BACTERIOLOGICAL AND IMMUNOLOGICAL STUDY OF *CLOSTRIDIUM CHAUVOEI* AMONGST CATTLE IN SRI LANKA

by

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ABSTRACT

This thesis titled "Bacteriological and immunological study of *Cl. chauvoei* amongst cattle in Sri Lanka" comprises a literature review on *Cl. chauvoei* and black quarter disease (BQ) and series of studies carried out on bacteriological and immunological aspects of BQ and occurrence of the disease in Sri Lanka, for the degree of Master of Philosophy. These studies were carried out at the Veterinary Research Institute (VRI), Gannoruwa, Peradeniya, Sri Lanka.

The main objective of this study was to develop a better vaccine against BQ for use in Sri Lanka. This was successfully achieved. A suitable local field isolate of *Cl. chauvoei* (399) was selected as the seed culture for BQ vaccine production from among 14 such isolates from the culture collection maintained at the VRI, based on their growth rates (cfu/ml/24 hours) in cooked meat medium. An attempt was also made to develop an improved vaccine medium for the production of BQ vaccine. In this study, four vaccine media were compared for their growth promoting capacity by growing three local isolates of *Cl. chauvoei* in each medium. Bacterial yields obtained in terms of cfu/ml/24 hours were statistically analyzed and the medium which favoured best bacterial yields was selected. The quality of this medium was further improved by altering its composition. This improved medium is cheaper, less laborious to prepare and better in growth promoting

capacity. Further, the vaccine production procedure was modified in order to minimize the risk of contamination. In the new procedure, the incubation period of the vaccine medium was reduced from 36-48 hours to 24-30 hours while ensuring adequate bacterial growth.

A vaccine was prepared using the selected local field isolate of *Cl. chauvoei* in the improved vaccine medium. The duration of immunity induced by this newly developed vaccine was compared with an imported BQ vaccine and the presently used vaccine. The new vaccine was found to maintain adequate potency up to 9 months of post-vaccination as against the 6 months of duration of immunity induced by the presently used vaccine. The shelf life of the newly developed vaccine was also determined and it was found that this vaccine could be stored at $+4^{0}C$ for a period of at least 12 months without loosing its potency.

The Enzyme-linked immunosorbent Assay (ELISA) as described by Crichton, Solomen and Barton (1990) was established at the VRI to evaluate the immune response to *Cl. chauvoei* in guinea pigs and cattle. This technique was used to monitor the immune response induced by three BQ vaccines i.e., the newly developed vaccine, an imported BQ vaccine and the presently used vaccine, for a period of 12 months in guinea pigs. This study revealed that ELISA could be used to measure the antibody response and thereby assess the potency of BQ vaccines effectively up to a period of 2 months of post-vaccination and that the titres detected

after about 6 months were not true indicators of the protective immunity. It was also revealed that this technique was successful in detecting antibodies in sera of immunized cattle and therefore it could be used to assess the efficacy of BQ vaccination in cattle.

A study was undertaken to characterize some of the local field isolates of *Cl. chauvoei* and to investigate whether *Cl. chauvoei* and *Cl. septicum* can be differentiated using biochemical tests. Biochemical tests described by Carter (1974) to differentiate pathogenic *Clostridia* were carried out with the local isolates of *Cl. chauvoei* along with a reference culture of *Cl. chauvoei* (NCTC 8070) and *Cl. septicum* (NCTC 574). According to Carter (1974), the only test which differentiate the two organisms based on biochemical reactions was that of fermentation of sucrose and salicin i.e., *Cl. chauvoei* ferment sucrose but not salicin whereas *Cl. septicum* ferment salicin but not sucrose. In this study, it was evident that all the local isolates of *Cl. chauvoei* and the reference *Cl. chauvoei* strain ferment both sugars as in the case with reference *Cl. septicum* strain. Therefore, it was understood that biochemical tests were not distinctive enough to differentiate the two organisms and therefore, of not much use for this purpose.

In addition, a study was also undertaken to differentiate *Cl. chauvoei* and *Cl. septicum* by active protection test in guinea pigs. It was revealed in this

study that guinea pigs immunized with either *Cl. chauvoei* or *Cl. septicum* vaccines were protected when they were challenged with *Cl. chauvoei* culture. However, when the challenge culture was *Cl. septicum*, guinea pigs immunized either with *Cl. chauvoei* or *Cl. septicum* vaccines could not withstand the challenge. Therefore, it can be postulated that if 2 unknown cultures of *Cl. chauvoei* and *Cl. septicum* are used to challenge groups of guinea pigs protected with *Cl. chauvoei*, deaths can be expected only if the challenge culture is *Cl. septicum*. Hence, the two organisms can be differentiated by using this technique. However, it was understood that the passive protection test described by Carter (1974) was a better choice than the active protection test carried out as in the present study, in order to differentiate the two organisms.

Further, a questionnaire based survey was also carried out in endemic regions of the country in order to collect information on some of the basic features of occurrence of the disease in Sri Lanka. This study was carried out during the period July to December in 1993 and covered the BQ outbreaks that occurred during the period of 1991-1993. In this study, it was found that cattle and buffaloes were equally susceptible to the infection. However, European crosses were found to be more susceptible than local and indian crosses. Local and Indian crosses of 6 months to 2 years of age and European crosses of 6 months to 4 years of age were found to be more susceptible to the

disease than the other age groups tested i.e., up to 6 months and over 4 years of age. The highest percentage of mortality due to BQ was observed in the driest month of the year i.e., August while a greater proportion of deaths occurred during July to November period accompanied by the North-West monsoon. It was also revealed that most of the farmers (64.2%) vaccinate their animals only after an outbreak and a considerable proportion (35.8%) of farmers never practice vaccination against BQ. This observation explains why BQ continues to be a problem to the livestock industry in Sri Lanka.