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Original Article

Serological Study on Mucosal Response of Cattle to Haemorrhagic Septicaemia Vaccine

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ABSTRACT

The purpose of this study was to compare the efficacies of systemic immune response to mucosal vaccination against *Pasteurella multocida* infection in cattle. Three groups of ten cattle each were immunized subcutaneously and intranasally with bacterin of *P. multocida* serotype B: 2. Group 1 and Group 2 were immunized subcutaneously and intranasally with 0.5 ml of bacterin mixed with mucosal adjuvant respectively. Group 3 served as a control group was immunized subcutaneously with 1 ml of final vaccine product of bacterin as recommended dose according to Central Veterinary Research Laboratories, two cattle for each group unvaccinated control, all three groups received a booster dose on day 24 post inoculation.The level of the antibody immune response in these three groups was measured by the indirect haemagglutination test. Serum and nasal antibodies of vaccinated animals increased after the second vaccination, and this difference was statistically significant. Concentration of serum antibody against *P. multocida* increased from primary vaccination (6.25 antibody titer) on day 7 to (82.5 antibody titer) on day 31 after boosting animal, similar levels of protection were obtained from group 3. The nasal antibodies were rised from (3.75 antibody titer) at first vaccination on day 7 to (20 antibody titer) on day 31 after the second inoculation. The mucosal level of antibody in the intranasally vaccinated group was less compared to subcutaneously vaccinated group.

Keywords: Immune response, *P. multocida*, vaccine, mucosal adjuvant.

INTRODUCTION

Pasteurella multocida (*P. multocida*) causes heavy losses, particularly in wet areas and when the animals are exposed to humid, chilly weather or are stressed by heavy work (Benkirane and Alwis, 2002). There is ample evidence that buffaloes are more susceptible than cattle, in both species, young and young adult animals are more susceptible than older animals (Alwis, 1990). Also *P. multocida* is etiological agent of fowl cholera in birds, atrophic rhinitis in pigs and rhinitis, pneumonia, otitis media and death in rabbits (Mannheim, 1984; Jarvinen, *et al*., 1998). The two common serotypes of *P. multocida* associated with disease in these species are types B: 2 (in Asia) and E:2 (in Africa). Infection occurs by inhalation or ingestion of *P. multocida* bacteria or by the ingestion of contaminated feed-

stuffs. The infection originates from healthy carriers or clinical cases, or possibly from ticks (Radostits *et al*., 2003) and biting insects. The control of pasteurellosis is depending on vaccination. Haemorrhagic septicaemia vaccine is produced from local strains at Central Veterinary Research Laboratories (CVRL), determinated by (Elbashir, 1993).
Based on the previously mentioned facts, the present study was delineated (i) to investigate the effectiveness of vaccination in cattle with (HS) bacterin, and to test the pea-nut oil and freund´s incomplete adjuvant as mucosal adjuvant for delivery of vaccine (ii) to evaluate the efficacy of intranasal (I/N) versus subcutaneous (S/C) administration of the vaccine in stimulating protective immunity against *P. multocida*.

MATERIALS AND METHODS

Bacteria

The freeze-dried strains of *P. multocida* (B and E) which were used in this study were obtained from the Department of Microbiology, faculty of Veterinary Science, University of Khartoum they had been isolated from outbreak of haemorrhagic septicaemia (Shigidi and Mustafa, 1979). Freeze-dried strains were reconstituted in nutrient broth and incubated at 37^{o}C for 24 hours, then checked for purity.

Antigen

Formalin treated whole cells 0f *P. multocida*, serotypes B:2, and E:2, were prepared according to the protocol of vaccine production in the Central Veterinary Research Laboratories, Khartoum (Elbashir,1993). The bacteria were propagated in the Gottingen bioreactor (Bio-Chem-Spezialgerate GmbH, Göttingen, Germany).

Mucosal adjuvant

Pea-nut oil mixed (I: 1 v/v) with Freund´s incomplete adjuvant was experimented as mucosal adjuvant to deliver the vaccine.

Experimental animals

Thirty head of local breed of cattle which were used in this study were located in a traditional farm in White Nile state, with no previous vaccination history. The calves were 6-9 months old; the calves were divided into 3 groups each of ten cattle. Pre-immunization sera and nasal swabs were collected.

