Improvement of Haemorrhagic Septicaemia Vaccine by Removing of Anaphylactic Agents

Short Communication

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Summary

The anaphylactic reactions in cattle and buffaloes following haemorrhagic septicaemia vaccination in different parts of Iran were reported. The free endotoxin as an allergic substance is a component of liquid phase of the vaccine. A dense culture of bovine *Pasteurella multocida* type B:2 was prepared in triptose phosphate broth supplemented by yeast extract. The bacterial cells were separated by centrifugation and alum gel precipitation methods. The safety trials of the vaccines conducted in mice, rabbits, guinea pigs, cattle and buffalos. The shock reaction did not occur either in laboratory or farm animals, which were tested in both controlled and field conditions.

Key words: haemorrhagic septicaemia, *Psteurella multocida*, vaccine, anaphylactic shock

Introduction

Haemorrhagic septicaemia (HS) is an acute, highly fatal, septicaemic disease principally affecting cattle and buffaloes. It is a primary pasteurellosis, which caused by two specific serotypes of *Pasteurella multocida*. The Asian and African sereotypes are designated B:2 and E:2, respectively (De Alwis 1993). The disease is distinctly different from some other pasteurellosis where pasteurella plays only a secondary role in cattle and buffaloes.

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In all countries where HS occurs, vaccination as a deliberate attempt to protect animals against diseases is adopted. Several types of vaccines including a plain bacterin whole, alum precipitated and aluminum hydroxide gel, oil adjuvant, multiple emulsion and live vaccines are used (Verma & Jaiswal 1998). In Iran HS occurs in humid and marshy area particularly in the North, along the coast of Caspian Sea, in the Northwest, near to the lake of Urmia and also in the Southwest towards the basin of the Karoun river in Khuzestan province. It has never been recorded on the plateau where the soil is usually very dry (Baharsefat & Firuzi 1977, Kaveh *et al* 1960).

An aluminum hydroxide gel vaccine is currently used for prevention of the HS in Iran. The vaccine is prepared from a local isolate of *P.multocida* serotype B:2 in fermentor (Razi Institute, Karaj). Although the potency of the vaccine has been claimed satisfied by the manufacturer, a postvaccination shock that considerably high up to 12% of vaccinated animal population occurs (Vesal & Maleki 2000, Moazeni Jula 2001). As it was shown in other researches that free endotoxin is the main anaphylactic agent in such kind of vaccine (Bain 1963, Wray & Thomlinson 1972a, Morrison & rayan 1987), the main objects of the present study were: 1) to separate the pasteurella cells from broth media (liquid phase) in *P.multocida* culture by precipitation and centrifugation, and 2) to evaluate the safety of improved vaccines in laboratory and field animals.

Materials and Methods

Laboratory Animals. Forty healthy male Balb/c mice, eight white male New Zealand rabbits and eight guinea pigs (Specific Pathogen Free Animals Research & Production Dept., Razi Ins., Karaj) were housed under clean homing conditions. All animals were fed *ad libitum*.

Preparation of dense culture. The *P.multocida* serotype B:2, that has been isolated from previous outbreaks of HS in Iran was used for production of dense culture. A

freeze dried ampoule of the bacterium was resuspended in 300ml triptose phosphate broth (TPB) and incubated at 37°C for 18h. This broth was subcultured into 8 liter TPB containing 2% yeast extract and incubated at 37°C for 6h. Formalin was added to the culture to make a final concentration of 0.4%.

Separation of whole cell by centrifugation and alum precipitation. The whole cell of *P.multocida* was separated by centrifugation and alum precipitation methods. The bacterial suspension was centrifuged in 5000rpm for 20min. The supernatant was discarded and the pellet was resuspended in a proper volume of 0.9% sodium chloride solution to make a concentration of 3×10^9CFU/ml *P.multocida*. Absorbencies were read at 540nm with spectrophotometer. A solution of 10% aluminum hydroxide gel was added to the bacterial suspension and mixed well. The prepared vaccine labeled as centrifuged aluminum hydroxide adjuvanted vaccine (CAHAV) and kept at 4°C until use. *P.multocida* (whole cell) was precipitated by adding a 10% alum solution to the 6h incubated dense culture for preparation of modified alum precipitated vaccine (MAPV). The suspension left overnight at room temperature for alum precipitation of bacterial cells. After discarding the supernatant, the volume was adjusted to have a 3×10^9CFU/ml *P.multocida* by sterile normal saline. The prepared vaccine was kept at 4°C until use.

Sterility test. Four tubes of each nutrient broth, nutrient agar, blood agar, Sabouraud's dextrose agar and thioglycolate (anaerobic medium) were inoculated with 1ml of each vaccine. Two tubes of each medium were kept at room temperature, while the rest were incubated at 37°C for 3 days.

Safety trials in laboratory animals. For the safety test of vaccines, healthy mice, rabbits and guinea pigs were injected. Mice were received 0.5ml of the vaccines IP or SC. The guinea pigs and rabbits were inoculated SC with 2 and 3ml of the vaccines, respectively.

