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THE INEFFICACY OF POLIVALENT *PASTEURELLA MULTOCIDA* VACCINES FOR SHEEP

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ABSTRACT

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Immunity assays on sheep sera using passive mouse protection tests showed that vaccines containing more than 4 strains of *Pasteurella multocida* did not give a good immunity. The immune response was not enhanced by the use of an oil adjuvant, and high concentrations of bacteria had only a partial positive effect.

Attempts to extract selectively the protection-inducing antigen(s) from *P. multocida* by veronal, phenol or potassium thiocyanate extraction were unsuccessful. Furthermore, it was found that sheep antisera to the recognized type strains of *P. multocida* afforded only limited protection against a number of field strains.

We concluded from this that successful immunization against ovine pasteurellosis will depend on either the identification of a strain of *P. multocida* that gives a wide spectrum of immunity or the discovery of a live mutant suitable for vaccine production and the definition of cultural conditions that promote the expression of a common immunizing antigen.

INTRODUCTION

In a previous publication (Cameron, Pienaar & Vermeulen, 1980), it was reported that only a limited degree of cross-immunity exists among *Pasteurella multocida* type A strains, despite their being serologically indistinguishable, and no correlation could be established between protection and antibody titres (Cameron, Engelbrecht & Vermeulen, 1978). In an effort to find a solution to the practical problems posed by the above findings, 3 avenues of investigation were pursued.

Firstly, the possibility of composing an effective polyvalent bacterin with different adjuvants was investigated. Such a bacterin should afford protection not only against a wide range of *P. multocida* Types A and D strains, but also against various serotypes of *P. haemolytica*, since this organism is very commonly found in pneumonia and other infections in sheep (M. M. Henton, personal communication 1982).

Secondly, should this approach prove to be unsuccessful or impractical, the possibility of using soluble extracts of the bacteria would be investigated. If selective extraction of the immunizing antigen(s) could be effected, a composite product that would contain the relevant antigens only and would avoid subjecting the host animal to an excessively large number of bacterial antigens that are irrelevant to the establishment of protective immunity could possibly be formulated.

Thirdly, should all attempts to establish a polyvalent vaccine fail, it was proposed to establish which immunotypes of *P. multocida* are the most prevalent in South Africa, as suggested by Tereszczuk (1965). For this purpose, antisera were prepared in sheep against all the recognized serotypes of *P. multocida* and the ability of these sera to afford protection against a number of field isolates was determined.

MATERIALS AND METHODS

Bacterial strains

All the type strains of *P. multocida* used in this study were referred to in a report by Cameron *et al.* (1980). The field isolates were all derived from cases of fatal ovine pneumonia.

Experimental animals

Mice: Conventional male albino mice (4–6 weeks old) were used throughout. They were housed in plastic cages and fed a commercial pelleted ration.

Sheep: Young adult Dorper rams (6–12 months of age) were used for testing the polyvalent vaccines and for preparing the antisera.

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Preparation and assay of vaccines

Formalin-inactivated, alum-precipitated bacterins were prepared in shake flasks as described previously (Cameron *et al.*, 1978). The density of the cells was adjusted according to the requirements of each particular experiment. Vaccines prepared from dried bacteria and extracts were prepared as described by Cameron *et al.* (1980). For the oil emulsion vaccine, the packed cell volume was increased fivefold and emulsified in a mineral oil, as outlined elsewhere (Cameron, 1982).

The vaccines that were prepared from dried material were assayed by active immunity experiments in mice (Cameron *et al.*, 1978). The various polyvalent products were assayed in sheep. For each vaccine 3 sheep were immunized by the administration of 2 doses of vaccine at 4-week intervals, using the dosages given in Table 1. The sheep were bled 14 days after the last injection and the pooled sera were assayed, as described elsewhere (Cameron *et al.*, 1978).

Bacterial extracts

Phenol and veronal extracts for *P. multocida* Strain A14 g (A:3) were prepared, as described previously (Cameron *et al.*, 1980), and the potassium thiocyanate (KSCN) extract was prepared, as described by Gaunt, Moffat & Mukkur (1977).

Hyperimmune sheep antisera

Hyperimmune sera were prepared in sheep against the *P. multocida* strains listed in Table 5 by the method of Perreau, Perreau, Botto & Vallée (1970), except that the dosages for the intravenous injections were halved, because some sheep showed shock reactions when the full dose was used. Three sheep were used for each strain. Their sera were pooled and the haemagglutination, agglutination titres and precipitating properties, using phenol extracts as antigen, were determined (Cameron *et al.*, 1980). The protective properties of the pooled antisera to the homologous and field strains of *P. multocida* were assayed in mice (Cameron *et al.*, 1978).

