Indian Journal of Animal Sciences **78** (4): 339–341, April 2008

Immune response of sheep to bentonite clay and alum—adjuvanted enterotoxaemia vaccines*

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Received: 14 February 2007; Accepted: 14 October 2007

ABSTRACT

An investigation was undertaken to evaluate the immune response of sheep to 2 types of enterotoxaemia vaccines prepared by incorporating bentonite clay and alum separately as adjuvants. *Clostridium perfringens* type D was used for preparation of vaccines. Two groups of sheep were immunized separately with both vaccine formulations, and the immune response of sheep against all the formulations was assayed by mouse neutralization test (MNT). It was evident from the result that both types of vaccines protected sheep against enterotoxaemia up to 120th day post vaccination (MNT titre of 2 IU/ml) without booster dose of vaccination. Bentonite clay adjuvanted enterotoxaemia vaccine was found superior to alum precipitated vaccine in terms of cost, availability and production of higher immune response at 30th day of post vaccination.

Key words: Alum, Bentonite clay, Enterotoxaemia, Mouse neutralization test (MNT), Vaccination

In sheep, enterotoxaemia leads heavy mortality due to epsilon toxin, a major lethal exotoxin produced by *Clostridium perfringens* type D. Hence, the protection of sheep against enterotoxaemia is mainly aimed at neutralizing the epsilon toxin by inducing the development of specific antitoxic immunity (Zemlyakova 1974). The available vaccines for the prevention of enterotoxaemia are having certain limitations such as, induction of local reaction at the site of inoculation, availability of vaccine and high cost factor. The currently available alum precipitated vaccines do not maintain immunity in sheep for longer than 6 months (Blackwell *et al.* 1983). Bentonite clay has been reported to be a better adjuvant compared to other adjuvant aluminium hydroxide, saponin and dextron. Due to the excellent adsorption activity of bentonite, it was used for virus concentration as well as an adjuvant (Bayramoglu and Yalim 1971, Bayramoglu and Yalim 1974).

In the present investigation 2 different vaccine formulations containing bentonite clay and alum as adjuvant were prepared and evaluated experimentally in sheep for their efficacy.

MATERIALS AND METHODS

Freeze-dried culture of *Clostridium perfringens* type D obtained from the Institute of Animal Health and Veterinary Biologicals, (IAH&VB), Bangalore, was used for preparation of vaccines in the present study.

Clostridium perfringens type D was inoculated into Robertson bullock heart media and then subcultured in Thioglycollate broth media. Two types of Thioglycollate media were prepared, viz. thioglycollate media without bentonite clay for preparation of alum adjuvanted enterotoxaemia vaccine and thioglycollate media with bentonite clay for preparation of bentonite clay adjuvanted enterotoxaemia vaccine. Bentonite clay was added @ 0.3% of media. Incubation was done at 37°C and pH was adjusted to 7.8 at every 2 h interval using 10% sodium hydroxide for 2 days. Then, trypsin was added up to final concentration of 0.25% and incubated at 37°C for $1^1/_2$h.

Production of epsilon toxin was confirmed by inoculation of 0.2 ml supernatant culture of *Clostridium perfringens* type D into mice and amount of epsilon toxin was estimated by mouse titration test (Gilroy 1967).

The toxin was inactivated by adding formalin up to the final concentration of 0.5% and incubated at 37°C for 11 days to convert epsilon toxin into toxoid (Jayaraman and

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Mallick 1961). Further, alum was added up to a final concentration of 1% at time of bottling to prepare alum precipitated enterotoxaemia vaccine.

Sheep (100) were divided into 2 groups of 50 each. In the present investigation 7500 MLD of toxoid was used per dose of vaccine. Group 1 was inoculated with 1 ml of bentonite clay adjuvanted enterotoxaemia vaccine and group 2 received 2.5 ml of alum precipated enterotoxaemia vaccine and animals vaccinated were observed for any adverse reaction and body temperature was then recorded periodically. Serum samples were collected from the experimental sheep just before and after vaccination at monthly intervals. These samples were stored in duplicate aliquots of 1 ml each at –20°C until further use.

