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Research Paper

# A study of immune response of calves given with varying doses of biofilm haemorrhagic septicaemia vaccine

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## ABSTRACT

A preliminary investigation was undertaken to examine whether the presently recommended dose of haemorrhagic septicaemia vaccine 5-10 ml needed any revision and also to evaluate the suitability of passive haemagglutination assay and complement fixation test for measuring immunity in comparison with direct potency test. From the above tests it concludes that the three different doses of biofilm haemorrhagic septicaemia vaccine in calves conferred satisfactory immunity. The complement fixation test titres unlike passive haemagglutination assay titres were closely related to direct potency test.

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**Key words :** Complement Fixation Test; Passive haemagglutination Assay (PHA), Biofilm vaccine

## INTRODUCTION

Haemorrhagic septicaemia (H.S.) in bovines is an acute septicaemic, infectious disease principally caused by *Pasteurella multocida* serotype B:2. In India, the disease is known to occur throughout the country, accounting for 46 to 55 per cent of the total mortality in bovines due to infectious diseases (Dutta *et al*., 1990). The disease is commonly encountered in the beginning of monsoon season. The *Pasteurella* organisms are known to be the normal inhabitants of upper respiratory tract of bovines and they assume a pathogenic role whenever resistance of the host is lowered due to "stress" resulting in precipitation of clinical disease hence it is very difficult to predict the exact time of out break of this disease. Due to peracute nature of the disease prophylactic mass vaccination of animals appears to be the only method of choice to control this infection in bovines, rather than treatment of individual animals. Presently, broth vaccine with potash alum adjuvant is widely used in India.

But, inspite of repeated vaccinations, still outbreaks of the disease occur due to poor immunogenicity of vaccine and changes in the expression of immunogens by the pathogen *in vitro* than those produced *in vivo* hence, there is a great concern to evolve an alternate vaccine to over come the above problems. Therefore, biofilm form of pathogen which mimics natural infection (Costerton *et al.,* 1987, Prakash *et al.,* 2003) was experimentally evaluated and Biofilm vaccine gives longer duration of protection than conventional vaccines for the control of haemorrhagic septicaemia in cattle.

## RESEARCH METHODOLOGY

**Vaccine**:

Biofilm, haemorrhagic septicaemia vaccine produced in the Institute of Animal Health and Veterinary Biologicals, Bangalore, was used.

**Calves:**

Three groups of three calves in each were used in the experiment and were vaccinated subcutaneously as follows:

| Group No. | Vaccine dose |
|---|---|
| I | 10.0 ml |
| II | 5.0 ml |
| III | 3.0 ml |

Also two calves were used as control group.

**Potency test**:

After three weeks of vaccination, all the vaccinated calves along with control calves were challenged with 1.0 ml of 1:10 dilution an 18 hour old $P_{52}$ (Phase-I) culture as per the method used for potency testing of haemorrhagic septicemia vaccine.

**Serological studies**:

Serum samples were collected from all vaccinated calves before vaccination, post vaccination, and first and second weeks post challenge. Serum samples were also collected from control calves just before challenge. Passive haemagglutination test and complement fixation test were used to measure the antibody titres in the above samples.

**Antigens:**

**Extraction of crude capsular polysaccharide (CCPS):**

The method of Carter (1955) was used for the extraction of CCPS.

**Sensitization of sheep erythrocytes:**

Done as per method described by Cruickshank (1965). and it is adsorbed on the stabilized sheep erythrocytes as described by Sawada *et al.* (1982).

**Passive haemagglutination assay (PHA)**:

To the two fold serially diluted serum sample in 0.5 ml quantity, an equal quantity of sensitized RBCs were added, kept at room temperature for 2 hours and 30 minutes and then at $4^0$C overnight before the results were recorded. (Table 1)

**Complement fixation test (CFT)**:

*Antigen preparation:*

*P. multocida* $P_{52}$ strain was grown in roux flasks. Pure growth of organisms was harvested, washed thrice with normal saline and then resuspended in 0.5% phenol saline and used as antigen.

Antigen titration was done and 1:10 dilution of antigen was used in the test. CFT was carried out as per Methods described by Cottral (1978).

## RESULTS AND DISCUSSION

The results obtained from the present studies as well as relevant discussion have been presented in detail as under:

**Potency test:**

All the vaccinated calves survived the challenge while the controls died with typical lesions of haemorrhagic septicaemia. Heart blood smears revealed bipolar organisms. Pure culture *Pasteurella* was isolated from heart blood inoculation on to blood agar plates (Table 2).

The potency test conducted on crossbred calves with three different doses Biofilm Haemorrhagic Septicaemia vaccine shows that, all the three doses were effective in conferring immunity. However, in an earlier study, reported by Iyer and Ranga Rao (1959) that the doses 5 ml of alum precipitated haemorrhagic septicaemia vaccine subcutaneously or 3 ml of oil adjuvant haemorrhagic septicaemia vaccine given intramuscularly conferred a satisfactory immunity in vaccinated animals. However, further studies employing more number of animals based on their body weight for each dose level are necessary to arrive at definite conclusions.