RESULTS

Efficacy of polyvalent vaccines

The results of a series of experiments designed to compare the efficacy of various *Pasteurella* vaccines in sheep are given in Table 1. Although the number of sheep that could be used to assay each vaccine was limited, useful information was nevertheless obtained.

TABLE 1 Immunity in sheep induced by various vaccines as determined by passive mouse protection tests

Vaccine	Dosage (mℓ)	Final concentration per strain % packed cells	Logs protection		
			A14g (A:3)	33191 (D:3)	P1059 (A:8)
A Alum-precipitated 10 strains	5	0,15	0,3	0,1	0,7
B Alum-precipitated 10 strains	5	0,3	1,2	1,0	0,5
C Alum-precipitated 6 strains	5	0,15	2,0	0,2	0,6
D Alum-precipitated 3 strains	5	0,3	4,6	1,2	4,8
E Oil emulsion 3 strains	2	0,3	2,6	0,9	1,7
F Alum-precipitated 4 strains	2	0,25	4,3	0,7	1,3

TABLE 2 Spectrum of immunity induced by a vaccine composed of 4 strains of *Pasteurella*

Vaccine composition	Challenge strain									
	A14g (A:3)	DII (D:3)	M4 (A:1)	TS8 (A:5)	A11 (A:7)	P1059 (A:8)	"Liver" (A:9)	M17 (D:4)	D6 (D:6)	
	Logs protection									
P. multocida A14(A:3) *P. multocida* DII(D:3) *P. haemolytica* I29 (1) *P. haemolytica* I28 (2)	1,8	1,8	2,0	0,3	0,0	0,6	0,1	0,1	1,2	
	Challenge strain									
	S1 (A)	7675A (NT)	4009 (A)	1676 (NT)	74 (NT)	135 (A)	17785 (NT)	8467 (A)	8473 (A)	NM (D)
	Logs protection									
	0,0	0,9	0,5	0,6	0,0	0,0	0,0	0,0	0,0	0,0

NT = Not typeable

Vaccine A, which contained 10 different strains at a final concentration of 0,15% packed cells, produced a poor response. Better results were obtained when the concentration of the vaccine was increased to 0,3% packed cells per strain (Vaccine B). A reduction in the number of strains to 6 (Vaccine C) gave a better response to Strain A14 (A:3). A further reduction in the number of strains to 3 resulted in a product (Vaccine D) which gave very good protection to the homologous vaccine strains. A similarly composed oil emulsion vaccine (Vaccine E), however, did not give comparable results. A product containing 4 strains (0,25% packed cells per strain) in a 2,0 mℓ dose (Vaccine F) also gave good results and is preferable both from a production and an application point of view.

A vaccine similar to the above but containing 2 strains of *P. multocida* and 2 strains of *P. haemolytica* was assayed for its ability to induce immunity to a series of heterologous type strains as well as a series of field isolates of *P. multocida*. The results given in Table 2 show that a good immunity was obtained against both the *P. multocida* vaccine strains and the strains A1 (A:1) and D6 (D:6) and a slight immunity was demonstrable to strain TS8 (A:5) and P1059 (A:8). A low level of immunity could be demonstrated to strain 7675A; 4009; and 1676 (untypeable), but no protection against the other 7 field strains that were tested was evident.

Evaluation of chemical extracts

The results presented in Table 3 show that a vaccine prepared from a veronal extract gave a degree of immunity equal to the immunity induced by whole cells. Incorporation of the extract in Freund's complete adjuvant (Difco) had no beneficial effect.

When lower doses of the extract were compared with equivalent concentrations of whole cells (Table 4), virtually identical results were obtained at each level that was tested. No selective extraction of the immunizing antigen was therefore obtained.

TABLE 3 Comparison of the immunogenicity of *P. multocida* strain A14(A:3) whole cells and extracts in mice

Vaccine*	Antigen concentration	Logs protection
Wet cells alum-precipitated	1% packed cells	4,0
Wet cells oil emulsion	1% packed cells	3,7
Dry cells alum-precipitated	2,5 mg/mℓ	3,4
Dry cells oil emulsion	2,5 mg/mℓ	3,0
KSCN extract alum-precipitated	2,5 mg/mℓ	0,5
KSCN extract oil emulsion	2,5 mg/mℓ	0,6
Veronal extract alum-precipitated	2,5 mg/mℓ	4,0
Veronal extract oil emulsion	2,5 mg/mℓ	3,6
Medium only alum-precipitated	—	0,4
Medium only oil emulsion	—	0,3

* Freud's complete adjuvant (Difco) was used to prepare the oil adjuvant vaccines

TABLE 4 Quantitative comparison of dry *P. multocida* strain A14(A:3) cells and veronal extract in mice

