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Evaluation of immuno efficiency of hemorrhagic septicemia vaccine strain (vaccine seed)

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PEER REVIEW

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Comments

This is a useful study. The authors compared the seed culture of hemorrhagic septicemia (HS) vaccine strain P-52 for its efficiency in mice before and after passage in natural host (calf) by using an endpoint dilution assays for calculation of LD_{50}.
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ABSTRACT

Objective: To compared seed culture of hemorrhagic septicemia (HS) bacteria which was used to produce vaccine for its antibody induction efficiency before and after passaging in natural host (calf) using laboratory animals.

Methods: Serial dilution of virulent bacteria was injected in to mice which were immunized with HS vaccine which was obtained from seed bacteria before and after back passaged in calf. Ratio of survived and dead was calculated by Reed-Meunch hypothesis and the LD_{50} value for each vaccine trial groups were calculated.

Results: The immunological study revealed that vaccine prepared from back passaged seed culture showed greater improvement in its immunopotency than seed vaccine (before back passage). Around 200 mice were used to study the immuno efficiency of vaccine. Each mouse was from the same source, which were free from the *Pastuerella* infection previous to expose to trial infection. The same broth culture of HS was used to induce infection in mice in both trials (vaccine before back passage and vaccine after back passage). The 0.2 mL of broth dilution from 10^{-1} to 10^{-10} was used, as dilution increases, death rate decreases. It indicates the minimum load of bacterium is required to induced infection.

Conclusions: Obtained results revealed that back passaged vaccine seed HS bacteria in its natural host had provided better immune efficiency to the culture than laboratory stock culture, and this findings recommended that regular annual back passage was mandatory for the vaccine seed culture of *Pastuerella multocida* bacteria for better establishment of immune potent vaccines.

KEYWORDS

Hemorrhagic septicemia, Laboratory animals, Natural host, LD_{50} values, Annual vaccination

1. Introduction

Heamorrhagic septicemia (HS) is an acute infectious disease of cattle, buffaloes, sheep and goats, caused by *Pasteurella multocida* serotype B (*P. multocida*). The disease occurs mainly during rainy season particularly in early monsoon. It spreads rapidly among herds of animals, causing morbidity and mortality between 50% to 100%. Immunization against infectious agent is the only available tool in prevention and control of the disease. Multivalent vaccines were used annually in the endemic area, in spite of annual vaccination the outbreaks of disease were recorded every year. This may be due to improper vaccination and low potent vaccine usage. Keeping potency of vaccine as target this experiment was designed to check the potency of vaccine seed culture of HS before and after back passaged in calf. Enhancement in potency of back passaged vaccine seed culture was determined in detail in this experimental study.

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2. Materials and methods

2.1. The preperation of vaccine strain of P. multocida (strain-P52)

The seed vaccine strain of *P. multocida* (strain-P52) was received from Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, the Drug/Vaccine Control Division of India for the purpose of vaccine production in Biological Production Division, Institute of Animal Health and Veterinary Biologicals, KVAFSU, Hebbal, Bangaluru. In this study, back passaging was done by injecting 1 mL of neat bacterial culture (vaccine strain) into calf (natural host). At 30 h post-injection blood was collected aseptically from jugular vein for preparation of seed bacterial culture for vaccine production. The vaccine prepared from before and after passage seed culture were checked for immune potency using laboratory mice.

2.2. Studies on the potency test of HS vaccine

2.2.1. Potency test of HS batch No: 5/08-09 vaccine (seed culture before back passage)

Potency test of HS batch No: 5/08-09 vaccine (seed culture before back passage) was inoculation to 50 mice, each with 0.2 mL dose by I/M on right thigh. After two weeks booster dose was administered. Tweenty one days after the vaccination, mice were divided into 10 groups of 5 mice each. Simultaneously unvaccinated 50 mice were divided into 10 groups of 5 mice each to serve as control. *P. multocida* P-52 strain bacteriological stock culture was tested for pathogenicity and virulence in mice. The stock culture was sub cultured in nutrient broth and inoculated for 8 h at 37 °C. The incubated culture was again checked for purity by smearing with grown strain smear and was found to be pure growth of P-52. The broth culture was serially diluted in log dilution in nutrient broth to get 10^{-1} to 10^{-10}. Theses culture dilutions were injected to group of 5 mice each of vaccinated and unvaccinated control mice. The groups were designated as A1 to A10 for vaccinated and B1 to B10 for unvaccinated control. These grouped mice were housed in different cages labeled appropriately. The inoculated mice were kept on observation for 5 d and the mortality ratio was recorded daily in both the groups in the morning and evening. Results were tabulated and interpreted as for standard procedure (Reed and Muench hypothesis).

2.2.2. Potency test of HS batch No: 8/08-09 vaccine (seed culture after back passage)

The same experimental procedure was repeated for the vaccine which was prepared from the back passaged culture. In this vaccine potency test the same number of mice were used and the mice are from the same source. Other experimental conditions were maintained the same as above. The inoculated mice were kept on observation for 5 d and the mortality ratio was recorded daily in both the groups in the morning and evening. Results were tabulated and interpreted as for standard procedure (Reed and Muench hypothesis).

3. Results

The experimental induction of HS disease was done with the broth culture of *P. multocida* bacteria and its purity and specificity were checked by growing it on specific selective agar medium. The overnight culture gave clear turbidity with smearing from it. And Giemsa stain indicated the presence of bipolar bacilli.

In each trials proportional distance and LD_{50} values were calculated by using below formula:

$$\text{Proportional distance for vaccinated mice} = \frac{\text{Mortality above 50\%} - 50}{\text{Mortality above 50\%} - \text{Mortality below 50\%}}$$

-Log of LD_{50} for vaccinated mice=-Log dilution above 50%+Proportionate distance

3.1. Studies of potency test of HS vaccine(P-52), before passage in calf (natural host)

Potency test result of HS seed vaccine strain (batch No: 5/08-09) (vaccinated group) (Table 1). The Proportional distance for vaccinated mice was 0.3, -Log of LD_{50} for vaccinated mice was 3.3 and LD_{50} value was $10^{-3.3}$.

Potency test result of HS seed vaccine strain (batch No: 5/08-09) in the unvaccinated control group was shown in Table 2. And the calculated proportional distance for vaccinated mice was 0.77, -Log of LD_{50} for vaccinated mice was 9.77 and LD_{50} value was $10^{-9.77}$.

The difference in LD_{50} between vaccinated and control was $10^{-6.47}$ ($10^{-9.77}$-$10^{-3.3}$) and the minimun is Log 4. The LD_{50} value indicated that HS vaccine was potent.

3.2. Potency test result of HS seed vaccine strain after back passaged in calf (natural host)

In the potency test result of HS back passaged vaccine seed strain (batch No: 8/08-09) (vaccinated group). The proportional distance for vaccinated mice was 0.2, -Log of LD_{50} for vaccinated mice was 3.2, LD_{50} value was $10^{-3.2}$.

For the Potency test result of HS seed vaccine strain (batch No: 8/08-09) (unvaccinated control group), the proportional distance for vaccinated mice was 0.77, -Log of LD_{50} for vaccinated mice was 9.77, LD_{50} value was $10^{-9.77}$.

The difference in LD_{50} between vaccinated and control was $10^{-6.57}$ ($10^{-9.77}$-$10^{-3.2}$) and the minimum is Log 4. The LD_{50} value indicateed that HS vaccine was potent.