**Table 1 : Passive haemagglutination assay (PHA) titres**:

| Group | Dose | Calf number | Before vaccination | 3 weeks after vaccination | One week after challenge | Two weeks challenge |
|---|---|---|---|---|---|---|
| I | 3 ml | 1 | 1 :32 | 1 :32 | 1 :32 | 1 :32 |
| | | 2 | 1 : 8 | 1 :16 | 1 :16 | 1 :32 |
| | | 3 | 1 :16 | 1 :16 | 1 :32 | 1 :32 |
| II | 5 ml | 1 | 1 :16 | 1 :32 | 1 :32 | 1 :64 |
| | | 2 | 1 :8 | 1 :32 | 1 :32 | 1 :64 |
| | | 3 | 1 :16 | 1 :64 | 1 :64 | 1 :64 |
| III | 10 ml | 1 | 1 :16 | 1 :32 | 1 :32 | 1 :64 |
| | | 2 | 1 :8 | 1 :32 | 1 :64 | 1 :64 |
| | | 3 | 1 :8 | 1 :64 | 1 :64 | 1 :64 |
| IV | Control | 1 | 1 :32 | | | |
| | (Before challenge) | 2 | 1 :16 | | | |

**Table 2: Complement fixation test (CFT) titres:**

| Group | Dose | Calf number | Before vaccination | 3 weeks after vaccination | One week after challenge | Two weeks challenge |
|---|---|---|---|---|---|---|
| I | 3 ml | 1 | No titre | 1 :16 | 1 :64 | 1 :256 |
| | | 2 | 1 : 4 | 1 :16 | 1 :32 | 1 :128 |
| | | 3 | 1 :4 | 1 :32 | 1 :64 | 1 :256 |
| II | 5 ml | 1 | 1 :2 | 1 :16 | 1 :32 | 1 :64 |
| | | 2 | 1 :4 | 1 :16 | 1 :32 | 1 :128 |
| | | 3 | 1 :4 | 1 :32 | 1 :164 | 1 :256 |
| III | 10 ml | 1 | No titre | 1 :32 | 1 :64 | 1 :256 |
| | | 2 | No titre | 1 :64 | 1 :64 | 1 :256 |
| | | 3 | 1 :4 | 1 :32 | 1 :64 | 1 :256 |
| IV | Control (Unvaccinated | 1 | No titre | | | |
| | before Challenge) | 2 | 1 :2 | | | |

Jerico *et al.* (1985) reported that passive haemaggulutination assay, direct and indirect bacterial agglutination tests, modified complement fixation test and ELISA do not measure protective immunity against virulence factors of *P. haemolytica.* In the present experiment also there was no correlation between passive haemaggulutination assay titre and the potency tests as both control calves and some vaccinated calves showed a titre of 1:16 or 1:32. but, the control calves died where as all vaccinated calves withstood the challenge. De Alwis *et al.* (1978) also reported that the sensitivity of passive haemaggulutination assay was insufficient to detect immunity in vaccinated cattle. However, Sawada *et al.* (1985) found that there was a close relationship between immunity and antibody titres due to passive haemaggulutination assay using capsular antigen.

The capscular extraction procedure has got a definite bearing over the evaluation of protective response elicited as mentioned by Tadayon and Lauerman (1981) where in Aimes (1951). Gaunt *et al.* (1977) and Mukkur (1977) also have indicated that KSCN antigen used in the present experiment is a saline extracted one. This could have influenced the sensitivity of passive haemaggulutination assay thereby causing a poor correlation with direct potency test.

Killed whole cell bacteria were used as antigen for complement fixation test. Since there was better complement fixing response in all vaccinated calves which withstood the challenge, it could be stated that complement fixation test using antigen was better for testing protective antibody titres.

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## LITERATURE CITED

Amies, C.R. (1951). *Br. J. Exp. Pathol.*, **32**:259-264.

Carter, G.R. (1955). Studies on *P. multocida*. Indirect heamagglutination test for the identification of serological types *Am. J. Vet. Res.*, **16**: 481-489.

Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M. and Marrie, T.J. (1987). Bacterial biofilms in nature and diseases. *Ann. Rev.Microbiol.*, **41**:435-464

Cottral, G.E. (1978). Manual of standardized methods in veterinary microbiology, Ed. I. pp. 61-62.

Cruickshank, R. (1965). Medical microbiology. 2nd Ed. Williams and Wilkins Co., *Baltimore,* 934 pp.

De Alwis, M.C.L., Gunatilake, A.D.P. and Wikramasinghe (1978). *Ceylon Vet. J.,* **26**:35-40.

Dutta, J., Rathore, B.S., Mullick, S.G., Singh, R. and Sharma, G.C. (1990). Epidemiological studies on occurrence of haemorrhagidc septicaemia in India. *Indian Vet. J.,* **67**: 893-899.

Gaunt, G.., Moffat, R. and Mukkur, T.K.S. (1977). *Avian Dis.,* **21**:543-547.

Jerico, K.W.F., Cho, H.J., Yates, W.D.G. and Kozub, G..C. (1985). *Am. J.Vet. Res.*, **46**:2457-2460.

Mukkur, T.K.S. (1977). *Infect immune*, **18**:583-587.

Prakash, B., Honnegowda and Krishnappa, G. (2003). Comparative evaluation of biofilm and free cell vaccines against *Salmonella gallinarum* in chicks. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.,* **23**: 49-52.

Sawada, T., Rimler, R.B. and Rhoader, K.R. (1982). Indirect haemagglutination test that uses glutaraldehyde fixed sheep erythrocytes sensitized with extract antigens for detection of pasteurella antibody. *J. Clin. Microbiol.,* **15**:752-756.

Tadyon, R.A. and Lauerman, L.H. (1981). *Vet. Mic.*, **6**:85.

Sawada, Takuo, Richard, B., Rimler, Keith and Rhodes (1985) *Am. J. Vet Res.*, **46**:1247-1250.

Iyer, Vancheswar and Ranga Rao, D.V. (1959). *Indian Vet. J.*, **36**:416-420.

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