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# *Immunization of Cattle and Buffaloes with a Combined Anthrax, Haemorrhagic septicaemin and Blackleg Vaccine in Iran.*

Aarabi, I., Sotoodehnia, A. Ardehali, M., Moosawi, M.

**Abstract:** A batch of combined vaccine composed of anthrax spore vaccine strain 34F2, haemorrhagic septicaemia (H.S) vaccine, strain Roberts type 1 of pasteurella multocida, and blackleg vaccine strain Clostridium chauvoei was prepared and tested in cattle. Vaccinated animals survived the challenge tests of virulent anthrax and Cl. chauvoei strains while the controls died due to infections. In the case of haemorrhagic septicaemia, development of antibody was measured by mouse protection test.

The combined vaccine effectively immunized the cattle, without serious side effects.

**Keywords:** *Iran / Vaccines / Cattle / Buffaloes / Combined vaccines/ Anthrax / Blackleg in cattle*

## Introduction

Haemorrhagic septicaemia, blackleg and anthrax are prevalent among cattle and buffaloes in Iran. These diseases have already been prevented by individual vaccines and subsequently by combined vaccines. The use of combined vaccines have been studied in some countries mostly with satisfactory results *(3,4,5)*.

In this study we attempted to make a triple combined vaccine and

standardize it for immunization of cattle and buffaloes, against haemorrhagic septicaemia, blackleg and anthrax.

## Materials and Methods

***VACCINE PRODUCTION:***

H.S and blackleg combined vaccine preparation was described earlier by Baharsefat et al 1976*(1)*. this vaccine was inactivated by 0.5 percent formaldehyde and incorporated with 0.2 percent saponin as adjuvant.

Anthrax live spore vaccine was prepared with 34F2 strain, according to W.H.O requirement*(6)* and definite quantity of this spore vaccine was mixed with the above H.S-blackleg combined vaccine (18 millions spore per vaccinal dose). Since the presence of formaldehyde in inactivated combined H.S-blackleg vaccine had deterious effect on anthrax live spores, it was neutralized by sodium meta-bisulfite ($Na_2S_2O_5$) in a concentration of 3 percent from a 50 percent W/V stock solution, Sotoodehnia et al 1988*(8)*, and anthrax spore vaccine was added afterwards.

*VACCINATION OF ANIMALS AND POTENCY TEST OF COMBINED VACCINE:*

***I - Mouse protection test of H.S vaccine.***

Twelve healthy calves about one-year-old were vaccinated with the above combined vaccine. No vaccine or medication had previously been used in these animals. 3 ml. vaccinal dose were inoculated S/C to each calf behind the shoulder. They received the same dose as booster a couple of weeks later. Two calves were also kept as unvaccinated control animals.

To ensure the lack of antibody against P.multocida, in calves' sera, blood was taken from all animals before vaccination. After vaccination the first bleeding was performed twelve days after the last vaccination and the bleeding was continued five more times, with about one or one and a half months time - intervals. Sera from two control calves were taken in each stage along with the vaccinated animals. Individual serum in each stage of bleeding, from either vaccinated or control calves was injected to seven healthy white mice weighing between 20-25 g.O.5 ml serum was inoculated S/C and challenge carried out next day by 18.hour old culture of P.multocida virulent strain. This culture was prediluted $10^{-7}$, then inoculated I/P to mice *(7)*. Challenge dose was adjusted to

contain approximately 260 P.multocida colonies by direct culture. The results of the mouse protection test in vaccinated and control groups are summarized in table 1.

***II - Rabbit vaccination test of H.S vaccine:***

Ten healthy rabbits were vaccinated with 1 ml. dose of combined vaccine and similar vaccinal dose was repeated a couple of weeks later. Twelve days after the last vaccination, all vaccinated rabbits along with three unvaccinated controls were challenged by a 22 hour-old culture of P.multocida virulent Roberts strain type 1. Culture was prediluted $10^{-7}$, and then 0.5 ml. from this culture was inoculated S/C to rabbits.

*POTENCY TEST OF ANTHRAX VACCINE:*

In order to evaluate the immunity of anthrax vaccine, eight guinea-pigs were vaccinated with 1 ml. of the combined vaccine. They were challenged three weeks post vaccination by anthrax virulent strain. 200 MLD of anthrax 17JB (pasteur No II) virulent strain which was inoculated to both vaccinated and five control guinea-pigs, contained 170000 live spores as a challenge dose.

*POTENCY TEST OF BLACKLEG VACCINE:*

Ten healthy guinea-pigs each weighing between 250-400 g. were injected subcutaneously with a quantity of 2ml. of the combined vaccine as the primary dose, followed four weeks later by a seconed injection of the same quantity of the vaccine as the secondary dose. After two weeks the vaccinated and two unvaccinated guinea-pigs were injected intramusculary with 4 and 1 MLD of 24 hrs. virulent culture of Cl. chauvoei respectively *(2)*.

