*VETERINARSKI ARHIV 71* (1), 37-45, 2001

# Serological response of bovines to combined vaccine containing foot and mouth disease virus, rabies virus, *Pasteurella multocida* and *Clostridium chauvoei* antigens

**Villuppanoor Alwar Srinivasan[1]*, Guddeti Srinivas Reddy[1], Koppunuru Ananda Rao[1], and Ueli Kihm[2]**

[1] *Indian Immunologicals, Rakshapuram, Gachibowli, India*
[2] *Federal Veterinary Office, Liebefeld-Bern, Switzerland*

**SRINIVASAN, V. A., G. S. REDDY, K. A. RAO, U. KIHM: Serological response of bovines to combined vaccine containing foot and mouth disease virus, rabies virus, *Pasteurella multocida* and *Clostridium chauvoei* antigens. Vet arhiv 71, 37-45, 2001.**

**ABSTRACT**

Five groups of ten animals were vaccinated with a combined vaccine containing foot and mouth disease (FMD), rabies, *Pasteurella multocida* and *Clostridium chauvoei* antigens and individual component vaccines containing FMD, rabies, *Pasteurella multocida* and *Clostridium chauvoei* antigens, respectively. Serological response of the calves was assayed on days 21 and 90 post vaccination. There was no significant variation in the serological response elicited by individual component vaccines and combined vaccine containing all four antigens.

**Key words:** food and mouth disease, rabies, *Pasteurella multocida, Clostridium chauvoei*, prophylaxis, combined antigens, serology, vaccination

## Introduction

Regular prophylactic vaccination of dairy animals against infectious diseases in developing countries has become an important input to maintain milk production and to reduce economic losses. In an exercise to reduce the cost of vaccination, many workers attempted simultaneous vaccination with foot and mouth disease (FMD) and haemorrhagic septicaemia (HS) (JOSEPH and HEDGER, 1984), FMD and rinderpest (RP) (HEDGER et al., 1986;

* Contact address:

Dr.Villuppanoor Alwar Srinivasan, Indian Immunologicals, Rakshapuram, Gachibowli, Hyderabad - 500 019, India, Tel: +91 40 3000 894, Fax: +91 40 3000 213, E-mail: raksha2@hd1.vsnl.netin

ISSN 0372-5480
Printed in Croatia

SRINIVAS et al., 1996) and vaccination with combined vaccines containing FMD and rabies antigens (DANNACHER et al., 1987; PALANISAMY et al., 1992). The use of combined vaccines depends on the epidemiological situation and vaccination regimes practiced in a particular region. In many South and South-East Asian Countries FMD, HS and Black Quarter (BQ) vaccines are commonly used with different vaccination regimes. Rabies in bovines is also a serious problem in many developing countries. Vaccination with rabies is advocated in bovines in endemic areas as many workers have recorded outbreaks in bovines (RAMANNA et al., 1991; MURIUKI et al., 1994; KIM et al., 1994; SINGH et al., 1995). The efficacy of combined FMD, HS and BQ antigens has been studied by REDDY et al. (1997). The present investigation was undertaken to study the serological response of bovines to combined vaccine containing FMD, rabies, *Pasteurella multocida* and *Clostridium chauvoei* antigens.

## Materials and methods

### Vaccines

FMD virus strains O, A, C and Asial grown on BHK 21 suspension cell cultures and inactivated with Binaryethylenimine (BEI) were used to formulate the vaccine. The vaccine contained aluminium hydroxide gel and saponin. Potency of the vaccine was determined as per ANONYMOUS (1993) and each valency had a potency of $\geq 3$ $PD_{50}$.

Rabies antigen containing CVS strain of rabies grown on BHK21 suspension cell cultures and inactivated with BEI. The vaccine contained aluminium hydroxide and tested for potency as per ANONYMOUS (1993). The antigen used for vaccine production had a potency $\geq 2.5$ IU per dose.

*Pasteurella multocida* (HS). Formalin inactivated, purified and concentrated bacterin with 3 mg dry mass was used to formulate the vaccine. The vaccine containing aluminium hydroxide gel was tested as per ANONYMOUS (1989).