Safety trials in field animals. When the sterility and safety tests in cultures and laboratory animals were found satisfactory, ten cattle in Razi Institute animal barn

were injected SC with 5-10ml of each vaccine. This amount was 2-3times more than vaccine dose in cattle. The injected animals were observed at least 3h for any signs of anaphylactic reaction. Also the animals were examined weekly for any local reactions at the injection site up to 4 weeks. The safety test of the vaccines was carried out in the field conditions. A total of 1994 cattle and buffaloes were injected SC with usual vaccine doses of MAPV and CAHV. This trial was done with cooperation of Iranian Veterinary Organization and agreement of the clients in Shadegan, Ramhormoz and Shoshtar districts in Khuzestan province. After injection of the vaccines, animals were under direct vision at least 3h for occurrence of any shock reaction.

Results and Discussion

In the present study after separating the liquid phase of HS vaccine and reconstitution with normal saline, the safety of the vaccine in laboratory and farm animals was demonstrated. Tables 1 and 2 show the results of safety trials in laboratory and field animals. Neither anaphylactic shock reaction nor noticeable local inflammations were seen between the laboratory and field animals.

Table 1. *Results of safety test of two different HS vaccines in laboratory animals*

Vaccine	Animal	Route of injection	Dose (ml)	No. of survived / No. of tested
MAPV	Mouse	SC	0.5	10/10
	Mouse	IP	0.5	10/10
	Rabbit	SC	3	4/4
	Guinea pig	SC	2	4/4
CAHV	Mouse	SC	0.5	10/10
	Mouse	IP	0.5	10/10
	Rabbit	SC	3	4/4
	Guinea pig	SC	2	4/4

MAPV: Modified Alum Precipitated Vaccine; CAHV: Centrifuged Aluminum Hydroxide adjuvant Vaccine

Table 2. *Results of safety trials in cattle and buffaloes vaccinated with two different HS vaccines*

Vaccine	Animal	RVSRI Farm	Shadegan	Ramhormoz	Shoshtar	Shock Reaction	Total
MAPV	Cattle	10	136	232	197	Nil	575
	Buffalo	-	231	-	142		373
CAHV	Cattle	10	142	305	187	Nil	644
	Buffalo	-	248	-	154		402
Total		20	757	537	680		1994

RVSRI: Razi Vaccine & serum Research Institute

It is well known that LPS (endotoxin) of gram-negative bacteria could be able to produce anaphylactic reactions in tested animals. Wray and Thomlinson (1972b) induced sever anaphylactic reactions by injection of 50-100μg/kg of *E.coli* endotoxin in calves. The dosage necessary to produce clinical signs and death differed considerably in individual animals. In another study they (1972a) showed that the severity of the shock reactions in response to a given dose of endotoxin was related to the degree of previous sensitization. Saban *et al* (1997) showed that histamine and prostaglandin were released from bovine lung tissue following administration of pasteurella LPS.

Aluminum potassium sulfate (alum) is a gel that adsorbs the pasteurella cells, make a thinly net and settle them. Then the liquid phase of the culture that consists of unused nutrients, different components of lysed cells and free LPS could be easily separated and discarded. As well as, the alum gel acts as an adjuvant for improving the immunogenecity of the vaccine (Pastorel *et al* 1997, Verma & Jaiswal 1998, Bunn 1993). Shock reactions following administration of HS aluminum salts adjuvant vaccines have been previously reported (Bain 1962, Rhoades & Rimler 1989). There are several reports on anaphylactic shock occurrence following use of the currently aluminum hydroxide gel vaccines between the cattle and buffaloes in different parts (Fars, Khuzestan, West Azarbaijan, Mazandaran and Gilan provinces) of Iran (Iranian Veterinary Organization Reports, 2000). The occurrence of shock

ranged from 3 to 12% of vaccinated animals. Vesal and Maleky (2000) reported the anaphylactic reactions in 6.2% (n=191) of vaccinated cattle in the Shiraz University farm. The latest occurrence of shock reaction happened in November 2001 among 12.8% (n=140) of animals vaccinated in Ramhormoz farms, Khuzestan province (Moazeni Jula 2001).

It is not well clear that why some of the vaccinated animals react to the sensitizing substance but others do not. In the farm conditions it was noticeable that shock reaction happened in both first vaccinated and booster received animals. However, how the first group became sensitized to the vaccine allergen is not well understood. As a conception it can be imagined that exposure to LPS of other gram-negative bacteria may play the role of sensitizing agent for inducing the IgE production. These antibodies fix onto the surface of mast cells and basophiles. When the sensitized animal is vaccinated, the allergen (free LPS) induces release of the anaphylactic mediators from provoked cells (Morison & Rayan 1987). Then the infections induce by gram-negative bacteria such as collibacillosis, salmonellosis and pneumonic pasteurellosis should be considered important in predisposing the animals to anaphylactic reactions following receiving the HS vaccine. The improved vaccines prepared in this study were safe in cattle tested with two injections by 3 weeks interval and 2-3 times more than vaccine dose.

Although the shock reaction was removed by using the new developed HS vaccines, the investigations have to be continued to optimize a large-scale cultural conditions to reduce the free LPS production in the broth media.

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