

LACK OF CROSS-IMMUNITY AMONG *PASTEURELLA MULTOCIDA* TYPE A STRAINS

C. M. CAMERON, LORRAINE PIENAAR and ALETTA S. M. VERMEULEN, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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Active and passive protection studies in mice using sheep antisera revealed that the immunological relationship among *Pasteurella multocida* Type A strains could not be correlated with their serological relationship as determined by a haemagglutination or an agglutination test. Furthermore, strains possessing similar phenol extractable antigens or heat stable antigens did not provide complete cross-protection.

The conclusion was reached that immunity to *P. multocida* Type A strains is induced by an antigen which is strain specific and not detectable by existing serological typing systems. The immunological relationship of strains can therefore not be predicted on the basis of their serological characteristics.

Résumé

ABSENCE D'IMMUNITÉ CROISÉE PARMI LES SOUCHES DE TYPE A DE PASTEURELLA MULTOCIDA

Des études de protection passive et active faites sur des souris en utilisant des antisérums de mouton ont révélé que la relation immunologique parmi les souches de type A de *Pasteurella multocida* ne pouvait pas être en corrélation avec leur relation sérologique comme il a été déterminé par un test d'haemagglutination ou un test d'agglutination. De plus, les souches possédant des antigènes extractibles au phénol ou des antigènes stables à la chaleur ne procurèrent pas de protection croisée complète.

La conclusion atteinte fut que l'immunité aux souches de type A *P. multocida* est induite par un antigène qui est spécifique de la souche et non détectable par des systèmes de type sérologique actuels. La relation immunologique des souches peut donc être prédite sur la base de leurs caractéristiques sérologiques.

INTRODUCTION

Appreciable convincing evidence has been published to show that, in the case of *Pasteurella multocida* Types B and E, immunological specificity resides in the polysaccharide capsule (Perreau, Petit, Bergeron, Gayt & Marquet, 1963; Penn & Nagy, 1974; Nagy & Penn, 1976) and that the passive haemagglutination test which detects antibodies to this antigen is a reliable criterion for assessing the immune status of immunized animals (Carter, 1964; Dua & Panduranga Rao, 1978).

In contrast to the above, Cameron, Engelbrecht & Vermeulen (1978) showed that determination of haemagglutinating antibodies is not a valid criterion for assessing the immune status of animals with respect to immunity to *P. multocida* Types A and D. Moreover, strains of *P. multocida* which, according to the haemagglutination test, are serologically related, do not necessarily afford cross-immunity, whereas unrelated strains may exhibit an appreciable degree of cross-protection. The deduction was therefore arrived at that immunity to *P. multocida* Type A strains is induced by an antigen or antigens other than those which are operative in the passive haemagglutination test.

As outlined previously (Cameron *et al.*, 1978), other serotyping systems have been developed for *P. multocida* and experiments were conducted to determine whether these would offer a closer correlation with actual immunological relationships in terms of cross-protection among *P. multocida* Type A strains. In this respect the somatic antigen typing system employing agglutination tests as developed by Namioka & Murata (1961) and applied by Perreau using hyaluronidase treated antigens (personal communication, 1978), was first investigated. Strong evidence has been presented by Louembe (1976) that agglutinating antibodies in rabbit antisera bear

a direct correlation with type specific immunity when tested in mice, but Alexander & Soltys (1973) found no apparent relationship between the titre of agglutinins and protection in turkeys.

The serotyping method devised by Heddleston, Gallagher & Rebers (1972) employs a heat-stable antigen in agar gel precipitin tests and is chiefly used for typing *P. multocida* strains isolated from poultry. Since it has been found that vaccines prepared from strains identified by this technique give only homologous immunity (Heddleston, Gallagher & Rebers, 1970), the significance of this antigen as an indicator of immunological specificity was also investigated. The value of crude phenol and veronal buffer extracts were also studied in this connection.

