

**DETERMINATION OF THE VIABILITY AT
+4°C OF FREEZE-DRIED ATTENUATED LIVE
AGALACTIAE VACCINE.**

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ABSTRACT. Studies on viability of attenuated live agalactiae vaccine prepared with M. agalactiae strain AIK40 have been carried out. The freeze-dried samples were examined for viability by the colony count technique. The results indicated that the surviving organisms in the live vaccine, when stored for four years at +4°C, were still sufficient enough to immunize the animals against the agalactiae disease.

Key words: AGALACTIA/LIVE VACCINES/ATTENUATION/ANIMAL DISEASES/VACCINES

INTRODUCTION

Arisoy, F. and Erdag, O. reported that attenuated live mycoplasma agalactiae vaccine culture survived for about one month at room temperature. The freeze-dried M. agalactiae attenuated vaccine kept at room temperature, survived for 35 days, whereas at +4°C the organisms survived for a long time(1).

In this study a batch of freeze-dried attenuated

live vaccine was prepared with strain AIK40 and its viability was checked by Miles and Misra colony count methods in a period of 4 years when it was stocked at +4°C (2) .

MATERIALS AND METHODS

Strain:

The attenuated strain AIK40 was used for preparation of the live vaccine.

Vaccine:

A freeze-dried ampule of strain AIK40 was reconstituted in 9ml. of PPL0 broth medium supplemented with 20% inactivated normal horse serum and incubated at 37°C for 48 hours. From this seed culture, 3000ml. batches of PPL0 broth were inoculated and incubated for 72 hours . The cultures were pooled and viable mycoplasma count showed the culture to contain 2.6×10^9 /ml . organisms . The vaccine culture was mixed with an equal volume of Mist desiccans (3) . The vaccine was checked for purity, and finally freeze-dried vials were kept at +4°C until used. Viability tests were made before and after freeze-drying.

RESULTS :

Results are summarized in table.1 which shows the falling curve of viable organisms of agalactiae vaccine during 4 years of storage at +4°C.

Table 1.**Results of viability tests**

Date of test	Viable mycoplasma per ml. at +4°C
1.11.1984	4.37 X 10 ⁸
20.4.1985	2.76 X 10 ⁸
21.11.1985	1.45 X 10 ⁸
20.1.1986	65.4 X 10 ⁶
20.4.1986	38.8 X 10 ⁶
8.9 .1987	37.4 X 10 ⁶
1.11.1988	29.9 X 10 ⁶

DISCUSSION:

The minimum number of viable organisms in the attenuated live agalactiae vaccine which is necessary for protecting sheep and goats has been reported to be about 4800 live mycoplasma. This is a vaccinal dose (4). Inoculation of larger doses of the live vaccine has been experienced with satisfactory results (4). However, it will be possible to keep the live freeze - dried vaccine in stock at +4°C for a long time if a suitable and rich culture is used in preparation of agalactiae live vaccine.

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