Use of Sodium Alginate Adjuvant in Preparation of a Formalin Killed Vaccine Against Infectious Bovine Rhinotracheitis

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Summary
A formalin inactivated vaccine was prepared with infectious bovine rhinotracheitis virus. This vaccine, combined with sodium alginate as adjuvant, was used for immunization of cattle. The neutralization index (NI) of circulating antibodies, produced to a single inoculation of the vaccine without adjuvant, was $log^{2.5}$. *However, NI to 1 and 2 inoculations of this vaccine mixed with adjuvant found to be significantly higher and reached* log^{4} *and* $log^{4.5}$, *respectively. Circulating antibodies persisted in the cattle for at least 4 months.*

Introduction

Viral respiratory diseases and, above all, infectious bovine rhinotracheitis, specially when complicated with other microorganisms, is of considerable economic importance to the cattle industries.

Inoculation of cattle with either monovalent or combined inactivated vaccines prepared with BVD or PI-3 have been widely used for prophylactic purposes. Annually, several million doses of inactivated IBR vaccine is manufactured and widely used in many countries of the world(1).

Inoculation of pregnant cattle with live attenuated vaccine against IBR is discouraged, since this vaccine may lead to abortion(2) and, furthermore, the virus may become latent and be shed, after reactivation, for lifelong. Consequently, development and production of a safe and non-infectious

vaccine that would immunise cattle of all ages is advocated by the veterinary authorities.

The present study was directed toward this goal. The data presented in this study is the serum antibody titers in cattle given 1 and 2 doses of inactivated IBR vaccine with and without sodium alginate adjuvant(3)

Materials and methods

Virus: A local strain of IBR virus, which had previously been isolated from an indigenous cow and adapted to tissue culture, was used for production of inactivated vaccine. Electron microscopy was exploited to confirm the morphology and homogenesity of the isolated virus in tissue culture.

Tissue culture: Bovine kidney cells, at 185th passage, were grown at 37°C in Roux bottles using YLE medium containing 10% foetal calf serum until complete monolayer were formed.

Titration: The virus titer was determined by observing cytopathic effect on the cultured cells when the changes were complete. The infectivity titer was calculated by Reed and Muench method.

Formalin killed vaccine: Suspension of propagated IBR virus amounting to $1 \times 10^{8.56}$ TCID 50/ml was inactivated with formalin, at final concentration of 0.4 percent , for one week at 4°C and tested for freedom from bacterial contamination and for absence of residual viral infectivity.

Since mineral oil adjuvants can induce amyloidosis and sarcoid like lesions, they were excluded from this experiment. For this reason, sodium alginate that had been successfully used in other inactivated viral vaccines (4) was considered to be a safe, effective and non-antigenic adjuvant.

The adjuvant contained 4% sodium alginate and 0.67% calcium gluconate. It was mixed with an equal volume of the vaccine prior to inoculation.

Experimental cattle: Ten 6-month old Holestin calves were used for testing the immunogenecity of the vaccine. They had no history of the disease and had not been vaccinated against IBR.

These animals were free of circulating antibodies against IBR, checked by seroneutralisation test as recommended by other investigators(5, 6) and were inoculated, intramucularly, as follows:

Calf No. 1, received 5 ml of the adjuvant and 5 ml of the medium (Group I).

Calves Nos. 2, 3 and 4 each received 5 ml of the vaccine without adjuvant (Group II)
Calves Nos. 5, 6, 7, 8, 9 and 10 each received 5 ml of the adjuvant and 5 ml of the inactivated vaccine (Group III) and calves Nos. 8, 9 and 10 were reinoculated 20 days later with 5 ml of adjuvant and 5 ml of inactivated vaccine (Group IV).

Samples of blood (10ml) were collected from all cattle at 15, 30, 45, 60, 90 and 120 days after the initial inoculation had been given.

Serologic tests: All sera, collected at different intervals, were incubated at 56°C for 30 min to remove the non-specific inhibitors and also for inactivation of complement.

The tube neutralisation technique was used for neutralisation test with IBR virus. The virus was tested against serum at 1:10 dilutions, equal volume of ten-fold dilutions of virus was mixed with 1:10 dilution of the antiserum and incubated at 37°C for 60 min.

A 0.2 ml volume of the mixture was then added to each of 5 monolayer tube cell culture of BK line with YLE medium containing 3% foetal calf serum. The tubes were incubated at 37°C for one week while examining them daily for cytopathic effects. The neutralisation index was calculated as the difference between the titer of the stock virus and the titer of the virus after incubation with the test serum.

