Potency Testing of Modified Alum Adjuvant Haemorrhagic Septicaemia Vaccine in Laboratory and Farm Animals

Short Communication

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Summary

The potency of a modified alum precipitated haemorrhagic septicaemia vaccine in mice, rabbit and cattle was evaluated. Active mouse protection test showed that the log of protection was 7.8. Passive mouse protection test on sera obtained from vaccinated cattle showed 67-100% protection up to four months and 25-67% protection 2 months post vaccination. Modified alum precipitated vaccine was potent to produce immunity against active challenge with virulent *Pasteurella multocida* in rabbits. The antisera collected from vaccinated rabbits conferred 83-100% protection in serummized mice.

Key words: haemorrhagic septicaemia, modified alum precipitated vaccine, potency

Introduction

Haemorrhagic septicaemia (HS) is an acute septicaemic disease principally affecting cattle and buffaloes caused by *Pasteurella multocida* (*P.multocida*). The disease is widespread in almost all parts of the world except for Oceania and usually associated with wet and humid weather. HS increased incidence is recorded during wet seasons (De Alwis 1999). In Iran the disease has an enzoo-epizootic nature and has been responsible for some bovine mortality. It is endemic in Khozestan, Gilan, Mazandaran, west and east Azarbaiojan (Kaweh *et al* 1960, Baharsefat & Firouzi 1977). Vaccination is the accepted method for HS control throughout the world.

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Currently an aluminum hydroxide gel adjuvant vaccine is used for prevention of HS in Iran. An anaphylactic shock reaction following administration of this vaccine was reported (Vesal & Maleki 2000). In previous study, we showed a component of liquid phase of the vaccine caused the post vaccination shock (Jabbari & Moazeni Jula 2002). This vaccine was improved by precipitation of bacterial cells with alum and substitution of the liquid phase by sterile normal saline solution. Also the safety of this modified alum precipitated vaccine (MAPV) was evaluated. The aim of the present study was to evaluate the potency of MAPV in laboratory and farm animals.

Materials and Methods

Vaccine preparation. The modified alum precipitated vaccine was prepared as explained previously (Jabbari & Moazeni Jula 2002). Briefly a dens culture (4.5×10^9CFU/ml) of *P.multocida* serotype B2 (local isolate) was prepared in triptose phosphate broth. The bacterial cell was precipitated by adding a 10% alum solution. The supernatant was discarded and replaced by sterile normal saline. The prepared vaccine was kept at 4°C until use.

Active mice protection test (AMPT). Fifty Balb/c mice were randomly divided into ten groups each of 5 that were intramuscularly inoculated with 0.2ml MAPV per mouse. Ten days after second injection, the groups of mice were challenged by ten-fold dilutions of 6h culture of *P.multocida* in sterile PBS (pH7.2). Challenge exposure consisted of intrapretonealy inoculation of 0.1ml of each dilution into one group of treated mice. They were observed daily for 7 days and mortality was recorded. The Median Lethal Dose (LD50) was calculated by the method of Ose and Muenster (1966).

Immunity trials in cattle. Ten cattle nearly one year old were intramuscularly injected with usual dose (3ml) of MAPV. Blood samples were taken before and up to 6 months post-vaccination. Sera were collected after coagulation and overnight keeping at 4°C for use in passive mice protection test (PMPT). PMPT was done with

0.5ml sera, which obtained from each cattle as explained above. Also one group of 5 mice was injected with a pooled sera composed of equal quantitative of each sample. The treated mice along with control group were challenged with 100LD50 of freshly *P.multocida* type B2. The mice were observed daily for 7 days and mortality if any, was recorded. Mice that died during the observation period were examined for presence of bipolar organisms in the blood smears and *P.multocida* in blood agar culture.

Immunity trials in rabbits. Ten healthy local reared rabbits weighing average 1.5kg were inoculated with 1ml of MAVP by intramuscularly route. The inoculation was repeated two weeks later. The treated rabbits along with two uninjected control rabbits were bled 3 weeks after the second injection. Two days after bleeding, each rabbit challenged by 1500CFU of virulent *P.multocida*. Sera were collected and evaluated in PMPT as explained above.

Results and Discussion

In AMPT difference of log units between LD50 of vaccinated and unvaccinated mice was 7.8. Dilutions of 10^1, 10^3 and 10^4 were shown 20%, 40%, and 20% mortality in vaccinated groups respectively. 100% mortality was shown in control groups except dilutions of 10^9 (60%) and 10^{10}(0%). According to this method a difference of at least 4 log units between the LD50 for vaccinated and unvaccinated mice is essential as the potency requirements of the vaccine (De Alwis 1992). In the present study, the MAPV produced a difference of 7.8 log units between the LD50 of immunized and control groups of mice, which is clearly above the minimum requirement for potency of the vaccine. The test was found a relatively inexpensive and easily reproducible method for evaluating alum precipitated vaccine.