*Clostridium chauvoei* (BQ). Fully grown cultures of *Clostridium chauvoei* are inactivated with formalin, purified and concentrated and used to formulate the vaccine. Antigens containing 200 haemolytic units per dose are incorporated. The vaccine containing aluminium hydroxide gel

was tested as per ANONYMOUS (1993).

Combined vaccine (FMD, HS, BQ, Rabies). The combined aqueous vaccine was formulated incorporating antigen payloads similar to that of individual component vaccines.

Five groups of 10 animals each were vaccinated with FMD+HS+BQ+Rabies, FMD, HS, BQ and Rabies vaccines in dose volumes of 4 ml, 3 ml, 2 ml, 2ml, and 1 ml respectively by intramuscular route. One group of five animals was kept as controls.

Five groups of 10 animals each were vaccinated with FMD+HS+BQ+Rabies, FMD, HS, BQ and Rabies vaccine in dose volumes of 4 ml, 3 ml, 2 ml, 2 ml and 1 ml respectively by intramuscular route. Animals vaccinated with FMD component were given a booster dose on the 21$^{st}$ day. One group of five animals was kept as controls.

Serum from blood samples collected on day 0, day 21 and day 90 post vaccination (dpv) were separated, inactivated at 56 °C for 30 min. and stored at -20 °C until further use.

Serological assay

*Food and mouth disease*. The serological response was measured by micro-serum neutralization test described by GOLDING et al. (1976) using IB-RS2 cells. Titres were calculated as per the method described by KARBER (1931) and expressed as $\log_{10}$ $SN_{50}$ values.

*Rabies.* Antibody titres were determined by rapid fluorescent focus inhibition test (RFFIT) as per the method described by SMITH et al. (1973). SAD strain of Rabies virus and $BHK_{21}$ monolayer cells were used in the test. Titres are expressed as International Units (IU) per ml after comparing the RFFIT titres of serum samples with International standard preparation.

*Haemorrhagic septicaemia.* ELISA test was performed on serum samples to determine the serological response elicited by all the vaccines as per the method described by NATALIA et al. (1992) with minor modifications – Flat-bottom PVC plates and orthophenylene diamine (OPD) as substrate were used in the test, and readings were taken at 492 nm wavelength. Titres were obtained by multiplying the corrected optical density (OD) values (sample OD - background OD (Ag+Conjugate) with serum dilution factor.

*Black quarter.* Antibody titres were measured by ELISA test described by CRICHTON et al. (1990) with minor modifications – HRPO-labelled rabbit anti-bovine conjugate (Sigma) was used at 1/3500 dilution OPD was used as substrate. OD was measured at 492 nm wavelength. Titres were calculated similarly to HS ELISA test.

Serological response to different vaccines was compared by using standard statistical methods described by SNEDECOR and COCHRAN (1980).

## Results and discussion

Serological response of calves to food and mouth disease virus antigens O, A, C, Asia in FMD vaccine and FMD, rabies, HS, BQ combined vaccine is presented in Table 1.

Immunity to FMD is conferred by serum neutralising antibody. Indirect estimation of the $\log_{10}$ $SN_{50}$ values which equates with 50% protection (PD) has been assessed, which is referred to as 50% protective antibody (PA). The $PA_{50}$ values for type O, A, C and Asia were calculated as 1.62, 0.99, 1.05 and 1.45, respectively (unpublished data). These values correlate well with observations made by PAY and HINGLEY (1992), using British cattle. The $PA_{50}$ values of type O in our studies (unpublished data) indicate lower

