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

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PEER REVIEW

Peer reviewer

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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}.
Details on Page S267

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.

Table 1

Studies of potency test of HS vaccine (P-52), before passage in calf (natural host) from June 12 to June 18, 2008.

Vaccinated group	Culture dilute	Mortality	Dead	Survive	Accumulated value		Mortality ratio	Percentage of mortality
					Dead	Survive		
A1	10^{-1}	3/5	3	2	16	2	16/18	88.88
A2	10^{-2}	4/5	4	1	13	3	13/16	81.25
A3	10^{-3}	3/5	3	2	9	5	9/14	64.24
A4	10^{-4}	4/5	4	1	6	6	6/12	50.00
A5	10^{-5}	2/5	2	3	2	9	2/11	18.18
A6	10^{-6}	0/5	0	5	0	14	0/14	00
A7	10^{-7}	0/5	0	5	0	19	0/19	00
A8	10^{-8}	0/5	0	5	0	24	0/24	00
A9	10^{-9}	0/5	0	5	0	29	0/29	00
A10	10^{-10}	0/5	0	5	0	34	0/34	00

Table 2

Potency test result of HS seed vaccine strain (batch No: 5/08) (Unvaccinated control group) from June 12 to June 18, 2008.

Vaccinated group	Culture dilute	Mortality	Dead	Survive	Accumulated value		Mortality ratio	Percentage of mortality
					Dead	Survive		
B1	10^{-1}	5/5	5	0	42	0	42/42	100
B2	10^{-2}	5/5	5	0	37	0	37/37	100
B3	10^{-3}	5/5	5	0	32	0	32/32	100
B4	10^{-4}	5/5	5	0	27	0	27/27	100
B5	10^{-5}	5/5	5	0	22	0	22/22	100
B6	10^{-6}	5/5	5	0	17	0	17/17	100
B7	10^{-7}	5/5	5	0	16	0	16/16	100
B8	10^{-8}	5/5	5	0	11	0	11/11	100
B9	10^{-9}	3/5	3	2	6	2	6/8	75
B10	10^{-10}	3/5	3	2	3	4	3/7	42.85

4. Discussion

The immunological study was conducted using laboratory mice to assess the immunopotency of HS vaccine (seed bacteria). Around 200 mice used in the study were from single source belonging to the same colony and were free from the *Pasteurella* infection by blood smear examination for bipolar organisms, previous to exposure to trial infection. The culture of hemorrhagic septicemia was used to immunized the mice in both trials (vaccine before back passage and vaccine after back passage). The virulent bacterium which was used to induce the infection was available in the form of broth culture. A volume of 0.2 mL of broth dilution from 10^{-1} to 10^{-10} was injected into each mice, as dilution increased death rate decreased which indicated the minimum load of bacterium was required to induce infection[1,2].

Results obtained indicated that potency test conducted for HS vaccine (strain-P52) has shown the increased immunity in the vaccinated animals with passaged vaccine (vaccine produced by the back passaged bacterial strain) than immunity imparted by unpassaged seed vaccine. Passaged strain had better efficiency because of the change in the membrane proteins. When bacteria is in the natural host it is under the iron restricted condition, *P. multocida* often expresses high molecular weight. Outer membrane proteins called iron regulated outer membrane proteins (IROMP) may be involved in induction of immunity and used as immunogen to produced subunit vaccines[3]. This indicated that back passaging in the natural host may help the bacterial to regain its potential to boost immune stimulation. This finding also supports that bacteria had changed its virulence and immunogenicity when it was grown out of its natural host system and it may become less immune competent in induction of antibody[2]. Two groups of seven animals were vaccinated with haemorrhagic septicaemia vaccine and black quarter vaccine. Pre and post vaccinated sera samples of cattle were collected and tested using Fourier transform infra red spectrometer. The variation observed in peaks indicating the change in protein and lipids levels in the animals was due to introduce of antigens[4]. Our findings also suggested that immune efficiency of vaccine strain may decrease when it was passaged repeatedly in laboratory condition (*in vitro*).

Immunological studies of HS vaccine (potency test) indicated that all mice were showing the immune response according to the level of antibody titre in them. Propotional distance value for the vaccine (before back passage) in experimental group is 0.30 and 0.20 for back passaged vaccine and proportional distance value for control group in both the trials is 0.77. The

difference between proportional distance value for before and after passaged vaccine was only 0.1 but in terms of log values of LD_{50} is $10^{-3.3}$ for before back passaged vaccine and $10^{-6.57}$ for back passaged vaccine. This LD_{50} log values had provided the huge difference in the immune potency of two vaccines used. After challenge studies using virulent HS strain, the obtained logarithmic (LD_{50}) values clearly indicated that back passaged vaccine was more potent than earlier seed vaccine which could be due to the changes in the antigen structures of bacteria. As a principle of attenuation when you grow any bacteria outside its host range for multiple generations it may reduce its virulence and may also altered in its immune efficiency. Such bacteria when you put back into its natural host, it suddenly regains all its virulence and immune efficiency.

