SHORT COMMUNICATIONS

Pasteurella multocida Infection in the Domestic Rabbit: Immunization with a Streptomycin-dependent Mutant

D.H. Percy, J.F. Prescott and J.L. Bhasin*

ABSTRACT

Fourteen *Pasteurella multocida*-free rabbits were inoculated intranasally with a streptomycin-dependent mutant of *P. multocida* serotype 12:A. Vaccinations with approximately 10^8 colony forming units were done on days 0, 14 and 28. Two weeks later the animals were separated into groups, which included 12 rabbits divided into two control groups of six unvaccinated *Pasteurella*-free animals. Seven vaccinated rabbits were challenged intranasally with the homologous virulent parent strain and the other seven vaccinates were challenged with a virulent strain of serotype 3:A. Rabbits were necropsied two weeks later. The vaccinated group challenged with the parent strain showed a more rapid nasal clearance of the organism than the vaccinated group challenged with the heterologous strain. However, the number of positive cultures of *P. multocida* recovered from tissues post-challenge were similar in vaccinated and control animals. In a significant number of animals, vaccination with serotype 12:A induced detectable antibody production to somatic antigens of both 12:A and heterologous strain 3:A.

Key words: *Pasteurella multocida*, immunization, rabbit, streptomycin-dependent mutant.

RÉSUMÉ

Cette expérience consistait à inoculer un mutant du sérotype 12:A de *Pasteurella multocida*, dépendant de la streptomycine, dans les voies nasales de 14 lapins exempts de *P. multocida*. La vaccination, avec environ 10^8 unités formatrices de colonies, se fit aux jours #0, #14 et #28. Deux semaines plus tard, on sépara les lapins en groupes, lesquels en incluaient 12, divisés en deux groupes de six témoins exempts de *Pasteurella*. Sept des 14 lapins vaccinés subirent une infection de défi intranasale avec la souche parente virulente homologue, tandis que les sept autres subirent le même genre d'infection, mais avec une souche virulente du sérotype 3:A. Deux semaines plus tard, on effectua la nécropsie de ces lapins. Ceux qui avaient subi l'infection de défi avec la souche parente affichèrent une clairance nasale plus rapide de la bactérie que ceux qui avaient subi l'infection de défi avec la souche hétérologue. Le nombre d'isolements de *P. multocida* réalisés à partir des tissus, après l'infection de défi, s'avéra toutefois semblable, chez les lapins vaccinés et chez les témoins. Chez un nombre appréciable de lapins, la vaccination avec le sérotype 12:A de *P. multocida* provoqua la formation décelable d'anticorps contre les antigènes somatiques de cette souche et de la souche hétérologue 3:A.

Mots clés: *Pasteurella multocida*, immunisation, lapin, mutant dépendant de la streptomycine.

Infections with *Pasteurella multocida* are an important disease problem both in the commercial rabbitry and the research laboratory (1). In surveys of the somatic and capsular types of *P. multocida* present in domestic rabbits, type 12:A is the most common isolate (2,3). Investigators have reported successful protection against homologous bacterial challenge following immunization with streptomycin-dependent mutants of *P. multocida* (4,5). We report on efforts to protect *Pasteurella*-free rabbits against challenge with a homologous or a heterologous strain following immunization with a streptomycin-dependent mutant of the 12:A parent strain.

Twenty-seven certified *Pasteurella*-free New Zealand white male rabbits each weighing approximately 2.5 kg were obtained from a commercial supplier (H.A.R.E. Rabbits for Research, Hewitt, New Jersey). In addition, nasal samplings and bacterial cultures for *P. multocida* were negative prior to beginning the vaccination trials. Animals were housed individually in stainless steel cages in two isolation rooms equipped with an anteroom and wash up area. Protective clothing, including surgical masks, were worn by all personnel while in the rooms. *Pasteurella multocida*, isolate R36, a serotype 12:A isolated from a case of *otitis media* in a rabbit was used for the production of SM-dependent mutants. The strain was passaged once on blood agar and then held frozen at -70°C. SM-dependent mutants were derived after mutagenizing the parent strain with n-methyl-n'-nitro-n-nitrosoguanidine

*Department of Pathology (Percy) and Department of Veterinary Microbiology and Immunology (Prescott), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 and the Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6 (Bhasin).

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(Sigma Chemical Co., St. Louis, Missouri) as previously described (6).

A selected stable SM-dependent mutant strain was inoculated on blood agar plates containing streptomycin at a concentration of 100 μg/mL of media. After 18-24 hours incubation, cells were harvested by suspension in 0.01 M phosphate buffered saline (PBS), pH 7.2 and diluted with sterile PBS to contain approximately 10^8 cells per mL. Vaccines were used within 30 minutes after their preparation. The 27 rabbits were divided into two groups on a random basis. The 14 rabbits in group 1 received 0.25 mL of the vaccine in each nostril on day 0, 14 and 28. The 13 rabbits in group 2 served as nonvaccinated controls. Nasal swabs were cultivated on blood agar containing no streptomycin post-vaccination for evidence of reversion.