MATERIAL AND METHODS

Bacterial strains

The following strains of *P. multocida* were kindly supplied by Dr P. Perreau*: Strain M.4 (Type A:1); Strain A.14 (Type A:3); Strain Ts.8 (Type A:5); Strain A.11 (Type A:7); Strain P-1059 (Type A:8) and Strain 'Liver' (Type A:9). Strain P-1059 (Type 3) was also obtained from Dr K. R. Rhoades**.

P. multocida Strain 33191 (sheep pneumonia) was typed as Type D:3 and *P. multocida* Strains 4009 (sheep pneumonia); SI (sheep septicaemia) and 7477 (bovine pneumonia) which were isolated at the Veterinary Research Institute, Onderstepoort, were all typed as Type A:3 by Dr P. Perreau (personal communication, 1979). Strain 125 was isolated from a case of porcine pneumonia and typed at the Institute as Type A:8, according to the method of Perreau (*vide infra*).

* Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, 10, Rue Pierre-Curie, 94700, Maissons-Alfort, France

** National Animal Disease Centre, P.O. Box 70, Ames, Iowa 50010, U.S.A.

Strains A.14, 33191, SI and 7477 were all typed by Dr K. R. Rhoades as Type 3 (Rhoades, personal communication, 1979).

The avirulent Strain 5727 was isolated from a case of rhinitis in a rabbit and could not be serotyped.

Experimental animals

Mice. Conventional male albino mice were obtained from the closed colony maintained at this Institute. They were housed in plastic cages and fed a balanced pelleted ration.

Rabbits. New-Zealand type albino rabbits were obtained from the colony maintained at this Institute. They were kept in wire cages and fed a commercial pelleted ration.

Sheep. Cross-bred Dorper Merino wethers were used for the preparation of hyperimmune sheep antiserum to Strains A.14 (A:3) and P-1059 (A:8).

Vaccines

Inactivated vaccines. Formalin inactivated, alum-precipitated vaccines were prepared as described previously (Cameron *et al.*, 1978). The packed cell volume was 1,0% and the dosage was 0,2 ml per mouse.

The composite polyvalent vaccine was prepared by growing each strain separately on B & J medium (Bain & Jones, 1958), adjusting the density to 1,0% packed cell volume and, after inactivation was completed, mixing equal volumes of each strain and precipitating with potassium alum. The following strains were included in the polyvalent vaccine: M.4 (A:7); A.14 (A:3); Ts.8 (A:5); A.11 (A:7); P-1059 (A:8); 'Liver' (A:9); 'Kobe 6' (D:2); M.17 (D:4); (D:6) and 33191 (D:3).

Live vaccine. *P. multocida* strain 5723 was grown as for the production of inactivated vaccine. The bacteria were harvested by centrifugation, resuspended in tryptone water and the density adjusted nephelometrically to give either $2,5 \times 10^7$ or 5×10^8 live organisms per ml.

Antigen extracts

Heat stable antigen. *P. multocida* strain A.14 (A:3) cells were produced in liquid culture. Extraction of heat-stable antigen was based on the procedure of Heddleston *et al.* (1972), modified as follows: 30 g of packed cells was suspended in 200 ml of 0,15 M NaCl, washed once and resuspended in 200 ml of phosphate buffered saline (pH 7,3) containing 0,5% formalin. The suspension was then autoclaved for 15 min at 120 °C, allowed to cool, and the bacterial cells sedimented by centrifugation. The supernatant fluid was dialyzed against 2 changes of 5 l of distilled water at 4 °C over 48 h and concentrated approximately tenfold by preevaporation. The concentrated extract was then lyophilized in 2,0 ml quantities.

Phenol extracts. The method used for extraction of Strain A.14 (A:3) was based on the procedure described by Sutherland & Wilkenson (1971) for capsular material.