Vaccine	Concentration mg/mℓ	Logs protection
Dry cells	0,5	1,7
Veronal extract	0,5	1,6
Dry cells	0,1	1,5
Veronal extract	0,1	1,0
Dry cells	0,02	0,4
Veronal extract	0,02	0,2

Immunological relationship among P. multocida *strains*

Since composite vaccines did not give a wide spectrum of immunity in sheep, an attempt was made to identify the prevailing immunotypes isolated from sheep pneumonia. To this end, the ability of hyperimmune sheep sera that were prepared against a series of type strains to afford protection to 5 field isolates was determined. The results given in Table 5 show that all the Type D antisera provided protection against the 2 type D

TABLE 5 Protective properties of *P. multocida* hyperimmune sheep antisera against heterologous field strains

Type strain antisera	Homologous serological titres			Homologous protection logs	Heterologous protection logs				
	Haemagglutination titre (reciprocal)	Agglutination titre (reciprocal)	Precipitin reaction		Field strains				
					33191 (D)	NM (D)	4009 (A)	135 (A)	15121 (A)
A:1	0	20	+	0,0	0,3	0,0	0,4	0,7	0,2
A:3	2	160	+	1,5	0,0	0,0	0,0	0,0	0,0
A:5	8	160	−	1,2	0,3	0,0	0,0	0,6	0,0
A:7	0	5	−	1,6	0,0	0,1	0,0	0,4	0,0
A:8	16	2 560	+	1,2	0,0	0,0	0,0	0,6	0,3
A:9	2	640	±	2,8	0,2	1,1	0,0	0,0	0,0
B:2	256	10	+++	5,0	0,0	0,0	0,0	0,0	nt
B:6	256	10	++	4,0	0,0	0,0	0,4	0,0	nt
D:2	0	5	−	4,0	1,8	2,6	0,0	0,0	0,0
D:4	16	10	−	1,7	3,0	3,1	0,0	2,7	1,9
D:6	0	10	−	1,6	3,0	4,2	0,4	0,2	0,0
E:12	32	40	−	5,5	0,0	0,0	0,0	0,0	nt

isolates (Strains 33191 and NM). None of the antisera provided protection against Strain 4009 (Type A), but mice that were challenged with Strains 135 (Type A) and 15121 (Type A) were protected by D:4 antiserum.

Discussion

The results of the experiments presented in this paper give rise to a particular predicament with regard to effective immunization of sheep against pasteurellosis. On one hand it was shown that vaccines containing 6 or more strains of *P. multocida* do not afford good protection, even against the vaccine strains. On the other hand, vaccines containing 3 or 4 strains do give good homologous immunity, but the spectrum of immunity to heterologous strains is poor. Moreover, attempts to extract selectively the immunizing antigen from bacteria failed, and a limited investigation into the immunological relationship among *P. multocida* strains revealed that non of the antisera against the recognized Type A strains afforded protection against the 3 Type A field isolates. However, it was found that a very distinct cross-immunity existed among the various Type D strains tested. Effective immunity to all type D strains should therefore pose no problem, provided that a vaccine of adequate potency is used.

Nevertheless, there are 2 avenues of investigation that may yet solve the problem of effective immunization against *P. multocida* Type A strains. Firstly, it has been found that *P. multocida* grown *in vivo* expresses antigens that are not found *in vitro*, and vaccines prepared from such bacteria will afford protection against heterologous strains (Heddleston & Rebers, 1972; Rimler & Rhoades, 1981). Similarly, in the case of *P. haemolytica*, a cytotoxin is only produced under specific and exacting conditions of cultivation (Baluyut, Simonson, Bemrick & Maheswaran, 1981; Chang, Richards & Renshaw, 1982; Himmel, Yates, Lauerman & Squire, 1982; Shewen & Wilkie, 1982). If the right cultural conditions could be established, it may thus be possible for *P. multocida* to express an antigen that could afford universal immunity.

In the second instance, it has been reported that a degree of cross-immunity does sometimes occur between serologically unrelated strains (Cameron, Engelbrecht & Vermeulen, 1978), and this is probably due to common antigens that are not reflected in the conventional serological tests. Such cross-protection has also been reported to occur between different species of *Pasteurella* (Makkur, 1977).

Live mutants have been used successfully (Linde, 1977; Wei & Carter, 1978), and Kucera, Wong & Eis (1981) recently showed that certain mutants of *P. multocida* can induce an immunity to various unrelated types. Also, in this study, it was found that hyperimmune sheep antiserum to Strain D4 protected mice against infection with a number of Type A strains. Therefore, if the ideal strain can be found and the precise cultural conditions can be defined, it may yet be possible to solve this tantalizing problem.

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