Study on Immunity of an Experimental Oil Adjuvant Haemorrhagic Septicaemia Vaccine in Cattle

Short Communication

Sotoodehnia*, A., Moazeni, G., Ataei, S., Omidi, B.
Aerobic Bacterial Vaccines Dept., Razi Vaccine & Serum Research Institute,
P.O.Box 11365-1558, Tehran, Iran
Received 24 Aug 2004; accepted 9 Feb 2005

Summary
An oil adjuvant vaccine (OAV) was prepared from a local strain of *Pasteurella multocida.* Strain 6:B was cultured and inactivated by formalin. Bacterial pellet was prepared by centrifugation and subsequently adjuvanted by Montannide oil ISA-70. A dose of prepared vaccine containing 3ml (2mg dry weight/ml) was injected into five calves by IM route. Animals were bled before and at 24, 90, 150, and 200 days post-vaccination. Collected sera were used in passive mouse protection test (PMPT). Active mouse protection test (AMPT) was carried out for OAV according to standard method. Results of PMPT showed 100% protection up to 150 days and 66-83% up to 200 days post-vaccination. In AMPT, 4 log of protection was gained. In this experiment the immunity induced by OAV adjuvanted by ISA-70 could protect the calves.

key words: haemorrhagic septicaemia, oil adjuvant vaccine, protection

Introduction

Haemorrhagic septicaemia (HS) is a primary pasteurellosis in cattle and buffaloes caused by *Pasteurella multocida (P.multocida)* serotypes 6:B and 6:E. Buffaloes are generally more susceptible than cattle, and young animals are more prone to the disease than adults(De Alwis *et al* 1981). The surface structure of *P multocida*, typical of a gram-negative bacterium, consists of an outer membrane made up proteins and lipopolysaccharide (LPS) surrounded by a capsular layer. Any of the

* Author for correspondence. E-mail:sotoodeh347@yahoo.com

component macromolecules of these structures could have the potential to confer protection (De Alwis 1999). An oil adjuvant vaccine for HS was first developed in the 1950s (Bain & Jones 1955). It consists of a water-in-oil emulsion, where the aqueous phase consists of a dense broth culture and the oil phase a light mineral oil. Various mineral oils have been used and some commercial products that have been used with success are Ondina 17(Shell) and Marcol 52 (Esso). The most economical emulsifying agent to use is probably purified anhydrous lanoline.

In all countries where HS occurs vaccination with local strains is adopted as the method of control. There are four different types of vaccines used against HS: broth bacterins; alum precipitated vaccine; aluminium hydroxide gel vaccine; and oil adjuvant vaccine (OAV) (De Alwis 1999). Bacterins are the simplest form of vaccine and consist of whole cells inactivated cultures.Antibody response to plain bacterin is poor and only provides rapid immunity for about six weeks. Alum precipitated vaccine is the most widely used vaccine in Asia and Africa. The disadvantages of this type of vaccine are that it only provides reliable immunity for three to four months and shock reactions can also occur. Aluminium hydroxide gel vaccine shares common properties with the alum precipitated vaccine and reliable immunity is almost similar to the protection obtained with Alum precipitated vaccine. OAV provides adequate immunity for 6-9 months if given at 4-6 months of age and repeated 3-6 months later, followed by annual revaccinations thereafter (OIE Manual 2000). In Iran HS is endemic in some parts of north, north-west, and south west of the country (Kaveh *et al* 1960). The disease is routinely prevented by aluminium hydroxide gel vaccine (Sotoodehnia *et al* 2000). In recent years, this vaccine was modified in a way that bacterial supernatant was discarded and replaced by saline (Jabbari & Moazeni 2002). Shock reaction was reduced and the modified vaccine is currently used throughout the country. In this study, an oil adjuvant vaccine was prepared against HS with a commercial mineral oil (ISA-70) and immunity conferred by the experimental vaccine was evaluated in cattle.

Materials and Methods

Vaccine production. *P.multocida* 6:B strain was used for vaccine production. Large scale bacterial culture was grown in fermentor containing triptose phosphate broth supplemented with yeast extract (Sotoodehnia *et al* 2000) at 37ºC and inactivated by 0.4% formalin. Bacterial cells were harvested by centrifugation at 5000rpm for 20min. The supernatant was discarded and the bacterial pellet, after three times washing, was finally resuspended in 20ml of saline. Final pellet was dried at 65ºC and bacterial dry weight was calculated 40mg/ml. OAV was emulsified after adding 0.5ml bacterial concentration to 2.5ml saline and 7ml Montanide ISA 70 at 16000rpm for 3min using drill 10G in mix emulsifier. It was estimated that one ml of the prepared vaccine has 2mg of dry bacteria.

