Indian Journal of Animal Sciences **78** (4): 367–368, April 2008

Evaluation of humoral and cell-mediated immune responses in cattle against experimental infectious bovine rhinotracheitis killed vaccine*

S B SONTAKKE[1], V V DESHMUKH[2] and M B GUJAR[3]

Maharashtra Animal and Fishery Sciences University, Parbhani, Maharashtra 431 402 India

Received: 22 August 2007; Accepted: 5 October 2007

Key words: Cell-mediated immune response, ELISA, Humoral immune response, SNT DLC, DNCB test

Infectious bovine rhinotracheitis (IBR), an important disease of bovine caused by bovine herpes virus (BHV-1), is characterized by rhinotracheitis and reproductive disorders. The ability of virus to survive in frozen semen makes it difficult to control the disease. The infection can be controlled by vaccination. Killed vaccine is safe, immunogenic and potent against IBR virus (Mehrotra and Shukla 1991, Kilari *et al.* 2000, Chinchkar 2000).

In present study, humoral and cell-mediated immune responses of the experimental IBR killed vaccine evaluated to study the potency of the vaccine.

Red Kandhari cattle (30) were divided into 5 groups each group comprising of equal no. of cattle. The HC group was kept as healthy control thoroughout the study period, The "PC" group was a IBR-positive group in which IBR seropositive cattle were kept unvaccinated control throughout the experiment. The "PV" group was a IBR seropositive and vaccinated group in which all the cattle were vaccinated with vaccine 2 ml each subcutaneously on '0' day of experiment. The PVb group comprises IBR seropositive cattle vaccinated by vaccine @ 2 ml each subcutaneously on the '0' day of the experiment and a booster vaccination was done on 28th day of the experiment. The "NVb" group comprises of IBR seronegative cattle vaccinated by vaccine @ 2 ml each subcutaneously on '0' day of experiment and a booster was given on 28th day of the experiment.

The experiment was planed for 56 days and observations were made on weekly intervals. An indirect plate enzyme linked immunosorbent assay (ELISA) and serum Neutralization test (SNT) were used for evaluation of humoral immune responses whereas differential lymphocyte counts (DLC) and dinitrochlorobenzene (DNCB) test were used for assessment of cell mediated immunity.

The method of Herring *et al.* (1980) was standardized and used for evaluation of the humoral immune responses. The method of Rai (1985) was used to standardize SNT. The DLC by Giemsa's staining of the blood smears was used to study cell-mediated immune responses of the experimental cattle (Jain 1986).

A method of Reddy *et al.* (1981) was used for the DNCB test in which the area over left side of the neck region of the experimental animal was shaved and cleaned. A 2% DNCB solution @ 0.5 ml animal was applied drop by drop over a circular area of 2 cm diameter for 3 consecutive days for sensitization of the skin. The right side of the neck region was used after a fortnight for challenge with the DNCB. The skin thickness was measured before challenge, 24, 48 and 72 h post challenge.

The 'HC' and 'NVb' groups did not show presence of IBR antibodies throughout the study period by ELISA and SNT. The PC, PV, and PVB groups were seropositive on the 'O' day of the experiment. The IBR antibodies were detected from 7 the day post-vaccination by ELISA and SNT. The ELISA and SNT titers rose thereafter till the end of the experiment. The titers were significantly higher in all vaccinated groups than 'HC' group at each interval of study. The ELISA and SNT titers wee significantly higher in all vaccinated groups from 21st day post-vaccination till the end of the experiment when compared with 'PC' group. The ELISA and SNT antibody titers of PVb and NVb groups were significantly higher from 14th day post booster vaccination till the end of the experiment when compared with PV group. Similar pattern of antibody response to the 'Ibrivax' vaccine was reported earlier by Kilari *et al.* (2000) and Chinchkar (2000).

Cell-mediated immune (CMI) response against experimental vaccine was successfully evaluated by using DLC and DNCB tests. DLC in vaccinated groups were significantly higher throughout the experiment as compared with 'HC' group. The DNCB test showed presence of additional cell-

*Part of the M.V.Sc. Dissertation submitted to the Maharashtra Animal and Fishery Sciences University by the first author.

Present address: [2]Scientist and Principal Investigator, ICAR, AINP on BT Disease, Animal Disease Research Centre, [3]Associate Dean and Principal, College of Veterinary and Animal Sciences.

mediated immunity in all vaccinated groups than HC group. The DLC was earlier used for evaluation of CMI by various authors (Coles 1986, Jain 1986). The DNCB test was used for evaluation of CMI in evaluation of killed viral vaccines (Chaudhari 1985, Deshmukh 1987). In the present study also the DNCB test was successfully used for the evaluation of the CMI. The vaccine was found to be highly immunogenic vaccine in the present study.

SUMMARY

Humoral and cell-mediated immune responses of the experimental IBR-killed vaccine, were evaluated to study the potency of the vaccine. ELISA and SNT were used for evaluation of humoral immune responses whereas DLC and DNCB tests wee used for assessment of CNI. The vaccine was found as highly immunogenic vaccine in the present study.

REFERENCES

Chinchkar S R. 2000. 'Studies on seroprevalence of infectious bovine rhinotracheitis and its immune response in vaccinated calves.' M.V.Sc. thesis.

Coles E H. 1986. *Veterinary Clinical Pathology*. 4th edn, pp. 68. W.B. Saunders Company, Philadephia.

Deshmukh V V. 1987. 'Evaluation of immune responses against Goat pox Infected / vaccinated goats.' M.V.Sc. Dissertation submitted to Marathwada Agricultural University, Parbhani.

Herring A J. Nettleton P F and Burrells. 1980. A micro-enzyme linked immunodies to infection bovine rhinotracheitis virus. *Veterinary Record* **107**: 155–56.

Jain N C. 1986. *Schalm's Veterinary Haematology*. 4th edn, pp. 33 and 823. Lea and Febiger, Philadelphia.

Kilari S Singh, S N Buche A V Sawarkar S and Ghosh S. 2000. Evaluation of immunogenicity of infectious bovine rhinotracheitis vaccine in Indian tropical climate. *Indian Veterinary Journal* **77**: 185–88.

Mehrotra M L and Shukla D C. 1991. Evaluation of immunogenicity of beta-propiolactone inactivated bovine herpesvirus-1 vaccine. *Indian Journal of Animal Sciences* **61**(3): 241–45.

Rai A. 1985. *Methods in Cell Culture and Virology*. Allied Publishers Pvt. Ltd., New Delhi.

Reddy M V. Rajan A and Sulochana S. 1981. Evaluation of cell mediated immune response in cattle by 2-4. dintrochlorobenzen. *Veterinary Immunology and Immunopathology* **2**: 483–90. (*fide Astrian Veterinary Bulletin* **52**: 7302.)