Pasteurella multocida isolate R36 (serotype 12:A) and isolate R105 (serotype 3:A) were used for challenge. R105 was a once-passaged strain isolated from the lung of a rabbit with acute pneumonia. It was stored in lyophilizing fluid at -70° C. The strains were thawed and streaked on blood agar plates. Following 18 hour incubation, the cells were harvested by suspension in sterile PBS and standardized to contain approximately 3 x 10^{10} cells per mL. The immunity of the rabbits was challenged two weeks after the third vaccination. Vaccinated rabbits were divided into two groups on a random basis. Group 1 received 0.25 mL of the homologous challenge strain intranasally in each nostril on day 0. In view of the relatively rapid nasal clearance of the homologous strain, they were rechallenged with R35 on day 7. Group 2 was inoculated intranasally with the heterologous strain on day 0 only. The unvaccinated controls were similarly divided into two groups and inoculated intranasally with the homologous or heterologous challenge inoculum using the same schedule as for the comparable vaccinated group. All rabbits were observed for 14 days and the survivors were killed. All serum samples were tested for antibody response to the capsular and somatic antigens of both homologous and heterologous challenge strains by the indirect haemagglutination test (7) and counter immunoelectrophoresis (8,9) respectively. Counter immunoelectrophoresis was performed according to Carter and Chengappa (8) using 1% agarose gel (Pharmicia agarose B, Pharmicia Chemicals, Denmark) and tris barbital-sodium barbitol buffer pH 8.6, ionic strength 0.03 as described previously (9).

Prior to challenge, the vaccine was tolerated well by the vaccinated rabbits. Food and water consumption were not altered and vaccinates showed no clinical evidence of respiratory disease. When challenged with either the homologous of heterologous strain, both the nonvaccinated and vaccinated animals generally remained asymptomatic. One nonvaccinated animal challenged with the heterologous strain (3:A) died on the fifth day postchallenge and *P. multocida* was isolated from the turbinates, and in pure culture from the lungs and heart blood. With the homologous challenge, there was a difference in the rate of clearance of the organisms from vaccinated and nonvaccinated animals. There was no significant difference in the rate of clearance of the heterologous challenge strain from the nasal passages of the vaccinated and nonvaccinated animals (Table I). The recovery of *P. multocida* serotype 12:A or 3:A from nasal passages, lungs and ears of nonvaccinated and vaccinated rabbits necropsied two weeks postchallenge is shown in Table II. *Bordetella bronchiseptica* was isolated from the respiratory tract of the majority of the vaccinated and nonvaccinated animals. Purulent rhinitis and *otitis media* were observed in a few animals and minimal to mild peribronchial and perivascular lymphoid hyperplasia was observed in all challenged animals (Table II). There was no significant correlation evident between the isolations of *B. bronchiseptica* and the presence of microscopic lesions in the lower respiratory tract.

In nonvaccinated rabbits, the preimmune sera lacked precipitating antibodies against the heat stable (somatic) antigens of *P. multocida* serotypes 12:A and 3:A (Table III). There were low antibody titers against the capsular antigens of 12:A in some samples (Table IV). In vaccinated rabbits, the prechallenged sera of these rabbits revealed precipitating antibodies against both serotypes 12:A and 3:A (Table III). The hemagglutination titers against the capsular antigens of both serotypes remained low or undetectable prior to challenge (Table IV). Following challenge with 12:A, rabbits developed antibodies against the somatic antigens of both 12:A and 3:A. Challenge of vaccinated rabbits with serotype 3:A showed elevated antibody titers to both serotype 12:A and 3:A (Tables III, IV).

To our knowledge, this is the first attempt to protect rabbits against challenge with a heterologous *P. multocida* serotype by vaccination with a streptomycin-dependent strain of *P. multocida*. It is evident from our serological data that vaccination of rabbits with the SM-dependent strain results in an elevated antibody response

TABLE I. Nasal Carriage of *Pasteurella multocida* in Controls and in Rabbits Vaccinated with the Streptomycin-Dependent Strain 12:A Following Challenge with Homologous 12:A or Heterologous 3:A Strains

Days Following Intranasal Challenge	Challenge Strain 3:A		12:A	
	Vacc(7)[a]	Nonvacc(6)	Vacc(7)	Nonvacc(6)
2	3.7[b]	3.7	1.4[d]	3.0[d]
4	3.6[c]	3.4[c]	0.6[e]	1.8[e]
6	2.9	2.6	0.7[e]	2.0[e]
10	3.0	3.3	0.7	1.7
12	1.9	1.4	0.0	0.7
14 (Necropsy)	0.7	0.2	0.6	0.6

[a]Number of rabbits in parenthesis

[b]Mean of colony numbers per rabbit. (Based on number of colonies of *P. multocida* per blood agar plate) 1 = up to 10 colonies; 2 = 11-20 colonies; 3 = 21-30 colonies; 4 = > 30 colonies