Bacteria were grown as for vaccine production, washed 3 times with 0,15 M NaCl and resuspended in distilled water to give a 10% concentration of packed cells. One volume of cell suspension, pre-heated to 65 °C, was mixed with 2 volumes of a 90% phenol solution also at 65 °C. The mixture was

intermittently shaken at 65 °C in a water-bath for 30 min and then cooled to approximately 10 °C. One volume of distilled water was added to the mixture and thoroughly mixed. The solution was then centrifuged in 500 ml glass centrifuge bottles at $3000 \times g$ for 30 min. The liquid phase was carefully removed and the extracted material precipitated by the addition of 5 volumes of cold ethanol containing 0,2% sodium acetate. The solution was kept at 4 °C for 30 min, after which the precipitate was recovered by centrifugation, dissolved in a small quantity of distilled water and dialyzed against 40 volumes of distilled water for 24 h. Any sediment which formed was removed by centrifugation and the supernatant fluid was lyophilized in 2,0 ml volumes.

An adaptation of the above procedure was used when only small quantities of extract were required for precipitin tests and when the bacteria could not be weighed.

Bacteria obtained from 100 ml of a shake culture were washed, suspended in 8 ml of 0,15 M NaCl and extracted with 16 ml of 90% phenol as above. After addition of 24 ml of distilled water, the mixture was centrifuged and the extract precipitated from the water phase by the addition of 125 ml of cold ethanol. The resultant precipitate was collected by centrifugation and dissolved in 5 ml of distilled water. This solution was used as such in the precipitin tests.

Veronal extract. The method used in this case was adapted from the procedure described by Barber & Eylan (1972) for preparing protein extracts.

A 5% suspension of washed bacteria of Strain A.14 (A:3) was prepared in 0,2 M veronal buffer pH 8,4 and stirred for 48 h at 37 °C. The cells were removed by centrifugation at $5000 \times g$ and tri-chloroacetic acid was added to the supernatant fluid to give a final concentration of 20% w/v. The precipitate was collected by centrifugation, dissolved in the smallest effective volume of 0,1 N NaOH and the pH adjusted to 7,3. The solution was dialyzed against distilled water as for the phenol extract and lyophilized.

Preparation of antisera

Rabbits. Antisera for serotyping purposes and for precipitin tests were prepared as suggested by Perreau (personal communication, 1978). Bacteria were grown on blood tryptose agar (Difco)* for approximately 18 h and washed off with 0,15 M NaCl containing 0,5% formalin and the density nephelometrically adjusted to correspond to Brown's opacity tube No. 10. The suspensions were kept overnight at room temperature and, after confirmation that the suspension was inactivated, they were emulsified with an equal volume of Freund's complete adjuvant (Difco)*.

Two rabbits for every strain were each injected subcutaneously with 1,0 ml ($2 \times 0,5$ ml at different sites) and rested for 1 month. The rabbits were subsequently given a series of intravenous injections of inactivated bacteria suspended in saline to a density corresponding to Brown's opacity tube No. 3. Two injections of 1,0 ml each were given per week for 3 weeks and the rabbits bled 7 days after the last injection. Sera were kept at -20 °C.

Sheep. Antisera for use in passive mouse protection tests were prepared in sheep as described by Perreau, Perreau, Botto & Vallée (1970).

* Difco Laboratories, Detroit, Michigan, USA

Serology

Haemagglutination. Passive haemagglutination tests were done as described previously (Cameron *et al.*, 1978), except that the supernatant fluid obtained from hyaluronidase-treated cells was used to sensitize the erythrocytes (*vide infra*).

Agglutination. Plate-agglutination tests for serotyping purposes were done with hyaluronidase-treated cells (Carter, 1972). The overnight growth from 1 Mason tube blood tryptose agar culture was suspended in 5 ml of phosphate buffered saline (0,15 M pH 6,0) and heated for 30 min at 60 °C. Subsequently, 112 units of hyaluronidase was added to each tube. After being incubating for 4 h at 37 °C, the cells were sedimented by centrifugation at 3000 × g and the supernatant fluid used for the haemagglutination tests. The cells were resuspended in 5,0 ml 0,15 M NaCl, washed once, resuspended in 5,0 ml 0,15 M NaCl and used for the plate agglutination tests.