Immune response of sheep to 2 types of vaccines was assayed by mouse neutralization test (MNT). MNT was performed according to procedure described by Jayaraman and Mallick (1961).

RESULTS AND DISCUSSION

In the present investigation, sheep vaccinated with alum precipitated vaccine and bentonite clay adjuvanated vaccine did not show any untoward reactions at the site of injection. On the contrary to this observation, Percival *et al.* (1954) observed local reaction which appeared within 24 h of inoculation of vaccine containing alum than the vaccine without alum and lesions persisted up to 8 days and then started regressing. No lesions were observed at 15th day. Green *et al.* (1987) observed swelling of about 2.5 cm in diameter in sheep and goat vaccinated with multivalent Clostridial vaccine and lesion was apparent even after 28 days of vaccination and were significantly larger in sheep than goats.

Humoral immune response against epsilon toxin plays an important role in protective immunity in enterotoxaemia (Kerry and Craig 1979). Hence, only humoral immune response was assayed by adopting MNT. The MNT using crude epsilon toxin as antigen had been acclaimed as the best test to assay the level of protective antibody titre in enterotoxaemia vaccinated animals (Jayaraman and Mallick 1961 and Dholakia *et al.* 1980).

The pre-immune sera collected from the experimental sheep showed an antibody titre of 1 IU/ml. This clearly indicated that the sheep were not protected against enterotoxaemia as the minimum level of antibody level required for protection was 2 IU/ml. This is in agreement with the report of Cristina de la Rosa *et al.* (1997), who reported that 2 IU/ml antibody titre was minimum protective level.

The serum samples of animals of group 1 administered with bentonite clay adjuvanted enterotoxaemia vaccine showed MNT titres of 8, 6, 4, 2, and 1 IU/ml of serum, at 30, 60, 90, 120, and 150 days post vaccination, respectively.

The serum samples of animals of Group II administered with alum adjuvanted enterotoxaemia vaccine showed MNT titres of 6, 6, 4, 2, and 1 IU/ml of serum at 30, 60, 90, 120, and 150 days post-vaccination, respectively.

From this, we could clearly state that booster dose of vaccination for the both types of vaccines is recommended at the 120 th day of primary vaccination. There was no significant difference between bentonite clay adjuvanted enterotoxaemia vaccine and alum precipitated enterotoxaemia vaccine as indicated by ANOVA (Analysis of variance) at $P<0.05$.

Thomson and Betty (1953) recommended 4 weeks interval, Jayaraman and Mallick (1961) recommended two injections of vaccine at the interval of 2–3 weeks, Bernath *et al.* (2004) recommended 8 weeks interval between first vaccination and booster vaccination.

Kerry and Craig (1979) observed MNT of 5 IU/ml in 13 sheep and 1–5 IU/ml in nine sheep at 42 days after first injection with multicomponent clostridial vaccine.

In the present study, alum and bentonite clay were used as adjuvants. Among the 2 adjuvants used, bentonite clay adjuvanted enterotoxaemia vaccine showed better immune response at the 30 th day post vaccination when compared to alum precipitated enterotoxaemia vaccine. Therefore, this could be effectively used as suitable candidate for vaccination of sheep against enterotoxaemia in endemic area as an alternate to alum adjuvanted enterotoxaemia vaccine. Tanto -O-Sonnie and Wilson (1960) described the important characteristic of bentonite clay that made it as adjuvant as follows: it was non toxic, inert, a good emulsifier, an adsorbent and then makes vaccine homogenous. Regarding the adjuvants effect it has been shown to improve immune response markedly against foot and mouth disease (Misra and Lal 1990), Brucellosis (Shailaja 1996) and Hemorrhagic septicemia (Shivaraj 1997, Veeragouda patil *et al.* 2004).

Considering the availability and handling, bentonite clay adjuvanted enterotoxaemia vaccine was found to be superior to alum precipitated enterotoxaemia vaccine.

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