## Results and Discussion

In mouse protection test, passive immunity was evaluated on the basis of the number of dead from the number of tested mice. Control mice in all stages of the test, died and pure P.multocida was isolated from the heart blood. They were all lacking antibody against P.multocida antigen. In immunized mice by calves' sera, the highest antibody level was found to develop at about one month post vaccination, then gradually disappeared up to six month later as, the end-point of this experiment.

All vacciinated guinea-pigs with combined vaccine, resisted the challenge

dose (4 MLD) of Cl.chauvoei virulent culture, while unvaccinated guinea-pigs which were injected with 1 MLD of the virulent culture died of typical lesions of Cl. chauvoei infection after 48 hrs.

Vaccinated rabbits survived the challenge but all controls died within 48 hrs. post challenge with systemic infection symptoms. pure P.multocida was isolated from heart blood.

Vaccinated guinea-pigs survived the challenge with anthrax strain 17JB., whereas all controls died within a period of five days post challenge. Anthrax virulent strain was isolated from dead animals.

Our conclusion indicated that combined vaccine could induce prote ctive antibodies against any of the incorporated antigens and no interaction seemed to occur in vaccine combination. In addition to this, safety, potency and induced immunity of the tested combined vaccine were found to be as much as the individual vaccines, which have already been confirmed in our laboratory.

## References


1 - *Baharsefat, M.Aarabi, I.Ardehali, M.Derakhshan, H.Hedayati, M.Mirkarimi, A.Immunization active des bovine par le vaccin associe antisepticemic hemorragique (pasteurellose) et anticharbon symptomatioque (quartier noirs) en Iran. Arch. Inst. Razi., (1976), 28,51-56.*

2 - *British pharmacopia (Veterinary) 1985,155 at 10 Appendix.*

3 - *Burdov, A.N. (1962)-simultaneous immunization of farm animals against anaerobic and aerobic infections, combined vaccine against anthrax, malignant oedema and blackleg. Trudy vsesoyuz. inst.eksp. Vet.26,139-144.*

4 - *Kadmov, R.A. (1975)-Combined immunization of sheep against anthrax, sheep pox and clostridial infection, Vet. Moscow, No.2, 50-52.*

5 - *Provost, A.and Perresu, P. (1978)-Combined vaccines for developing, J. Developments in Biological Standardization, 41,349-360.*

6 - *Requirement for anthrax spore vaccine (Live-for veterinary use) .W.H.O. Technical report series (1967), No.361.*

7 - *Roberts, R.S. (1947). J.Comp. Path,57:261.*

8 - *Sotoodehnia, A. and Aarabi, I. Neutralization of excess formalin by sodium Meta-bisulfite in combined anthrax & clostridial vaccine. Arch.Inst. Razi, (1988), 38,39,89-91.*

*Table 1.* Assessment *of induced antibody in vaccinated calves's sera by mouse protection test at different times post vaccination.*

| Calf's No | Date of vaccination | Date of calves bleeding | | | | | |
|---|---|---|---|---|---|---|---|
| | 25 April 1989 | 20 May 1989 | 25 June 1989 | 10 Aug 1989 | 9 Sept 1989 | 14 Oct 1989 | 15 Nov 1989 |
| 1 | " | 0/7* | 0/7 | 1/7 | 0/7 | 0/7 | 2/7 |
| 2 | " | 1/7 | 0/7 | 2/7 | 0/7 | 2/7 | 2/7 |
| 3 | " | 0/7 | 0/7 | 0/7 | 1/7 | 4/7 | 7/7 |
| 4 | " | 1/7 | 1/7 | 1/7 | 5/7 | 6/7 | 7/7 |
| 5 | " | 1/7 | 0/7 | 1/7 | 0/7 | 2/7 | 7/7 |
| 6 | " | 0/7 | 1/7 | 2/7 | 1/7 | 4/7 | 7/7 |
| 7 | " | 1/7 | 1/7 | 1/7 | 3/7 | 7/7 | 7/7 |
| 8 | " | 0/7 | 0/7 | 1/7 | 3/7 | 7/7 | 7/7 |
| 9 | " | 0/7 | 0/7 | 0/7 | 0/7 | 3/7 | 5/7 |
| 10 | " | 0/7 | 1/7 | 1/7 | 1/7 | 5/7 | 7/7 |
| 11 | " | 0/7 | 1/7 | 1/7 | 2/7 | 5/7 | 7/7 |
| 12 | " | 1/7 | 1/7 | 1/7 | 0/7 | 5/7 | 4/7 |
| $Control_1$ | | 7/7 | 7/7 | 7/7 | 7/7 | 7/7 | 7/7 |
| $Control_2$ | | 7/7 | 7/7 | 7/7 | 7/7 | 7/7 | 7/7 |

* Number of mortalities from number of tested mice (mortality ratio).