THE STABILITY OF WET T₁ CONTAGIOUS BOVINE PLEUROPNEUMONIA VACCINE

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SUMMARY

THE stability of wet T₁ contagious bovine pleuropneumonia (CBPP) Vaccine stored at 4°C was studied. Two large batches of the vaccines were stored at 4°C for up to 5 months. From the third month of storage, the vaccines were used to vaccinate CBPP-free animals. The immune levels of the animals were monitored by complement fixation test (CFT) and challenge trial. The results indicated that all the vaccinated animals produced reasonable antibody titres even after 5 months storage. Also, all the animals vaccinated with vaccines stored between 3 to 5 months resisted infection by contact while all the incubated and control animals came down with the disease. The results indicated that the vaccine could actually be stored for up to 5 months at 4°C before use.

INTRODUCTION

It had been a matter of controversy on how long the wet T₁ CBPP vaccine prepared from *Mycoplasma mycoides* subsp. *mycoides* (strain T₁) could be stored (shelf-life) at 4°C. Brown et. al (1965) and Gilbert and Windsor (1971) indicated that T₁ CBPP vaccine could only be stored at 4°C for one month. However, Garba (1980) pointed out that using the results of viable counts and pH measurements, the T₁ vaccine produced at Vom Laboratories could be stored at 4°C for up to 4 months and retain the minimum viable count of 10⁷ cfu/ml required to give satisfactory immunity (Gilbert and Windsor, 1971).

Following findings by Garba (1980), it is necessary to determine the potency in cattle of such vaccines that have been stored at 4°C. Therefore, this study was conducted to evaluate the level of immunity illicited by the vaccines. The result

of this study is expected to be of immense help to the users of wet vaccines especially in the developing countries where immediate application of this vaccine is hampered by lack of good roads and adequate transportation system.

MATERIALS AND METHODS

Strain of Mycoplasma:

Mycoplasma mycoides (strain T₁) was obtained from a pathogenic strain which had undergone 48 passages in eggs followed by 3 passages in broth.

Culture media:

The media and method used for the vaccine production were as described by Brown, et. al. (1965) except that both Potassium di-hydrogen orthophosphate (0.13% w/v) and di-sodium hydrogen orthophosphate were included in the broth culture as buffers. The pH was adjusted to 7.6.

Assay of Viable Mycoplasma:

The method used in determining the number of viable organisms was that described by Miles and Misra (1983).

pH Determination:

The pH of the wet vaccine was determined using pye 291 meter during the monthly viable count.

Source of Cattle Used:

Zebu cattle were used in this study. They were obtained from CBPP-free zone of Nigeria. The animals were quarantined for 3 months before being used for the experiment.

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The cattle were between the ages of 2—3 years and they weighed between 156—260 kilogrammes.

Serological Study:

The complement fixation test (CFT) was used in determining the antibody response of the cattle to vaccination (Chima and — Onoviran, 1975). The cattle were tested three times for CF antibodies during the quarantine period. After vaccination, the cattle were bled weekly for 8 weeks.

Grouping of Animals:

The vaccinated group, endobronchially intubated group and the control group, each included twelve cattle. The vaccinated animals received a dose of 0.5ml of the vaccine at the tail-tip.

Challenge Trial:

Twelve cattle were endobronchially intubated with broth culture containing 10% ground infected lungs (Lloyd and Trehewie, 1970) of the virulent mycoides subsp. mycoides. They were then mixed with the vaccinated and control animals

after 24 hours of intubation. This was to prevent false transfer of Gladysdale organisms to the vaccinated or control animals. The intubated animals became infected. They in turn spread the disease to other animals by contact. The surviving animals were killed and examined after 3 months of challenge.

Temperature taking:

A daily temperature of the vaccinated group was taken for one week before the vaccination and two weeks after the vaccination.

The temperature of the animals in the three groups were taken daily for one week before the challenge and for four weeks during the challenge period.

RESULTS

The initial viable count of both batches of vaccine and viable count at the time of vaccination are stated in Table 1. Batch B26/81 gave a viable count of 6.8×10^7 cfu/ml at the end of 5 month of storage at 4°C while Batch B27/81 gave 7.9×10^{10} cfu/ml. The pH of the vaccines remained at 7.6 till the end of 5 months.

