterotoxaemia affected unvaccinated animals confirmed renal inflammation. Kidney sections from dead unvaccinated sheep showed areas of coagulative necrosis with slight cellular infiltration surrounded by fibroblasts affecting the cortex and medulla. Kidney sections from moribund unvaccinated sheep killed *in extremis* showed hemorrhages, edema and congestion with dilation of the blood vessels and fibrin deposits in the interstitial spaces. Kidney sections from vaccinated (normal animal) killed for comparison showed no pathological lesions.

DISCUSSION
The sodium alginate toxoid, a bivalent *C. perfringens* vaccine, offered a significant protection to vaccinated sheep in the 3 farms as demonstrated by its high efficiency index. The X^2 tests on the association of survival rates of animals protected with the vaccine in all 3 farms were significant, indicating a high pattern of protection. When the farms were paired, a similar significant association was observed except between farms 1 and 3 where there were 11 deaths (vaccinated), 56 deaths (unvaccinated) in the first farm and 7 deaths (vaccinated), 89 deaths (unvaccinated) in farm 3.
The finding implicating *C. perfringens* type *D* as the major cause of death confirms earlier reports (8, 11). The presence of sugar in the urine of unvaccinated sheep which came down with the disease coupled with the typical symptoms is indicative of the disease manifestation in sheep (15). The protein in the urine is associated with kidney inflation (acute nephritis) which led to the loss of plasma protein in urine as seen in this study.
Epizootiological analysis (crude morbidity, crude and cause-specific mortality and incidence rates of fatal disease) for determining vaccine efficacy in feedlot animals presents several problems.
Crude morbidity can be recorded and differences between vaccinated and unvaccinated groups determined. Given the acute nature of enterotoxaemia among sheep in the field and our present level of diagnostic technology the measure is very crude. Therefore, mortality may be a better measure of vaccine effect. For this study, mortality due to enterotoxaemia was easy to diagnose without argument and a specific cause of mortality can be determined with far greater accuracy than for morbidity. From the results the average mortality rate for the control group was approximately 13.3 times higher than that of the vaccinated animals. Over the entire study period the vaccinated group had a significant lower mortality rate. The vaccine's overall protective effect was reduced by the deaths among the vaccinated animals. The reason for this could be that these subjects developed low protective antibody due to some immunosuppressive phenomenon. Another reason could be that due to genetic variation in individual responses to vaccination (12). Although sheep appear to respond satisfactorily to vaccination with *C. perfringens* toxoid, the lowest levels that give protection appear to be of the order of 0.1 units per ml for type D and twice that much for type C (16).
The bivalent *C. perfringens* vaccine used in this study is protective and efficient enough to be recommended for large scale use in the prevention of *C. perfringens* related enterotoxaemia in Nigeria.

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Table 1: Results of natural exposure to enterotoxaemia of sheep vaccinated (A) with sodium alginate *Clostridium perfringens* types C and D toxoid and unvaccinated control (B).

Farm Percentage	No. of animals		No. of animals		(mortality rate)	
	A	B	A	B	A	B
1	500	200	11	56	2.20	28.00
2	320	470	4	126	1.25	26.81
3	350	400	7	89	2.00	22.25
Total	**1170**	**1070**	**22**	**271**	**1.88***	**25.33***

* Average percentage mortality

Appendix 1: Calculation of vaccine efficiency (Orienstein et al., 1988).

	Sick	healthy
Vaccinated	22(a)	1148(b)
Unvaccinated	271(c)	199(d)

Odds ratio = ad/bc
= 22x199/271x1148 = 0.0141
VE (%) = (1-0.0141) x 100
= 98.59 %