Immunization of cattle

The antigen bacterin was mixed with the mucosal adjuvant and the mixture was placed in a water bath at 37^{o}C for 15 minutes. A dose of 0.5 ml of the antigen described above was injected subcutaneously in group (I) and the same dose inoculated intranasally in group (II), cattle in two both groups received a second dose of vaccine on day 24 post initial immunization. Control group of two cattle of two groups were received the normal saline via the same route. The group (III) was served as control group, received 1 ml of the bacterin subcutaneously as recommended dose according to Central Veterinary Research Laboratories. The group was vaccinated with the bacterin without the mucosal adjuvant. 1ml of the bacterin was administered subcutaneously; the cattle received a booster dose at day 24 post inoculation. A control group of two cattle received normal saline through the same route of treated group.

Laboratory Assay

Antibodies against the *Pasteurella multocida* were measured by using indirect haemagglutination test (IHA) using human blood "O" (RBC's Bain *et al*., 1982) , but sheep red blood cells have been used according to (Sawada et al., 1982).

Test Procedure

The test was carried out in microtitre plates of Flow Laboratories, each having 96 U shaped wells, arranged in 8 rows and 12 columns designated as A-H and 1-12, respectively. Two fold dilutions of the test sera starting from 1: 5 to 1: 640 were made in normal saline solution and added in 25µl amounts to all the wells of plate except those of column 11 and 12 which were maintained as controls. First four wells (A-D) of column 11 were added with known negative serum and last four wells (E-H) with the known positive serum. All the wells of the column 12 were added with normal saline solution. Sensitized RBC's (1%) were added in equal amounts (25µl) to all the wells of the plate, so that column 12 served as control for the RBC's. The plates were incubated at room temperature for two hours and the observations were recorded. Thereafter the plates were kept under refrigeration for overnight shake lightly, allowed to resettle and read again. Results were interpreted as under:

- **Positive:** No button formation, clumping occurring in an unordered and ragged pattern.
- **Negative:** Button formation, RBC's clumping in an organized and regular pattern.

Interpretation

Both IgM and secretary IgA classes of antibody are produced in response to any of vaccinated animals. IgM and IgA antibody is first detectable within 1 week after immunized animals and peaks at 4 weeks.Titers of 1: 5 are considered borderline and follow-up samples should be tested for serology.

RESULTS

Mucosal adjuvant

Pea-nut oil mixed with Freund´s incomplete adjuvant experimented as mucosal adjuvant in cattle proved to be safe and hence it was used to deliver the vaccine**.**

Immune response of cattle

Results of samples of vaccinated and control animals, the serum and nasal swabs collected from cattle in all groups were tested for pasteurellosis antibodies by indirect haemagglutination test IHA, no results recorded on day 0, the small levels observed on day 7, 14, 21 and 24 before the booster dose, the peak of antibodies level was recorded in all groups after the second dose of vaccine. The results of pasteurellosis antibodies of all animals in the three groups are shown in table 1.

Table 1. Mean average of antibody titers in three cattle groups vaccinated intranasal and subcutaneous with bacterin HS vaccine by IHA (n=8)

Route of administration	No. Cattle	day0	Day7	Day14	Day21	Day24	Day31
I/N (0.5 ml)	8	0	6025	12.5	17.5	28.75	82.5
S/C (0.5 ml)	8	0	3.75	5	7.5	11.25	20
S/C (1 ml)	8	0	8.75	12.5	21.25	36.25	92.5

DISCUSSION

P. multocida causes heavy losses, particularly in low-lying areas and when the animals are exposed to wet, chilly weather or are stressed by heavy work (Benkirane and Alwis, 2002). There is ample evidence that buffaloes are more susceptible than cattle and that, in both species, young and young adult animals are more susceptible than older animals (Alwis,1990b). The two common serotypes of *P. multocida* associated with disease in these species are types B:2 (in Asia) and E:2 (in Africa). All age groups are affected with *P. multocida*, but in cattle the most susceptible age group is between 6 months and 2 years.