TABLE 1
The pattern of vaccination and viable counts.

<i>Vaccin Batch</i>	<i>Storage Time (In months) at 4°C.</i>	<i>Initial Viable Count on day of production</i>	<i>Viable count on day of vaccination.</i>	<i>Period between vaccination and Challenge</i>
B26/81	3	3.1×10^9 cfu/ml.	4.68×10^8 cfu/ml.	15½ months
	4	"	1.2×10^8 cfu/ml.	4½ months
	5	"	6.8×10^7 cfu/ml.	3½ months
B27/81	3	8.7×10^9 cfu/ml.	8.6×10^8 cfu/ml.	5 months
	4	"	2.1×10^8 cfu/ml.	4 months
	5	"	7.9×10^7 cfu/ml.	3 months

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TABLE 2:

The reciprocal of antibody titre.

Vaccine	Period of Storage at 40°C	Animal Vaccinated (Ear Number) tag	Weekly CF antibody titre								
			Initial	1	2	3	4	5	6	7	8
B26/81	3 months	411	Negative	Neg.	10	20	20	40	20	10	Neg.
		415	"	"	20	40	80	40	20	20	10
	4 "	402	"	10	10	20	40	40	20	10	10
		407	"	20	20	40	80	40	20	20	10
	5 "	423	"	20	20	40	40	20	20	10	10
		417	"	10	20	40	40	80	40	10	10
	3 "	296	"	Neg.	10	40	80	40	20	20	10
		450	"	"	20	40	80	40	20	10	Neg.
	4 "	418	"	"	10	20	40	80	40	20	10
		422	"	10	20	40	80	40	20	20	10
B27/81	5 "	338	"	10	20	40	80	40	20	20	10
		354	"	10	10	20	40	20	10	10	10

The details of the individual animal antibody responses to vaccination as measured by rise of CFT titres are shown in Table 2.

The daily temperatures of the 12 vaccinated animals taken for two weeks after vaccination did not show any remarkable change from those taken before vaccination. After challenge there was no appreciable change in daily temperature of vaccinated cattle. The average temperature of intubated cattle rose from 38.8°C to 41.1°C, while those of the control animals rose from 38.3°C to 41.6°C.

The challenge trials showed that the twelve vaccinated animals resisted the infection while all the intubated and 10 of the control animals died of CBPP (Table 3). The scoring method used was modified from Hudson and Turner (1963) and Karst and Mitchell (1972).

DISCUSSION

Although some authors have reported

that *Mycoplasmas* still remained viable after storage of 4°C for 3 to 4 months (Black, 1973) and that V5 vaccine retained a high viable count after 2 months of storage (Hudson, 1964), only very little work has been carried out on the potency of these mycoplasma cultures in cattle.

This study has shown that refrigeration of wet T₁ CBPP vaccine could store much longer than was previously indicated (Brown, *et al.*, 1965). The vaccines stored for 5 months gave sufficient immunity to protect the vaccinated animals during challenge trial, and that the viable count is a reliable test of the potency of the vaccines.

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TABLE 3
Scoring of the Findings During Challenge Trials.

	<i>Intubated</i>	<i>Controls</i>	<i>Vaccinated</i>
Number challenged	12	12	12
Clinical signs			
(a) Nil	0	0	10
(b) Mild	0	0	1
(c) Moderate	0	0	1
(d) Severe	0	2	0
(e) Died	12	10	0
Serological (CF) response			
(a) Nil	0	0	8
(b) Low (titre = 10)	0	0	3
(c) Moderate (titre = 20 to 80)	2	4	1
(d) High (titre 80)	10	8	0
Pathological findings			
(a) Nil	0	0	11
(b) Healed	0	0	1
(c) Extensive, acute	6	5	0
(d) Sequestration			
(i) Small	2	4	0
(ii) Large or multiple	4	3	0
Total Score =	82.0	68.0	6.0
Mean Score per animal =	6.83	5.67	0.50

Scoring System: Clinical signs: None = 0, Mild/Moderate = 1, Severe = 2:

Died = 3

CFT + ve 1, - ve = 0; Pathological findings: None/healed. = 0,

Extensive/acute = 1, Small sequestrum = 2 Large or multiple sequestrum = 6.

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