Table 1. Serological response of calves to FMD antigen ($\log_{10}$ $SN_{50}$ values)

| Virus types | | Mean titres ± SE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | O | | | A | | | C | | | Asia 1 | | |
| Vacc. | dpv / N | 0 | 21 | 90 | 0 | 21 | 90 | 0 | 21 | 90 | 0 | 21 | 90 |
| 1 | 10 | <0.61 | 2.4 ±0.21 | 2.34 ±0.4 | <0.61 | 1.98 ±0.21 | 2.20 ±0.3 | <0.61 | 1.86 ±0.21 | 1.86 ±0.3 | <0.61 | 2.31 ±0.30 | 2.40 ±0.21 |
| 2 | 10 | <0.61 | 2.36 ±0.34 | 2.41 ±0.34 | <0.61 | 1.86 ±0.3 | 2.11 ±0.2 | <0.61 | 1.75 ±0.32 | 1.86 ±0.11 | <0.61 | 2.2 ±0.3 | 2.46 ±0.36 |
| 3 | 10 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 |
| 4 | 10 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 |
| 5 | 10 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 |
| 6 | 5 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 | <0.61 |

1 = FMD+HS+BQ+Rabies; 2 = FMD; 3 = HS; 4 =BQ; 5 = Rabies; 6 = Control

values when compared to the figures mentioned by PAY and HINGLEY (1992). This could be attributed to animal variation. PAY and HINGLEY (1992) suggested global $PA_{50}$ values of 1.26 for all virus strains. Although $PA_{50}$ values provide some indication regarding the indirect measure of protective levels, an attempt was made to derive a logical conclusion by comparing challenge test data and SN titres in our laboratory. Comparison of SN titres and challenge test results indicate that more than 80%, of animals with SN titres above l.41 and 1.61 were protected when challenged with type Asial and type O, respectively, whereas challenge using type C and type A viruses showed that more than 95% of animals with SN titres above 1.01 were protected. These tests were carried out on 85, 53, 101 and 129 animals using type O, C, A and Asia challenge viruses, respectively. The above data also indicate that animals with SN titres for type O above 1.61,

Table 2. Serological response of calves to rabies antigen (IU/ml)

| Vacc. | dpv / N | Mean antibody titres ± SE | | |
|---|---|---|---|---|
| | | 0 | 21 | 90 |
| 1 | 10 | < 0.12 | 8.5±2.2 | 9.06±3.1 |
| 2 | 10 | < 0.12 | 8.35±1.5 | 8.61±2.3 |
| 3 | 10 | < 0.12 | < 0.12 | < 0.12 |
| 4 | 10 | < 0.12 | < 0.12 | < 0.12 |
| 5 | 10 | < 0.12 | < 0.12 | < 0.12 |
| 6 | 5 | < 0.12 | < 0.12 | < 0.12 |

1 = FMD+HS+BQ+Rabies; 2 = FMD; 3 = HS; 4 = BQ; 5 = Rabies; 6 = Control

and Asial above 1.41, can be considered as protective titres, and that SN titres above 1.01 for type C and A can be considered as protective titres.

In the present study, the SN titres for all four valences are higher than the protective level on 90 dpv, and both groups of animals administered with FMD vaccine and combined vaccine showed no significant differences (P>0.05) (Table 1).

There was no significant differences (P>0.05) when the results of 0.21 and 90 dpv antibody titres of animals vaccinated with rabies vaccine alone and FMD, HS, BQ, rabies combined vaccines were compared (Table 2). DANNACHER et al. (1987) and PALANISAMY et al. (1992) reported that there was no difference in the serological response to rabies antigen when cattle were injected with rabies + FMD antigens.

Table 3. Selorogical response of calves to *Pasteurella multocida* and *Clostridium chauvoei* antigens (ELISA titres)

| | | Mean titres ± SE | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Pasteurella multocida* | | | *Clostridium chauvoei* | | |
| Vacc | dpv / N | 0 | 21 | 90 | 0 | 21 | 90 |
| 1 | 10 | 10.4±1.6 | 72±5.0 | 63.0±4.5 | 8.6±1.6 | 66±3.8 | 54.0±4.0 |
| 2 | 10 | 8.0±2.0 | 8.9±2.4 | 7.0±1.6 | 10.0±2.8 | 8.0±2.0 | 8.8±1.5 |
| 3 | 10 | 8.6±1.6 | 68.0±4.8 | 65.0±6.0 | 7.0±1.0 | 7.6±1.2 | 7.0±1.0 |
| 4 | 10 | 9.0±1.6 | 8.2±1.2 | 8.0±1.0 | 7.6±0.8 | 69.0±4.8 | 60.0±6.0 |
| 5 | 10 | 7.6±0.8 | 8.0±2.0 | 7.0±1.6 | 8.0±1.0 | 10.6±1.6 | 9.0±1.2 |
| 6 | 5 | 7.0±1.6 | 7.0±2.0 | 7.0±0.6 | 8.6±0.8 | 8.0±1.0 | 7.2±1.2 |