Results

None of vaccinated cattle showed any evidence of hypersensitivity or untoward reactions to the vaccine. The sera from individual cattle in all groups were assayed and the results were recorded as the average antibody titres for each group.

Antibodies were not observed, throughout the test period, in calf No. 1. The results for the 1:10 serum dilutions are presented in Fig. 1. The mean values of the NI against IBR are presented in Fig. 1. The NI of $\log^{1.2}$ was first observed on the 15th day after vaccination with IBR plus adjuvant (Group IV), serum titers gradually increased and reached a maximum NI of $\log^{4.5}$ on the 60th day post vaccination. The highest NI of $\log^{2.5}$ and $\log^{4}$ were observed in Groups II and III, respectively. Inoculation of the vaccine with adjuvant led to significantly higher antibody responses.

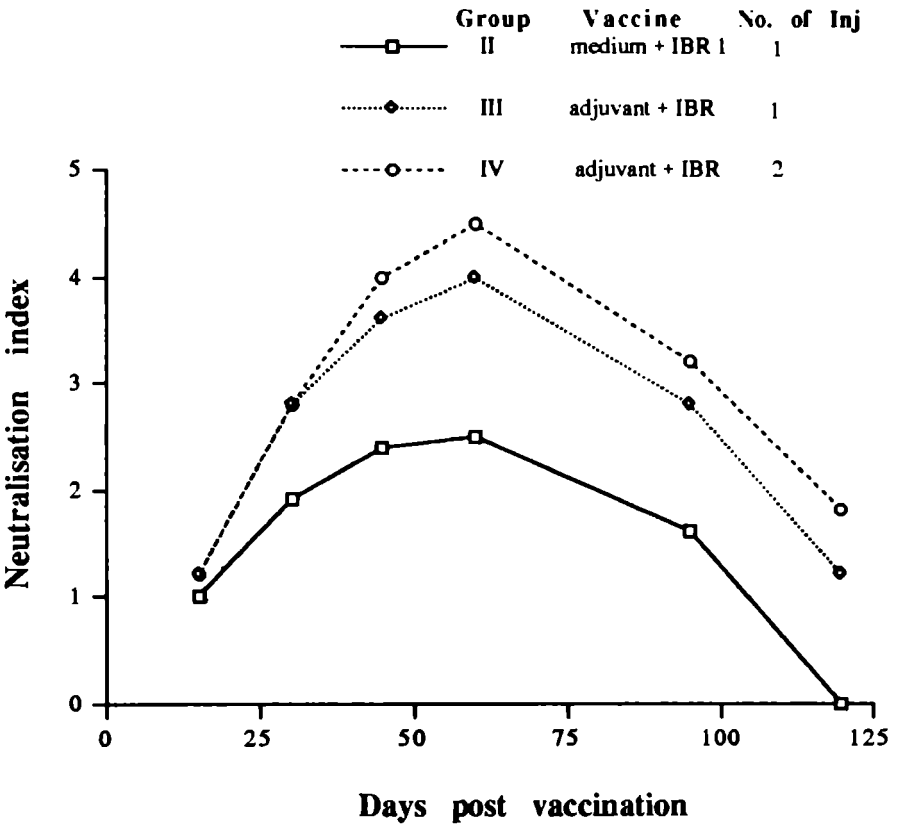

Fig. 1: Average antibody response of cattle inoculated with inactivated IBR vaccine with and without adjuvant, expressed as neutralisation index at a 1:10 antiserum dilution against IBR virus.

Discussion

Since the present study was carried out in a herd free from cirulating antibodies to IBR , the increase in titres of the relevant antibodies after vaccination was interpreted as a response to the formalin killed viral vaccine. The antibody titres in cattle inoculated twice with the virus plus adjuvant vaccine were not significantly higher than those vaccinated once, with the same vaccine, but the antibody titre were lower in those calves that received the vaccine without adjuvant. The antibodies, in cattle that received 2 doses of the vaccine (20 days apart), lasted about 4 months. For

this reason, it is recommanded to inoculate all susceptible cattle in feedlots, every 4 months.

Due to the experimental design, it was not possible to determine whether a correlation existed between a high NI and the protection against the disease, since this can be adequately ascertained only by challenge experiments or epizootiologic studies. It has been reported, however, that cattle having a serum NI of log $10^{2.0}$ or higher were protected against challenge doses of IBR virus(1). The NI in the present experiment was greater than the minimal protective titre.

At present, reliance is placed on subunit and live modified infectious bovine rhinotracheitis virus vaccine (7, 8, 9).

Although such vaccines provide a certain degree of protection in cattle, they are not commertialy available. Therefore, an effective killed virus vaccine would be of considerable importance to the cattle industry.

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