Somatic typing sera were rendered specific by absorption with hyaluronidase-treated bacteria. Cells obtained from 1 Mason tube were used to absorb 5 ml of 1:2, 1:5, 1:10 or 1:20 dilutions of antiserum. The cells were mixed with the sera which were kept for 2 h at 50 °C. After absorption, the bacteria were removed by centrifugation, the serum tested and, if necessary, re-absorbed with the appropriate strains.

Tube agglutination tests on sheep sera were also carried out, using hyaluronidase-treated cells. The antigen was nephelometrically adjusted to a density equivalent to standard *Brucella abortus* antigen. Twofold serial dilutions of the sera were prepared in 1,0 ml volumes and an equal volume of antigen added to each tube. The tubes were incubated overnight at 37 °C and the titre was taken as the highest serum dilution showing complete agglutination.

Gel precipitin tests. Precipitin tests were done on microscope slides or in Petri dishes with 1% Ion agar on the slides and 0,9% Ion agar in the plates. The preparations were kept in a humid atmosphere for 24 or 48 h at 28 °C, during which the precipitin reactions were recorded photographically.

Active immunity experiments

Active immunity experiments in mice using whole cell vaccines were conducted according to the method of Ose & Muenster (1968) and assessed as described previously (Cameron *et al.*, 1978). Briefly, a nephelometrically standardized suspension of bacteria was simultaneously titrated in groups of immunized and non-immunized mice. The LD50 in both groups was calculated and the difference taken as the logs protection afforded by a particular vaccine or antiserum. For determining the immunogenicity of extracts, the particular product was dissolved in Bain & Jone's broth (2,5 mg/ml) containing 0,5% formalin and the solution precipitated with alum as for vaccine preparation. The mice were immunized by 2 subcutaneous injections of 0,2 ml of the material at 4-week intervals and challenged 14 days later as for whole cell vaccines. Control mice received formalinized alumprecipitated broth without any antigen extract.

Passive mouse protection test

The protective value of sheep antisera was assayed in mice as described previously (Cameron *et al.*, 1978).

RESULTS

Lack of cross-immunity among *P. multocida* Type A strains

The results of an experiment in which the immunological relationship of some Type A strains was examined are shown in Table 1. From the data it is evident that with the exception of Strain Ts.8 (Type A:5) all the strains gave a good homologous active immunity in mice but that, apart from Strain P-1059 (A:8), which gave some protection to Strain A.14 (A:3), there was no evidence of any cross-protection among the strains. It is thus apparent that the capsular antigen A is generally not involved in the induction of immunity.

TABLE 1 Lack of active cross-immunity in mice among *P. multocida* Type A strains

Vaccine strain	Logs protection				
	Challenge strain				
	A.14 (A:3)	Ts.8 (A:5)	A.11 (A:7)	P-1059 (A:8)	125 (A:8)
A.14 (A:3)...	4,0	0,14	0,13	nt	nt
Ts.8 (A:5)...	1,3	0,1	0,13	nt	nt
A.11 (A:7)...	0,9	0,0	2,6	nt	nt
P-1059 (A:8)	2,7	0,4	nt	5,0	0,7

nt = not tested

Lack of cross-immunity among *P. multocida* Type A:3 strains

To investigate the role of the somatic antigen in immunity, an examination was made of the immunological relationship among a number of serologically identical strains.

TABLE 2 Lack of active cross-immunity in mice among *P. multocida* Type A:3 strains

Vaccine strain	Number of injections	Logs protection			
		Challenge strain			
		A.14 (A:3)	4009 (A:3)	SI (A:3)	7477 (A:3)
A.14 (A:3)	1	1,9	0,8	1,04	0,0
	2	4,0	0,8	0,0	0,5
4009 (A:3)	1	0,8	1,2	0,3	1,5
	2	2,6	1,3	0,0	2,3
SI (A:3)	1	0,9	0,06	0,8	0,16
	2	1,5	0,0	0,9	0,5
7477 (A:3)	1	0,8	0,6	0,3	4,4
	2	2,1	0,9	0,0	6,0

It is evident from an examination of the results given in Table 2, that none of the strains examined was able to produce effective protection against all the strains and, although there is evidence of cross-protection in some instances, the immunity afforded by the homologous strain was usually the best. It is further noteworthy that Strain SI did not give a good immunity even against itself and, although it is a highly pathogenic strain, it is apparently poorly immunogenic.