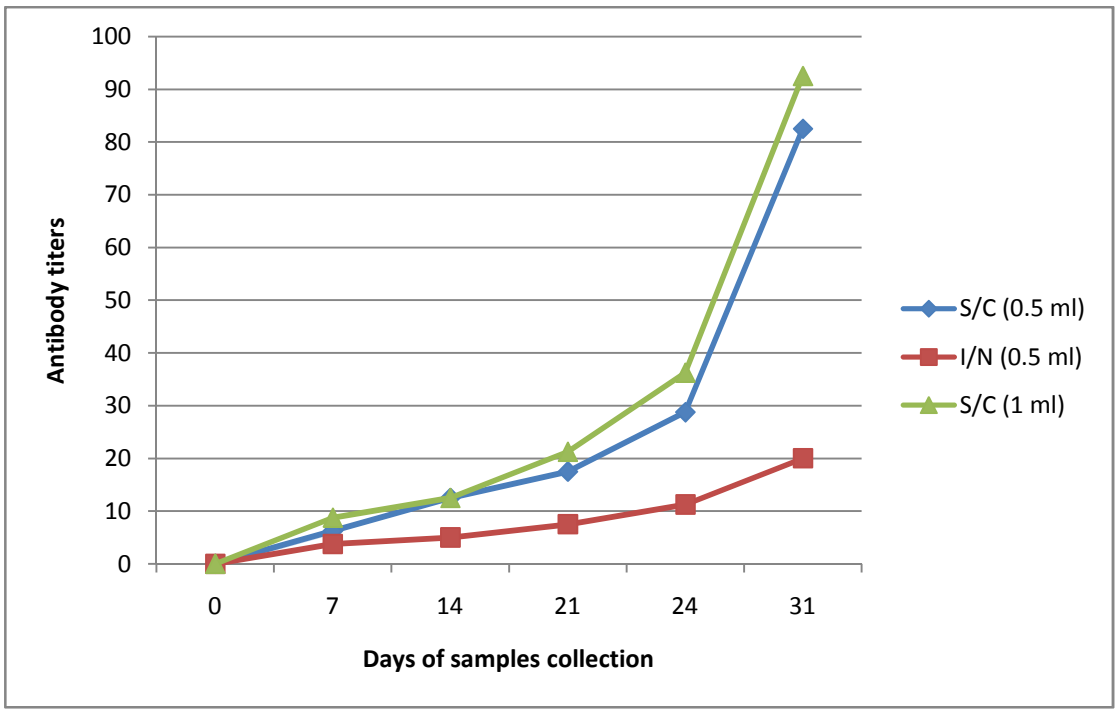

Figure 1. Antibody titers at 0, 7, 14, 21, 24 and 31 days post immunization

The present study was conducted to compare the subcutaneous vaccination to the intranasally inoculated in protecting cattle against pasteurellosis with haemorrhagic septicaemia vaccine. In this study the Freund´s incomplete adjuvant was mixed with the pea-nut oil (1:1 v/v) was used as mucosal adjuvant to vaccinate groups of cattle via the subcutaneous and intranasal route. The results of indirect haemagglutination in group 1 and group 3 were vaccinated subcutaneously were greater, these two groups were developed a serum anti-pasteurellosis response that dependent on dose volume and mucosal adjuvant when compared with the titer of group 2 was immunized intranasally, this group also developed secretory IgA antibody against *P. multocida* that was due to the dose volume is not cover all the nasal area or may be the mucosal adjuvant is not effective on nasal route. The finding of the bacterin vaccine gave a better protection when injected subcutaneously is in consistency with work of (Elbashir, 1993), who used a bacterin vaccine produced in a continuous cultivation system. In this study mucosal response to *P. multocida* resulted by vaccination with a bacterin, the average results of laboratory assay the IHA, the low titer levels were recorded on day 7 (3.75 antibody titer), and high level was obtained on day 31 (20 antibody titer) post booster dose this results were less protective than subcutaneous route, but bearing in mind that we used adjuvant might necessitate the experimentation of other mucosal adjuvant which might confer higher protection than the pea-nut freund´s incomplete adjuvant used in the present study. Even though intranasal immunization with bacterin is not an effective way to control infection, the method of vaccine delivery is not necessarily practical, especially when vaccinating a large number of animals. However, the efficacy of mucosal vaccination suggests that, it may eventually by possible to deliver vaccine by alternative routes, such as orally, to induce mucosal immunity in respiratory tracts.

The titration of nasal swabs samples in cattle represents only small level titers (0 on day 0, 3.75 on day 7, 5 on day 14, 7.5 on day 21, 11.25 on day 24 and 20 on day 31) because the titers of the nasal samples may not reflect the total of respiratory tract surfaces. Nasal swabs are not suitable technique for measuring secretary IgA, because the collection of nasal samples limited in nasal mucosa area of respiratory tracts.

CONCLUSION

A bacterin HS vaccine stimulates antibody activity to and protective immunity against *P. multocida* in cattle. The efficacy of mucosal vaccination suggests that, it may eventually by possible to deliver vaccine by alternative routes, such as orally, to induce mucosal immunity in respiratory tracts. The peak level of antibody titers against *P. multocida* was obtained after boosting animals.

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