Passive mouse protection test (PMPT). 5 calves of 7 months age were vaccinated with the experimental OAV. Each calf was received a dose of 3ml by IM route. One calf was also kept as unvaccinated control. The calves were bled prior to vaccination, and at 24, 90, 150, and 200 days post-vaccination. Sera were collected and subjected to PMPT according to the method of (Bain *et al* 1982). Mice in groups of 5-6 each were given 0.5ml of the calf sera by SC route. 24h later they were challenged with $100LD_{50}$ of viable *P.multocida* serotype 6:B by IP route. Groups of mice that received 24 and 90 days post-vaccine immune sera were also challenged with additional dose of 10^3LD_{50}.

Active mouse protection test (AMPT). AMPT was carried out using the method of Ose and Muenster (1968). Briefly, six groups of mice each of 6 were inoculated twice with 0.2ml OAV by SC route in two weeks interval. Control groups of mice were given two doses of 0.2ml oil adjuvant without bacteria. One week after the second inoculation, all groups of mice were challenged with 10^1-10^6LD_{50} of 6:B virulent strain (LD_{50}=3CFU).

Field trial. 500 calves more than three months old including light pregnant in three calf breeding centers in Tabriz, the Eeastern Azarbaijan province were selected and received 2-3ml of the OAV by IM route. Animals were observed for any side effects such as shock reaction and inflammation due to vaccination.

Results and Discussion

One ml of the vaccine had 2mg of dry bacteria. It has been reported that at least 1.5-2.0mg of dry whole bacteria should be incorporated in a dose of vaccine (De Alwis 1999). To achieve this standard, dense cultures are produced by using enriched media and aeration techniques such as vortex tanks or modern fermentors.

Results of PMPT showed that a 100% protection in groups of mice or cattle (table1) up to 150 days post-vaccination. Immunity level was declined between 66.6-83.3% at 200 days post-vaccination. The PMPT results evaluated by different workers were vary based on number of mice used, volume of serum and etc (Bain *et al* 1982, De Alwis *et al* 1978, Gomis *et al* 1989, Johnson *et al* 1993, Natalia *et al* 1993).

Table 1. ***Results of PMPT on sera of cattle immunized with ISA-70 oil adjuvant vaccine***

Cattle No.	Days post-vaccination							
	24		90		150		200	
	S/C	P (%)	S/C	P (%)	S/C	P (%)	S/C	P (%)
1	**5/5**	**100**	**6/6**	**100**	**5/5**	**100**	**5/6**	**83.3**
2	**5/5**	**100**	**6/6**	**100**	**5/5**	**100**	**5/6**	**83.3**
3	**5/5**	**100**	**6/6**	**100**	**5/5**	**100**	**4/6**	**66.6**
4	**5/5**	**100**	**6/6**	**100**	**5/5**	**100**	**5/6**	**83.3**
5	**5/5**	**100**	**6/6**	**100**	**5/5**	**100**	**4/6**	**66.6**
control	**0/5**	**0**	**0/6**	**0**	**0/5**	**0**	**0/6**	**0**

S/C: Number of survived/number of challenged, P (%): Percent of protection

Results of AMPT showed that all mice were protected against at least 10^4LD_{50} and less or 4 logarithmic units (Table 2). AMPT using the method of Ose and Muenster (1968) is a practical test for HS vaccines (Nagarajan *et al* 1972, Gupta & Sareen 1976, Chandrasekaran & Yeap 1978, Vipulasiri *et al* 1982). A well-prepared OAV should give 4-6 log units protection in mice. It was found that the AMPT alone was not a reliable index of cattle immunity and the vaccines containing lower antigen contents, which insufficiently protective for cattle gave

good results in mice. Thus the study of immunity of HS vaccines should be based on the results of both PMPT and AMPT.

Table2. ***Results of AMPT of oil adjuvant vaccine (ISA-70)***

Challenge dose(LD_{50})	Vaccinated mice		Control mice	
	S/C	P (%)	S/C	P (%)
10^1	6/6	100	0/4	0
10^2	6/6	100	0/4	0
10^3	6/6	100	0/4	0
10^4	6/6	100	0/4	0
10^5	5/6	83.3	0/4	0
10^6	4/6	66.6	0/4	0

S/C: Number of survived/number of challenged, P (%): Percent of protection

In the field, no shock reactions occurred in calves vaccinated with experimental OAV. Some cases of inflammation due to deep injection of the vaccine (10%) in the muscle were recorded. This adverse reaction was gradually disappeared within a month post-vaccination period. In present study, our findings showed that OAV prepared with Montanide ISA-70 gave a reliable immunity in vaccinated calves similar to study of Reddy *et al* (1996). In the study, cattle were immunized up to one year along with positive ELISA titers. In Vietnam, Montanide ISA-50, incorporating formalin-killed vaccines was evaluated in cattle and buffalo. This vaccine with a low viscosity did not produce local swelling (Phuong 1992).