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TABLE 3 Failure of *P. multocida* Strain A. 14 (A:3) sheep antiserum to protect mice against different heterologous *P. multocida* Type A:3 strains

		Challenge strain			
		A.14 (A:3)	4009 (A:3)	SI (A:3)	7477 (A:3)
Antiserum, Sheep 8121.....	Haemagglutination titre.....	256	256	128	64
	Agglutination titre.....	128	128	auto aggl.	32
	Logs protection.....	3,7	0,6	0,6	0,3
Antiserum, Sheep 8117.....	Haemagglutination titre.....	64	64	16	16
	Agglutination titre.....	256	128	auto aggl.	256
	Logs protection.....	3,9	nt	nt	0,0

nt=not tested

This observation was further supported by the results of an experiment in which the ability of sheep antisera to Strain A.14 (A:3) to protect mice against other Type A:3 strains was assayed (Table 3).

Despite the existence of high agglutination titres (which primarily reflect antibodies to somatic antigens), Serum 8121 did not protect against Strains 4009, SI or 7477 and Serum 8117 gave no protection against Strain 7477.

Therefore, as in the case of the capsular A antigen, antibodies to the somatic 3 antigen do not appear to play a prominent role in mediating immunity.

Immunogenicity of antigen extracts

In a further attempt to elucidate the immunological role of specific antigens, the immunogenicity of 3 different extracts as well as the protective value of their respective antisera were investigated. All 3 extracts produced multiple precipitin lines with a mixture of selected rabbit antisera and are obviously not pure (Fig. 1).

Table 4 shows that all 3 extracts produced active immunity in mice and their respective antisera were able effectively to protect mice.

Since the phenol extract gave both a good active immunity and revealed a clear dominant precipitin line, its possible role in mediating cross-immunity among strains was further investigated. For this purpose 4 strains, whose phenol extracts reacted with Strain A.14 (A:3) rabbit whole-cell antiserum (Fig. 2a), were selected and the ability of Strain A.14 (A:3) sheep antiserum to protect mice against them was determined. The common phenol extractable

antigen possessed by these strains is shown clearly in Fig. 2b in which antisera to these strains were tested against Strain A.14 (A:3) phenol extract.



FIG. 1 Antigenic composition of various extracts of *P. multocida* Strain A. 14 (Type A:3)

Well 1—Heat-extracted antigen 5 mg/ml; Well 2—Phenol-extracted antigen 10 mg/ml; Well 3—Veronal-extracted antigen 5 mg/ml; Well 4—Heat-extracted antigen 5 mg/ml; Well 5—Phenol-extracted antigen 10 mg/ml; Well 6—Phenol-extracted antigen 10 mg/ml; Well 7—Pooled rabbit antiserum to *P. multocida* Strain A.14 whole cells.

TABLE 4 Immunity afforded by vaccines and antisera prepared from whole cells and various extracts of *P. multocida* A.14 (A:3) to challenge with *P. multocida* A.14 (A:3)

Antigens used to prepare vaccines and antisera	Logs protection			
	Vaccine		Antisera	
	1 injection	2 injections	Prepared in rabbits	Prepared in sheep
Whole cells.....	2,1	2,6	6,0	3,3
Phenol extract.....	1,1	2,0	4,2	2,5
Veronal extract.....	0,3	1,4	4,4	3,4
Heat extract.....	0,7	1,1	nt	4,6
Hyaluronidase-treated whole cells.....	0,2	2,7	nt	nt