1 = FMD+HS+BQ+Rabies; 2 = FMD; 3 = HS; 4 = BQ; 5 = Rabies; 6 = Control

The immune response of calves to *Pasteurella multocida* and *Clostridium chauvoei* antigens measured by ELISA test is shown in Table 3. Comparison of antibody titres of calves vaccinated with HS vaccine alone, BQ vaccine alone, and FMD, HS, BQ, rabies combined vaccine showed no significant differences (p>0.05). Similar observations were made by REDDY et al. (1997) when HS and BQ antigens were combined with FMD virus antigens. An identical serological response was recorded by JOSEPH and HEDGER (1984) when HS vaccine was injected simultaneously with FMD vaccine. Serological response to FMD antigens when injected simultaneously with HS (JOSEPH and HEDGER, 1984), RP (SRINIVAS et al., 1996) or HS and BQ antigens (REDDY et al., 1997) remained altered and there was no evidence of antigenic competition.

Serum antibody titres (ELISA) for animals vaccinated with *Pasteurella multocida* vaccine and combined vaccine containing FMD+HS+BQ+ +Rabies antigens indicated that both tile vaccines induced similar response. CHANDRASEKARAN et al. (1994) compared serum antibody titres as measured by ELISA, and protection of buffaloes challenged with virulent organisms. Serum antibody titres above 54 ELISA units appeared to be indicative of protective titres. In the present report the serum antibody titres in animals vaccinated with HS combined vaccine and individual component vaccine showed protective titres.

Results of the present study indicated that the combined vaccine containing antigens of, FMD virus, rabies virus*, Pasteurella multocida* and *Clostridium chauvoei* produced and formulated according to our procedure can be used safely in countries where all these diseases are endemic, and that this procedure is likely to reduce the cost of vaccination.

## References

ANONYMOUS (1989): Recommended Diagnostic Techniques and requirement for Biological products. Vol. I. Office International Des Epizooties. Paris.

ANONYMOUS (1993): British Pharmacopoeia (Veterinary), HMSO, London.

CHANDRASEKARAN, S., L. KENNET, P. C. YEAP, N. MUNIANDY, B. RANI, T. K. S. MUKKUR (1994): Relationship between active protection in vaccinated buffaloes against haemorrhagic septicaemia and passive mouse protection test or serum antibody titres. Vet. Microbiol. 41, 303-309.

CRICHTON, R., J. SOLOMON, A. M. BARTON (1990): The development of an enzyme-linked immunosorbent assay for measuring the potency of vaccines containing *Clostridium chauvoei* antigens. Biologicals 18, 49-54.

DANNACHER, G., J. M. BLANCOV, M. COUDERT, M. FEDIDA (1987): Report session of Research Group Standing Technical Committee on European Commission. Control of FMD (FAO), Lyon, France.

GOLDING, S. M., R. S. HEDGER, P. TALBOT, J. WATSON (1976): Radial immunodiffusion and serum neutralization techniques for the assay of antibodies to swine vesicular disease. Res. Vet Sci. 20, 142-147.

HEDGER, R. S., W. P. TAYLOR, I. T. R. BARNETT, R. REIK, D. HARPHAN (1986): Simultaneous vaccination of cattle against foot and mouth disease and rinderpest. Trop. Anim. Hlth. Prod. 18, 21-25.

JOSEPH, P. G., R. S. HEDGER (1984): Serological response of cattle to simultaneous vaccination against foot and mouth disease and haemorrhagic septicaemia. Vet Rec. 114, 494-496.

KARBER, G. (1931): Beitrag Zur Kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Pathol. and Pharmakol. 162, 480-483.

