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An experimental study on the immunization of mice and rabbits with *Pasteurella multocida* toxoid

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ABSTRACT

A toxigenic strain of *Pasteurella multocida* serotype A: 12 of goat origin was selected on the basis of its toxin yield amongst other 3 toxin producing strains with a view to study its immune response. A crude *Pasteurella multocida* toxin (PMT) was prepared by cell sonication method. The immune response of inactivated PMT (toxoid) was studied in mice and rabbit model. The immune status of vaccinated animals was determined by ELISA and challenge test with crude toxin and live culture. Mice vaccinated with 2 doses schedule comprising 10 mg and 100 mg by s/c route gave a protection level of 60.0% and 73.3%, respectively, on challenge with 200 mg of crude toxin. Almost same level of protection was observed when challenged with live culture, 0.2 ml s/c (10^{-9}). While rabbits immunized with 200 mg of toxoid by s/c routes were protected when challenged with 400 mg of crude toxin by intranasal route and also did not show any dermonecrotic effect of PMT when 300 mg of crude toxin was given by I/D route.

Key words: Immunization, *Pasteurella multocida* toxoid, Rabbit

Pasteurella multocida toxigenic strains of serotypes A and D usually cause swine atrophic rhinitis (Chanter *et al.* 1986, Dominick and Rimler 1986). The occurrence of atrophic rhinitis in goats was not known until Baalsrud (1987) reported this disease. During the present study, 4 goat strains of *P. multocida* serotype A:3; A:3, 4; A:12 and one untyped isolated from goat pasteurellosis cases were screened for their ability to produce PMT, and to study its immune response in mice and rabbit model. The PMT obtained from serotype A:12 was inactivated and its immune response was studied in mice and rabbits model.

MATERIALS AND METHODS

Screening of Pasteurella multocida *strains for PMT production*

The four *P. multocida* strains belonging to serotype A:3, A:3, 4, 12, A:12 and one untyped strain of goat origin were obtained from the Division of Bacteriology and Mycology, IVRI, Izatnagar. The method described by Nakai *et al.* (1984) was followed to determine the lethal effect and dermonecrotic activity of PMT in mice and rabbits respectively. Lethal toxicity was expressed as the number of dead mice/number of mice injected. The dermonecrotic lesions in rabbits >5 mm in diameters were read as positive reaction. Dermonecrotic titre was expressed as the reciprocal of the highest dilution showing positive reaction.

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Preparation of toxoid

The PMT toxoid was prepared as per Frandsen *et al.* (1991). The PMT was treated with formaldehyde to a final concentration of 0.35% and incubated for 2 hr at 37°C. The formaldehyde-treated toxin was adsorbed on aluminium hydroxide gel (adjuvant grade) to a final concentration of 20.0%. A 2-dose schedule was followed for immunization of mice and a single dose for rabbits.

Sterility and safety of the vaccine

The sterility of the vaccine was tested on blood agar plates and in RCM (Robertson Cooked Meat) media and incubated at 37°C for 24 hr and 48 hr, respectively, for any aerobic and anaerobic contamination. Safety test was carried out in 6 mice and 2 rabbits by inoculating 1.0 ml of the vaccine by s/c route.

Immunization schedule

The primary and secondary immunization in mice were done in 4 separate groups, comprising 15 mice in each group. A 2-dose vaccination schedule consisting 10 µg and 100 µg of *Pasteurella multocida* toxoid alongwith adjuvant was given to each mice by s/c route. Blood samples were collected on days 0, 14 and 27. While a single dose of 200 µg of toxoid alongwith the adjuvant was given to 2 groups of rabbit comprising 3 rabbits in each group by s/c route. The secondary vaccination was done with the same dose of vaccine on 14th

day. Blood samples were collected on days 0, 7, 14, 21 and 27.

Assessment of immunity

The immune responses in the vaccinated mice and rabbits were assessed by enzyme-linked immunosorbent assay (ELISA). ELISA test was performed as per Engvall and Pearlmann (1971). The reaction was arrested with 5 NH_2SO_4 and OD readings were taken at 492 mm in ELISA reader. The absorbance value of a dilution of the test serum which was more than double of the value obtained in the antibody control was considered as positive reaction and was taken as criterion for determining ELISA titres in different serum samples.

Challenge test

The challenge test was carried out in mice on 28th day of post inoculation with crude PMT containing 200 µg/ml and with live bacterial culture of serotype A:12 diluted to 10^{-9} by I/P and S/C route respectively. The control mice were also challenged as above. Whereas rabbits were challenged by 2 different routes, viz. I/D and I/N routes. One group of 3 rabbits received 400 µg of PMT by I/N route, the second group consisting of 2 rabbits received 300 µg by I/D route. The control animals were inoculated with the same dose and concentration. The challenged mice were observed for 7 days for any untoward reaction or death. Rabbits challenged by I/D route were observed for 7 days. While animals challenged by I/N route were observed for 1 month.

Statistical analysis

The titre values of ELISA obtained on different days post vaccination were transformed into $\log_{10}$. The transformed data were analysed statistically by adopting method of analysis of variance (ANOVA) and student 't' test for comparing different days values. The mice were eye bled along with the control animals on 27th day of post vaccination. The sera samples were pooled together for further test. Each vaccinated rabbit was bled on day 0, 7, 14, 21 and 27.