Results of PMPT on sera collected from vaccinated cattle up to 6 months post-vaccination is given in table 1. All of the sera obtained before vaccination were negative. The sera which was obtained up to 4 month post-vaccination, showed 67-

100% protection in groups of serummized mice. The PMPT revealed 50-67% and 25-60% protection on sera obtained in fifth and sixth months post-vaccination respectively.

Table 1. *Results of PMPT on sera obtained from cattle immunized with MAPV up to 6 months*

No. Cattle	Months post-vaccination											
	First		Second		third		fourth		fifth		Sixth	
	S/C	P (%)	S/C	P (%)	S/C	P (%)	S/C	P (%)	S/C	P (%)	S/C	P (%)
1	5/5	100	5/5	100	6/6	100	5/6	83	4/6	67	2/5	40
2	5/5	100	5/5	100	5/6	83	5/6	83	3/6	50	2/5	40
3	5/5	100	5/5	100	6/5	100	6/6	100	3/6	50	1/4	25
4	5/5	100	5/5	100	5/6	83	5/6	83	4/6	67	2/5	40
5	5/5	100	5/5	100	6/6	100	6/6	100	4/6	67	3/5	60
6	5/5	100	5/5	100	6/6	100	4/6	67	4/6	67	2/5	40
7	5/5	100	5/5	100	5/6	83	4/6	67	3/5	60	2/5	40
8	5/5	100	4/4	100	6/6	100	4/6	67	3/6	50	2/5	40
9	5/5	100	5/5	100	5/6	83	5/6	83	3/5	60	2/6	33
10	5/5	100	4/4	100	6/6	100	6/6	100	3/6	50	2/6	33
Pooled 1	0/5	0	0/5	0	0/6	0	0/6	0	0/6	0	0/5	0
Pooled 2	5/5	100	5/5	100	5/6	83	5/6	83	3/6	50	2/5	40

S/C: Number of mice survived/number of challenged. P (%): percent of protection. Pooled 1: The pre-vaccination pooled sera from ten cattle. Pooled 2: The pooled sera from ten vaccinated cattle

Results of PMPT and direct challenge in rabbit are shown in table 2. All rabbits (n=10) vaccinated with MAPV survived challenge but both of unvaccinated ones died. The sera obtained from immunized and control rabbits before challenge were used in PMPT. 85-100% of mice immunized by sera from vaccinated rabbits survived challenge, whereas all of mice inoculated with control sera (unvaccinated rabbits) died.

It is generally agreed that direct challenge of immunized host animals is not feasible in potency testing of HS vaccine (Bain *et al* 1982, Jaiswal *et al* 1982, OIE Manual 2000). On the other hand, the PMPT has been known as a very satisfactory

test for immunity either in vaccinated or naturally immune ruminant (De Alwis 1992, Bain *et al* 1982). Vaccination of host animals elicits humoral response and the presence of circulating antibody in vaccinated animals correlated with immunity. Inoculation of immune sera from vaccinated animals protects the mice against challenge with virulent *P.multocida* (De Awis 1999, Rimler & Rhoades 1989). According to results of this study, MAPV produced a satisfactory immunity up to 4 months post-vaccination. However, the sera collected during 4 to 6 months post-vaccination showed an acceptable protection in mice.

Table 2. *Results of potency test of MAPV by PMPT and direct challenge with virulent P.multocida in rabbit*

Rabbit study code	Direct challenge by 1500CFU/ rabbit	PMPT	
		S/C	P(%)
VR-1	Survived	4/4	100
VR-2	Survived	4/4	100
VR-3	Survived	5/5	100
VR-4	Survived	5/6	83
VR-5	Survived	4/4	100
VR-6	Survived	3/3	100
VR-7	Survived	4/4	100
VR-8	Survived	5/5	100
VR- 9	Survived	4/5	80
VR-10	Survived	5/5	100
Pooled 1	-	5/6	83
Pooled 2	-	0/6	0
UR-1	Died	0/4	0
UR-2	Died	0/5	0

Pooled 1: The pooled sera from ten vaccinated rabbits, Pooled 2: The pre-vaccination pooled sera from ten rabbits

Previous works showed that survival of even one mouse of a group of five (20%) indicated the protection in host animal (De Alwis 1999, Bain *et al* 1982). According to our finding, since the immunity conferred by the vaccine lasts for a period of

about 6 months, vaccination of all suspected animals in the HS endemic areas before rainy seasons (2 times per year) is recommended. This vaccination program produces sufficient immunity in the animals to withstand the disease in one year. As a potency testing procedure, active immunization and challenge tests as well as PMPT on sera obtained from vaccinated rabbits have been used previously (Jaiswal *et al*, 1983). In this study, MAPV was enough potent to produce immunity against direct challenge and passive mouse protection test in rabbits.

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