KIM, J. E, E. K. HWANG, Y. J. LEE, J. W. PARK, S. M. BAE, H. J. SOHN, Y. D. YOON (1994): An outbreak of rabies in cattle in Korea. Korean Journal of Veterinary Public Health 18, 365-371.

MURIUKI, S. K., J. G. WANDERA, M. J. NJENGA, E. R. MUTIGA, A. THAIGAH, O. N. NJAGI, A. M. UTURI (1994): An outbreak of rabies in a diary farm in Kiambu district, Kenya. Bulletin, Animal Health and Production in Africa 42, 265-266.

NATALIA, L., B. PATTEN, A. SYAMSUDIN (1992): Evaluation of' bovine antibody responses to Haemorrhagic Septicaemia vaccine using ELISA and PMPT. Pasteurellosis in Production animals. Australian Centre for International Agricultural Research, Canberra.

PALANISAMY, R., B. C. RAMANNA, K. ANANDA RAO, V. A. SRINIVASAN (1992): Combined vaccination of cattle against FMD and rabies. Microbiologica 15, 45-50.

PAY, T. W. F., P. HINGLEY (1992): A potency test method for foot and mouth disease vaccine based on the serum neutralising antibody response produced in cattle. Vaccine 10, 707-713.

RAMANNA, B. C., G. S. REDDY, V. A. SRINIVASAN (1991): All outbreak of rabies in cattle and use of tissue culture rabies vaccine during the outbreak. J. of Comm. Dis. 23, 283-285.

REDDY, G. S, P. X. ANTHONY, V. A. SRINIVASAN (1997): Serological response to combined vaccination of cattle against foot and mouth disease, haemorrhagic septicaemia and black quarter diseases. Indian J. Anim. Sci. 67, 585-586.

SINGH, R., D. C. SHUKLA, P. N. KHANNA, K .P. SINGH, M. L. MEHROTRA (1995): An outbreak of rabies in cattle and buffaloes in Uttar Pradesh. Indian J. Anim. Sci. 65, 166-168.

SMITH, J. S., P. A. YAGAR, G. M. BAER (1973): Laboratory techniques in Rabies. 3rd edition. WHO, Geneva.

SNEDECOR, G. W., W. G. COCHRAN (1980): Statistical Methods, 7th edition. The Iowa State University Press, Iowa USA.

SRINIVAS, K., N. G. RAMESHBABU, RAMAKANT, M. RAJASEKHAR, M. S. VIJAYAKUMAR, T. M. LAXMINARAYANA, V. A. SRINIVASAN (1996): Simultaneous vaccination against foot and mouth and rinderpest diseases. Indian J. Virol. 12, 33-37.

Received: 19 August 1998
Accepted: 20 February 2001

**SRINIVASAN, V. A., G. S. REDDY, K. A. RAO, U. KIHM: Serološki odgovor goveda na cijepljenje polivalentnim cjepivom protiv slinavke i šapa, bjesnoće, hemoragijske septikemije i šuštavca. Vet. arhiv 71, 37-45, 2001.**

**SAŽETAK**

Pet skupina po deset životinja cijepljeno je različitim kombinacijama vakcina. Prva je skupina cijepljena polivalentnim cjepivom koje je sadržavalo antigene virusa slinavke i šapa, virusa bjesnoće te bakterija *Pasteurella multocida* i *Clostridium chauvoei*. Ostale su skupine cijepljene pojedinačnim antigenima, pa je tako druga skupina cijepljena antigenima virusa slinavke i šapa, treća antigenima virusa bjesnoće, četvrta antigenima bakterije *Pasteurella multocida,* a peta antigenima bakterije *Clostridium chauvoei.* Serološki odgovor svih životinja provjeren je 21. i 90. dana nakon cijepljenja. Na osnovi serološkoga odgovora nisu utvrđene značajne razlike među različitim načinima cijepljenja.

**Ključne riječi:** slinavka i šap, bjesnoća, *Pasteurella multocida,Clostridium chauvoei,* profilaksa, polivalentna vakcina, serologija