[c]No significant difference in rate of bacterial clearance between vaccinated and nonvaccinated rabbits at any stage postinoculation (challenge with heterologous strain)

[d]No significant difference in rate of bacterial clearance between vaccinated and nonvaccinated rabbits at 2, 10, 12 and 14 days postchallenge

[e]Significant difference in rate of bacterial clearance between vaccinated and nonvaccinated rabbits at the 10% level (challenge with homologous strain)

TABLE II. Experimental *Pasteurella multocida* Infection in Rabbits: Bacteriological and Pathological Findings

Challenge Strain of *P. multocida*	Number in Group	Number of *P. multocida* Isolates[c]			Pathology[c]			
		Nasal	Lung	Ear	Rhinitis	Peribronchial Cuffing	Focal Alveolitis	*Otitis media*[a]
12:A (v)	7	1	3	0	3	7	3	0
12:A (nv)	6	1	2	0	3	6	2	0
3:A (v)	7	2	2	1	6	6	3	0
3:A (nv)	6[b]	2	2	3	4	5	2	3

(v) Vaccinated
(nv) Nonvaccinated
[a]Macroscopic evaluation
[b]One rabbit in group died with acute pasteurellosis at five days postchallenge
[c]No significant differences in % of positive *P. multocida* isolates and % with lesions in vaccinated and nonvaccinated groups at the 5% level

TABLE III. Comparison of Detectable Antibody Response to Somatic Antigens of Serotypes 12:A and 3:A in 12:A-Vaccinated and Nonvaccinated Rabbits

	No. Tested	No. with Detectable Antibody[c] to 12:A	to 3:A
Prechallenge			
Nonvaccinated	12	0[b]	0[b]
12:A Vaccinated	13	9	7
Postchallenge with 12:A			
Nonvaccinated	6	3	0[b]
12:A Vaccinated	6	5	5
Postchallenge with 3:A			
Nonvaccinated	5[a]	2	1
12:A Vaccinated	7	6	5

[a]One death due to acute pasteurellosis at five days postchallenge
[b]Proportion of animals with detectable antibodies significantly different between vaccinated and nonvaccinated groups at the 5% level (Fisher's exact test)
[c]Based on counter immunoelectrophoresis technique

TABLE IV. Comparison of Antibody Response to Capsular Antigens of Serotype 12:A and 3:A in Vaccinated and Nonvaccinated Rabbits

		Reciprocal Antibody Titers[a]								
	Antigen	0	20	40	80	160	320	640	1280	Total Positive
Prechallenge										
Nonvaccinated	12:A	8	2	2	—	—	—	—	—	4/12
	3:A	12	—	—	—	—	—	—	—	0/12
12:A Vaccinated	12:A	7	1	5	—	—	—	—	—	6/13
	3:A	12	1	—	—	—	—	—	—	1/13
Postchallenge with 12:A										
Nonvaccinated	12:A	—	—	1	1	—	2	1	1	6/6
	3:A	3	1	2	—	—	—	—	—	3/6
Vaccinated	12:A	—	—	—	3	1	1	1	—	6/6
	3:A	5	1	—	—	—	—	—	—	1/6
Postchallenge with 3:A										
Nonvaccinated	12:A	1	1	—	—	—	1	—	2	4/5
	3:A	4	—	—	1	—	—	—	—	1/5
Vaccinated	12:A	1	—	—	—	—	1	2	3	6/7
	3:A	5	—	1	1	—	—	—	—	2/7

[a]Based on indirect hemagglutination technique

against the somatic antigens of both the homologous and heterologous serotypes of *P. multocida*. Lipopolysaccharide (10) and lipopolysaccharide protein complexes (11) which are the parts of the somatic complex of the organism (9), have been shown to elicit protective antibody response in certain animal species. In the present study, the demonstration of antibodies against the heat stable (lipopolysaccharide) antigens of the homologous and heterologous serotypes indicates that the SM-dependent mutant of *P. multocida* may induce an immune response to a heterologous strain. Whether this will induce cross-protection has not been determined. However, there were no significant differences in the number of isolations of *P. multocida* from tissues of vaccinated and control groups at necropsy following challenge with serotype 12:A or 3:A. There was essentially no difference in the immune response of the vaccinated or nonvaccinated rabbits to the capsular antigens of the parent serotype 12:A or 3:A, while an elevated response to these antigens was observed in some animals after challenge with parent serotypes 12:A or 3:A. This finding suggests that capsular antigens may be lacking in the SM-dependent mutant strain of *P. multocida*. Whether capsular antigens are essential in affording protection or cross-protection against *Pasteurella* in rabbits (12) currently has not been clearly defined. Regarding the presence of low titers of antibody to the capsular antigens of *P. multocida* in some nonvaccinated animals prior to challenge, cross-reactions have been detected between *P. multocida* and other Gram-negative organisms (13). In summary, it is evident that further studies are required to develop better methods of challenge and to define the protective antigens of the organism in more detail before vaccination can be endorsed as a means of control of lapine pasteurellosis.

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