nt=not tested

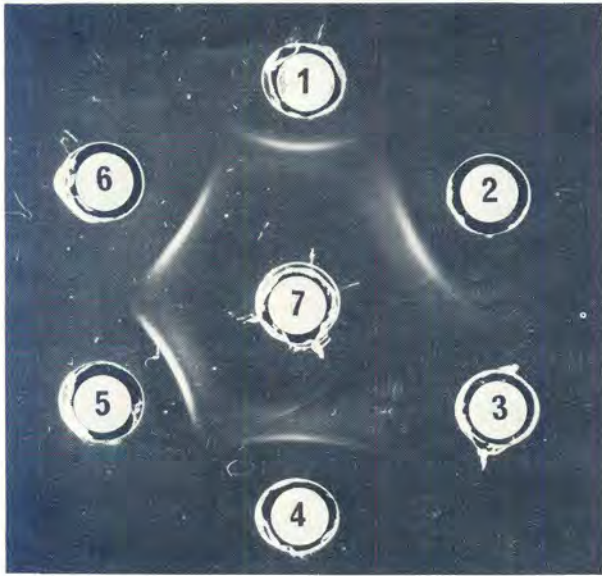


FIG. 2a Precipitin reactions of phenol extracts prepared from various strains of *P. multocida* with *P. multocida* Strain A.14 (Type A:3) rabbit antiserum

Well 1—phenol extract of *P. multocida* Strain M.4 (Type A:1);
 Well 2—phenol extract of *P. multocida* Strain A.14 (Type A:3);
 Well 3—phenol extract of *P. multocida* Strain Ts.8 (Type A:5);
 Well 4—phenol extract of *P. multocida* Strain P-1059 (Type A:8);
 Well 5—phenol extract of *P. multocida* Strain Liver (Type A:9);
 Well 6—phenol extract of *P. multocida* Strain 125 (Type A:8);
 Well 7—whole cell rabbit antiserum to *P. multocida* Strain A.14 (Type A:3) 2× concentrated

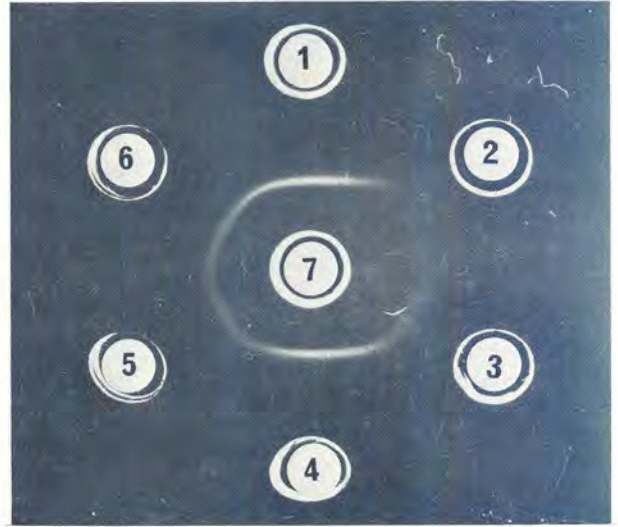


FIG. 2b Cross reactions of *P. multocida* Strain A.14 (Type A:3) phenol extract with whole cell rabbit antisera to various heterologous strains

Well 1—whole cell rabbit antiserum to Strain A.14 (Type A:3) 2× concentrated; Well 2—whole cell rabbit antiserum to Strain Ts.8 (Type A:5) 2× concentrated; Well 3—whole cell rabbit antiserum to Strain A.11 (Type A:7) 2× concentrated; Well 4—whole cell rabbit antiserum to Strain P-1059 (Type A:8) 2× concentrated; Well 5—whole cell rabbit antiserum to Strain Liver (Type A:9) 2× concentrated; Well 6—whole cell rabbit antiserum to Strain M.4 (Type A:1) 2× concentrated; Well 7—phenol extract of *P. multocida* Strain A.14 (Type A:3) 10 mg/ml

Similarly, 2 strains which possess the same heat extractable antigen as Strain A.14 (A:3) (Rhoades, personal communication, 1979) were also examined.

The results of these experiments are given in Table 5.