The protective efficiency of purified protein (outer membrane proteins) fractions of *P. multocida* serotype B:2 (P-52) strain were studied in mice[5]. Highest ELISA Log10 titre was observed in mice injected whole outer membrane proteins where as mice injected with purified protein fractions showed low levels of ELISA Log10 titres. It revealed that whole organism was required in its pure form to induce high level of antibody titre in the infected host. This study also indicated that under iron restricted conditions, *P. multocida* often expresses high molecular weight outer membrane proteins called IROMP which may be involved in inducing the cross protective immunity[3,6] and when grown in the natural host *P. multocida* also expresses 18 Kda fimbrial subunit protein which processes potential property and could be potential immunogen in inducing protective immunity[7,8]. These fimbrial protein which are expressing in the natural host well than in other unnatural host could be the reason to change in the immunostimulation by the vaccine seed before and after back passage into its natural host system. These fimbrial proteins and IROMP may be changed when seed bacterial culture was put in to natural host from the test tube. These altered protein might be the reason for increased immune potency of back passaged vaccine. Biofilm HS vaccine which was used in claves gave satisfactory antibody titre after the third booster dose[9]. It might be due to lack of immune potency inducible dosage in a second dose of vaccine. Although several vaccine formulations including alum precipitated, oil adjuvant and multiple emulsion vaccines are commercially available, the quest for suitable broadly protective HS vaccines with long-lasting immunity is on the upsurge[10].

Current finding indicated that back passage might be useful to *in vitro* adopted bacteria in getting membrane proteins with altered motifs and even chances of mutation such as single nucleotide polymorphism and definitely there is increased fimbrial activity in natural host than *in vitro*. These hypothetical queries has to be addressed by analyzing molecular level changes in bacteria by genomic study of bacterial seed used for vaccine production[11]. Information available on HS vaccines developed from time to time using whole bacteria or their components. Kinetics and isotype of antibody and cell-mediated immune responses have also been poorly understood so far. Hence information on their role in protection against HS is reviewed[12]. However, detailed laboratory mice experiment indicated that vaccine which was produced by back passaged bacteria strain had better and improved immune potency than unpassaged *in vitro* culture.

HS is one of the most important bacterial diseases of cattle and buffalo in India. The disease occurs mainly during the rainy season. It spreads rapidly among groups of animals, causing morbidity and mortality between 50 to 100%. Vaccination is the only available preventive measure for the control of disease, so regular annual vaccination is recommended in the endemic areas. The vaccine produced by the seed vaccine strain may lose its potential or may decrease in its immune potential to stimulate high antibody titre in the vaccinated animal because of repeated passaging in the unnatural host and stressful laboratory conditions (growth factors and environmental conditions). When we back passaged the vaccine seed culture in its natural host, it improves the immune potency of bacterial culture. The molecular and genomic level changes are needed to be resolved by proper genomic analysis and analysis of genomic level mutations which could reveal the reason for improvement of immune potency. So this study strongly recommend back passaging of the seed vaccine strain at least once in six to eight months in its natural host system for the induction of better immunity in the vaccinated animals.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

HS is an acute, fatal septicemic disease of cattle and buffaloes caused by *P. multocida*. Effective vaccination is a key tool in prevention and control of the disease. Although several vaccine formulations including alum precipitated, oil adjuvant and multiple emulsion vaccines are commercially available, the quest for broadly protective HS vaccines with long-term immunity is on the upsurge.

Research frontiers

The authors compared the seed culture of 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 median lethal dose (LD_{50}). The LD_{50} was estimated by the arithmetical method of Reed and Muench (1938).

Related reports

P-52 strain is used for HS vaccination in India. P-52 is a highly virulent field strain isolated from a disease outbreak. Formalin inactivated whole organism adjuvanted with alum or oil is used for control of HS. The vaccine has the disadvantage of providing short-term immunity and requiring repeated administration. Live vaccines have been used in some area. However, there are problems in low protection, high virulence and unsafe for primary vaccination in young calves. Potency tests in mice are more feasible than other methods in testing vaccine efficiency. LD_{50} determination is calculated by the arithmetical method of Reed and Muench (1938).

Innovations and breakthroughs

In 2008, "OIE, the Manual of Standards for Diagnostic Tests and Vaccines" stated the standard procedures of HS vaccine. A calf was infected with the bacteria cultured on casein/sucrose/yeast blood agar, and the blood of the infected calf was collected. A fresh aliquot of the infected blood was used for each new batch of vaccine. The manuscript confirms that the procedure is necessary for inducing better immunity in the vaccinated animals.

Applications

The authors recommend for back passage of the seed vaccine strain annually or at 6-8 month intervals in its natural hosts for better immune efficiency.

Peer review

This is a useful study. The authors compared the seed culture of 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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