RESULTS

Lethal effect of cell sonicates

Among the 4 serotypes studied only cell sonicate preparation from *Pasteurella multocida* serotype A:12, A:3, 4, 12 and untyped strains proved lethal to mice on inoculation through I/V and I/P route in a dose of 0.2 ml. Mice died within 15 hr to 7 days.

Assay of dermonecrotic effect in rabbits

The toxin from cell sonicate of serotype A:12 produced dermonecrotic lesion in rabbits within 15 hr after intradermal injection. The titre of DNT was determined to be 1:128 containing 22 mg of the crude cell sonicate (PMT).

Determination of antitoxin titre in mice by ELISA

The mice vaccinated with 10 µg and 100 µg of toxoid showed a titre of 1 024 and 4 096 on 27th day at 492 mm in ELISA reader.

Determination of antitoxin titre in rabbits by ELISA

The results of post-immunization antibody titre in rabbits as determined by ELISA test are presented in Table 1.

The statistical analysis of the result showed an increase in serum titre with mean $\log_{10}$ values of 2.107±0.098, 3.087±0.110, 3.538±0.95 and 3.614±0.099 on days 7, 14, 21, and 27th day post vaccination with maximum titre on day 27.

Result of challenge test in mice

The mice vaccinated with 10 µg and 100 µg of toxoid gave protection up to 60.0 and 73.3%, respectively, when challenged with *Pasteurella multocida* toxin. Control mice died within 7 days. While mice vaccinated with the same 2 dose schedule on challenge with live culture gave a protection level of 46.6 and 53.33% respectively. Control mice died within 24-28 hr.

Result of challenge test in rabbits

Rabbits withstood the challenge dose of 400 µg and 300 µg of *Pasteurella multocida* toxin by I/N and I/D route respectively. While control rabbits showed fever, reduction in body weight and dermonecrosis.

DISCUSSION

Pasteurella multocida toxin (PMT) is associated with the atrophic rhinitis in swine and goat (Chanter *et al.* 1986, Dominick and Rimiler 1986 and Baalsrud 1987). The immune response in mice model when determined by ELISA indicated rise in the antibody titre with higher dose of vaccine. Foged *et al.* (1989) also reported direct relationship between ELISA titre and vaccine dose in sows. The titre was highest on day 27 after the booster dose on day 14.

Table 1. ELISA titre observed in the sera of immunized rabbits with *P. multocida* serotype A:12 toxoid

Rabbit no.	Days post immunization			
	7	14	21	27
1	64	256	4096	2048
2	256	2048	8192	4096
3	256	2048	8192	4096
4	65	1024	4096	2048
5	256	2048	2048	8192
6	128	2048	2048	8182
7	128	1024	2048	8192
8	64	1024	2048	2048

Animals were challenged on day 28th.

The challenges test result indicated that the mice vaccinated with a 2-dose schedule of 10 µg/dose and 100 µg/dose gave 60.0 and 73.3% protection when challenged with 200 µg of crude PMT by I/P route on day 28 of vaccination. The challenge test with live culture (0.2 ml of 10^{-9} dilution) gave a protection level of 53.33%. The challenge test result with live culture indicates that antitoxin in the serum may prevent infection from toxigenic strain of *P. multocida.* The result correlates with the findings of Nielsen *et al.* (1991).

Nakai *et al.* (1985) reported that PMT is located intracellularly, however, it is not clear how anti PMT antibody protects against challenge with organism lacking toxin surface antigen. It has been further suggested that PMT directly or indirectly enhances the colonization a process that would be inhibited in the presence of anti PMT antibodies (Foged *et al.* 1989).

The ELISA titre in rabbits on different days of post vaccination ranged from geometric mean of 127.94 to 4111.50. This suggests that the rabbits were having a high antibody titre from 21st day of post inoculation. The difference in the geometric mean was significant at $P<0.01$ level.

The vaccinated rabbits survived the lethal effect of toxin when given intranasally. The control rabbits showed a reduction in body weight and died after 15 days. Chrisp and Foged (1991) also reported mortality in rabbits when challenged with PMT through I/N route. The second group of vaccinated rabbits did not show any dermonecrotic effect when challenged with PMT by I/D route. While the third group of vaccinated rabbits did not show any dermonecrotic lesions when challenged with PMT by I/D route.

Frymus *et al.* (1989) also observed that serum antitoxin neutralized the PMT either given through I/N or I/M. These findings indicate that circulating antibodies are capable of protecting piglets against PMT produced either in the nasal cavity or in other parts of the body.

The above observation indicate that antibody against PMT is not localized in the nasal septum but also remain circulating in the blood.

The results accrued in the present study on vaccination suggest that animals vaccinated with a 2-dose schedule are more protective when challenged with crude PMT and with live culture in mice while rabbits withstood the challenge test by I/N and I/D route.

Therefore, a vaccine (toxoid) prepared from a highly toxigenic strain with a suitable adjuvant may induce a high level of protective antibody titre in animals from infection of toxigenic strains of *P. multocida.*

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