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INTRODUCTION

STUDIES ON THE GROWTH OF PASTEURELLA MULTOCIDA
IN DIFFERENT MEDIA FOR VACCINE PRODUCTION

BY

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INTRODUCTION

Haemorrhagic septicaemia is a major cause of death particularly of water buffaloes and also of cattle in South East Asia. It also occurs to a lesser extent in the near and the middle east and Africa. The disease is caused by specific serotypes of *Pasteurella multocida*. In Asia the predominant serotype is 6 : B and in Africa type 6 : E predominate. All countries where the disease occurs have accepted vaccination as the only method of control. Some countries with better organized Veterinary Services adopt routine prophylactic vaccination, whilst others resort to vaccination as and when outbreaks occur.

Many types of vaccines have been used against Haemorrhagic septicaemia. The simplest vaccine used consists of plain broth bacterin i. e. broth culture inactivated by adding 0.5% formalin. Such a vaccine confers only a low grade immunity of short duration not exceeding 2 months. The alum precipitated vaccine is believed to protect animals for periods ranging from 3 - 5 months. (Bain 1963, De Alwis, Gunatilleke and Wickremasinghe 1978) and is the predominant vaccine used in most Asian Countries. In Thailand an Aluminium hydroxide gel vaccine is used and is reported to protect animals for 4 - 6 months (Anon 1979 e). A vaccine containing saponin (Delpy's vaccine) is reported to have been used in Iran (Bain 1963). The oil adjuvant vaccine (OAV) is reported to give the longest



period of immunity, but it is used as the predominant vaccine only in few Asian countries. The duration of immunity conferred by the vaccine is very variable and range from 6 - 9 months in Sri Lanka and Indonesia, (De Alwis et al 1978, Anon 1979 a) 12 months in Malaysia (Anon 1979 b), 21 months in Bangladesh (Anon 1979 C) and 28 months in India (Anon d). Such wide variation are probably due to the absence of standardisation in both the preparation and evaluation of the vaccine.

Various other vaccines have been tried out from time to time. These include the Sodium Alginate vaccine (Bhatty 1973), Freund's Adjuvant vaccine (Rao and Sambamurthy 1972), the capsular antigen vaccine (Naggy & Penn 1976) the streptomycine dependent live vaccine (De Alwis & Carter) but none of these have so far been satisfactory enough for regular large scale use in place of the established vaccine.

The Oil Adjuvant Vaccine.

The vaccine consists of a bacterin emulsified with a mineral oil using Lanoline as an emulsifying agent to produce a stable water in oil emulsion. It has been found that at least 2 mg. bacteria (dry weight) is required to immunise cattle or buffaloes. In the process of emulsification the bacterin gets diluted in an equal volume of the oil - Lanoline mixture. Thus if a single dose of the vaccine should not exceed 2-3 ml. by volume, for convenience of administration a dense bacterial



suspension is required for the purpose of oil adjuvant vaccine production. A dense bacterial suspension can be obtained either by growing the organism on the surface of solid medium and harvesting or by producing a dense broth culture. The former is not practicable on a large scale hence the necessity arises to produce dense broth cultures for large scale vaccine production.

Production of dense cultures.

A dense culture can be produced by :-

- (A) Growing the organism in a well balanced medium
- (B) by aeration.

Bain (1963) reported to have obtained bacterial yields ranging from 0.5 mg/ml. to 4.5 mg/ml. in aerated culture by altering the composition of the medium with various additives added to a basic caesin digest medium.

H. S. Vaccine Production in Sri Lanka.

Sri Lanka has a cattle population of 1.5 million and a buffalo population of 0.8 million. Nearly 75% of these animals live in areas where the risk from H.S. justifies prophylactic vaccination. At present about 0.6 million doses of H.S. vaccine are produced annually. Of this at its best, only one third has been of the oil adjuvant type, whilst the balance has been the Alum precipitated vaccine. Thus if Sri Lanka's total need of an effective vaccine is to be realised a complete switch over to the oil adjuvant vaccine coupled with an increased



production (at least doubled) is an urgent requirement.

Equipment.

Vortex tanks made of metal and of 40 litre capacity containing 25 litres of medium are used. The medium is whirled into a vortex by a impellar rotating on a shaft at a speed of about 1000 r.p.m., connected to a motor. A current of air is pumped through a filter and passed over the medium in the tank. Some of the problems encountered with this apparatus are the frequent contamination of the tanks. This system has the advantage that minimum froth is formed.

Medium.

The medium presently used consists basically of a caesin digest to which is added autodigest of pancreas, yeast extract, sucrose and buffer. Depending on the circumstances it may be reconstituted using imported dehydrated media or the ingredients may be prepared from raw materials. The former may be expensive and the latter laborious.

In the present study an attempt was made to study the various factors which effected the density of aerated cultures in order to develop a suitable and economical medium and a simple culture system to obtain reasonable yields of bacteria for vaccine production, within the facilities available for vaccine production in this country.