References

Bain, R.V.S., Jones, R.F. (1955). Studies on haemorrhagic septicaemia of cattle. III production of adjuvant vaccine. *British Veterinary Journal* 111:30-34.

Bain, R.V.S., De Alwis, M.C.L., Carter, G.R. and Gupta, B.K. (1982). *Haemorrhagic septicaemia*. FAO Publication.

Chandrasekaran, S. and Yeap, P.C. (1978). Safety and potency testing of haemorrhagic septicaemia oil adjuvant vaccine by mouse protection test. *Kajian Veterinar* 10:28-34.

De Alwis, M.C.L., Gunatileke, A.A.P. and Wickremasinghe, W.A.T. (1978). Duration of immunity to haemorrhagic septicaemia in cattle and buffaloes following immunization with alum precipitated and oil adjuvant vaccines. *Ceylon Veterinary Journal* 26:35-41.

De Alwis, M.C.L. (1981). Mortality among cattle and buffaloes in Sri Lanka due to haemorrhagic septicaemia. *Tropical Animal Health and Production* 13:195-202.

De Alwis, M.C.L. (1999). Haemorrhagic septicaemia. *ACIAR Monograph* No.57.

Gomis, A.I.U., De Alwis, M.C.L., Radhakrishnan, S. and Thalagoda, S.A. (1989). Further studies on stability and potency of the haemorrhagic septicaemia oil adjuvant vaccine. *Sri Lanka Veterinary Journal* 36:9-20.

Gupta, B.K., Sareen, R.L. (1976). Evaluation of haemorrhagic septicaemia oil adjuvant vaccine by mouse protection test. *Indian Veterinary Journal* 53:489-492.

Jabbari, A.R., Moazeni Jula, G.R. (2002). Improvement of haemorrhagic septicaemia by removing of anaphylactic agents. *Archives of Razi Institute* 54:85-92.

Johnson, R.B., Castro, L.V., Sleep, J., Houge, M.M., Dawkins, H.J.S. and Spencer, T.L. (1993). The antibody response of rabbits and cattle to *pasteurella multocida*: Assessment of cross reactivity between serotypes. An international workshop held at Bali, Indonesia, 10-13 August 1992. *ACIAR Proceedings* No.43:159-164.

Kaveh, M., Sohrab, V. and Baharsefat, M. (1960). Bovine pasteurellosis in Iran-methods of vaccination. *Archives of Razi Institute* 12:99-105.

Nagarajan, V., Sunderam, S. and Ramani, K. (1972). Evaluation of the potency of haemorrhagic septicaemia alum precipitated vaccine by mouse protection test. *Indian Veterinary Journal* 49:1080-1083.

Natalia, L., Patten, B. and Syamsndin, A. (1993). Evaluation of bovine antibody responses to haemorragic septicaemia vaccine using ELISA and PMPT.An international workshop held at Bali, Indonesia, 10-13 August 1992. *ACIAR Proceedings* No.43,219-223.

OIE Standard Commission. (2000). *Manual of standard for diagnostic tests and vaccines*. Pp:446-456.OIE Publication.

Ose, E.E., Muenster, O.A. (1968). A method of evaluation of vaccines containing *Pasteurella multocida*. *Journal of Veterinary Research* 29:1863-1866.

Phuong, P.T. (1992). Haemorrhagic septicaemia in vietnam and its control. *ACIAR Proc.* No.43, Bali.

Reddy, G.S., Anand Raq, K. and Asrinivasan, V. (1996). Immunity conferred by oil-adjuvant haemorrhagic septicaemia vaccine. *Indian Journal of Animal Sciences* 66:703-704.

Sotoodehnia, A., Aarabi, I., Ataei, S., Naserirad, A. (2000). Preparation and standardization of haemorrhagic septicaemia gel adjuvant vaccine for calf and buffalo using *Pasteurella multocida* local strain6:B. *Pajouhesh & Sazandegi* 47:111-113 (in Persian).

Vipulasiri, A.A.,Wijewardana Thula, G. and De Alwis, M.C.L. (1982). A note on the effect of storage temperature and time on the potency of haemorrhagic septicaemia oil adjuvant vaccine. *Sri Lanka Veterinary Journal* 30:19-21.