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Effect of Storage Conditions on the pH of Blackleg Vaccines

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Abstract: Six batches of blackleg vaccines produced at the National Veterinary Research Institute (NVRI), Vom, Nigeria were stored at different temperatures for varying durations in amber and transparent bottles and pH values were observed to be lowered (in the transparent bottles), even at the recommended shelf life of the vaccines. Since the use of transparent bottles for packaging blackleg vaccines was accompanied by a drastic change in pH compared to amber coloured bottles, it is suggested that the use of transparent bottles to package the vaccines should be discouraged. Also, prior to bottling, it is suggested that the pH should be adjusted to 7.7-8.0, to modulate any pH drops that may occur subsequently, during storage.

Key words: Storage conditions, pH, blackleg vaccines, NVRI, transparent bottles

INTRODUCTION

Blackleg is a disease of cattle and sheep caused by *Clostridium chauvoei* (Radostits *et al*., 1994; Useh *et al*., 2003). There is no consensus on the pathogenesis of the disease, but toxins and neuraminidase (sialidase) produced by the aetiologic agent have been reported to play significant complimentary roles in the mechanisms of blackleg (Useh *et al*., 2006a). The economic losses of Nigeria's Zebu cattle to the disease have been estimated at approximately US dollars 4.3 million annually (Useh *et al*., 2006b). Vaccination is the most reliable preventive strategy against the disease (Kijima *et al*., 1998) and the use of combined clostridial vaccines using *C. chauvoei*, *C. perfringens* and *C. novyi* have been recommended (Cortinas *et al*., 1994). In Nigeria, formalized *C. chauvoei* vaccines have been used successfully to control blackleg (Useh, 2006).

The keeping quality of vaccines remain a major determinant of their efficacy, yet the problem of unpredictable potency due to poor storage conditions has remained an issue of major concern in the control of blackleg in Nigeria. In this study, we investigated the effect of various storage conditions on the pH of various batches of blackleg vaccines produced at the NVRI, Vom, Nigeria and report here that transparent bottles if used to store blackleg vaccines produce a drastic drop in pH, leading to decreased efficacy of the vaccines.

MATERIALS AND METHODS

Preparation of blackleg vaccines: Blackleg vaccine is prepared by growing *C. chauvoei* strain NCTC 3764 in reinforced clostridial medium (pH 8.2) inactivated with 0.62% formaldehyde as described by Hirsch and Grinsted (1954). Thereafter, the vaccines are dispensed in 100 mL amber coloured bottles and diluted at 1:10 dilutions using sterile distilled water before use.

Storage conditions: In this study, six batches of blackleg vaccines were used. Each batch was dispensed in 100 mL capacity amber coloured and 200 mL capacity transparent bottles. The vaccines were kept at three different temperatures namely, 4°C, 25°C and 37°C for 7 days, 1 month, 6 months, 8 months, 12 months and 14 months periods.

Viable counts: Prior to inactivation, viable counts were conducted for each batch as described by Miles and Misra (1938) using cysteine glucose peptone water as diluent.

pH measurement: pH measurement of inactivated cultures was carried out before each batch was dispensed and later adjusted to optimal pH (pH 7.0) using Lovibond comparator and 10 N NaOH. The pH of the stored vaccines at the intervals mentioned earlier were measured using ROTA indicator (M and BH Deganham, England).

RESULTS

The average viable count obtained from the 6 batches prior to inactivation ranged from 2.5×10^7-3.0×10^9 cfu mL^{-1}, whereas the pH ranged from 6.2-7.6 (Table 1). Lower pH values were accompanied by higher viable counts.

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Table1: Viable count and pH of *C. chauvoei* cultures prior to inactivation

Batch No.	Viable count (cfu mL^{-1})	pH	Adjusted pH prior to bottling
2/2001	2.5×10^7	7.6	7
3/2001	2.0×10^9	6.4	7
4/2001	1.6×10^8	6.8	7
5/2001	6.2×10^8	6.8	7
1/2002	2.6×10^9	6.2	7
2/2002	3.0×10^9	6.2	7

Table 2: pH values of blackleg vaccines stored at different temperatures and durations

Duration		*pH at 4°C	*pH at 25°C	*pH at 37°C
7 days	A	7	6.76	6.70
	T	7	6.76	6.70
1 Month	A	7	6.76	6.53
	T	6.17	6.17	6.10
6 Months	A	6.73	6.53	6.53
	T	6.17	6.17	6.00
8 Months	A	6.47	6.43	6.47
	T	6.13	<6.00	<6.00
12 Months	A	6.45	6.27	<6.00
	T	<6.00	<6.00	<6.00
14 Months	A	6.40	6.20	<6.00
	T	<6.00	<6.00	<6.00

Amber coloured bottles, T-Transparent bottles,*-Average pH value of 6 batches

The pH dropped faster in the vaccines packaged in transparent than amber coloured bottles at the different temperatures investigated (Table 2).

DISCUSSION

The shelf life of blackleg vaccines produced at NVRI, Vom, Nigeria is about 12 months at 4°C. In this study, it was observed that the pH of the vaccines continued to drop as from day 7 after packaging (both in amber coloured and transparent bottles), although the drop was more drastic in the transparent than amber coloured bottles. It is recommended that vaccines which will not be administered immediately should be stored in the dark in amber coloured bottles. It is however difficult to achieve this, especially that most vaccine shops do not bother about most professional issues related to vaccine storage and potency.

From the findings in this study, it is impossible to use transparent bottles for packaging blackleg vaccines, due to their tendency to produce drastic drops in pH, even within the normal shelf life of the vaccines. Amber coloured bottles also produced drops in pH and it is suggested that, although they are best for use in packaging blackleg vaccines, the pH should be adjusted to 7.7-8.0 in order to modulate the pH drops, in accordance with previous report (Mhoma, 1985).

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