Although sheep antiserum to Strain A.14 (A:3) was efficacious in giving homologous protection, it did not effectively protect mice against infection by Strain M4 (A:1), 125 (A:8), Liver (A:9), P-1059 (A:8) or 33191 (D:3). This indicates that neither the phenol extract antigen nor the heat extractable antigen is responsible for cross-protection.

Immunological spectrum of Strain P-1059 (A:8)

Since Strain A.14 (A:3) was apparently unable to afford protection against any of the strains tested, the possibility of finding a more suitable strain for study was investigated and Strain P-1059 (A:8) was selected for this purpose.

Sheep antiserum to Strain P-1059 (A:8) did afford protection to Strains A.11 (A:7) and 'Liver' (A:9) but not to any other strain tested, including Strain 125 which is also a Type A:8 (Table 6). This finding further supports the contention that the absence or presence of cross-protection among strains cannot be predicted by serological relationships which can be determined at this stage.

Immunizing effect of live and polyvalent vaccines

Since it appears that vaccines prepared from single strains of *P. multocida* give an unpredictable and limited spectrum of immunity, the possibility of compensating for this defect by using either a live vaccine or a composite polyvalent vaccine was investigated.

The results of this experiment are shown in Table 7.

TABLE 5 Failure of *P. multocida* A.14 (Type A:3) antiserum (Sheep 8121) to protect against strains with identical phenol extractable antigens or which are serologically identical, according to Rhoades (personal communication, 1979)

		Challenge strains					
		A.14 (A:3)	M.4 (A:1)	125 (A:8)	Liver (A:9)	P-1059 (A:8)	22191 (D:3)
Logs protection afforded by A.14 (A:3) antiserum.....		3,9	1,1	0,3	0,9	0,1	0,0
Antigenic relationship among strains	Identical phenol extractable antigens.....	+	+	+	+	-	-
	All Rhoades type 3.....	+	-	-	-	+	+

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TABLE 6 Spectrum of protection afforded by *P. multocida* P-1059 (Type A:8) sheep antiserum to heterologous *P. multocida* Type A strains

		Challenge strain									
		M.4 (A:1)	A.14 (A:3)	Ts.8 (A:5)	A.11 (A:7)	P-1059 (A:8)	Liver (A:9)	4009 (A:3)	SI (A:3)	7477 (A:3)	125 (A:8)
P-1059 (A:8)...	H. A. titre.....	16	32	16	16	16	16	128	256	32	32
Sheep antiserum	Agglutination titre	256	2048	64	4096	4096	auto	1024	auto	64	4096
No. 106.....	Logs protection	0,0	0,5	0,0	3,0	5,0	4,2	0,0	0,5	0,0	0,9

TABLE 7 Immunity induced by an avirulent live vaccine and a composite polyvalent inactivated vaccine to various *P. multocida* serotypes

	Logs protection									
	Challenge strain									
	A:1	A.14 (A:3)	Ts.8 (A:5)	P-1059 (A:8)	A.11 (A:7)	Liver (A:9)	4009 (A:3)	SI (A:3)	7477 (A:3)	
Live strain 5727, 5 × 10 ⁸ /mouse.....	0,0	0,6	0,0	3,0	0,1	0,5	nt	nt	nt	
Live strain 5727, 1 × 10 ⁸ /mouse.....	0,0	1,2	2,0	0,0	0,0	0,7	nt	nt	nt	
Composite polyvalent vaccine, 0,2 ml/ mouse.....	nt	2,1	0,8	0,4	nt	nt	0,2	0,1	0,0	

nt=not tested

The live vaccine gave some protection to certain strains, but the results were erratic and immunity is apparently dependent on the dosage. The polyvalent vaccine did protect against Strain A.14 (A:3), but it was ineffective (less than 1,0 logs) against 2 other Type Strains Ts.8 (A:5) and P-1059 (A:8) as well as against 3 field isolates (Strains 4009, SI and 7477).

DISCUSSION

The results presented in this paper show that the immunological relationship among *P. multocida* Type A strains cannot be predicted on the basis of their antigenic properties as determined by haemagglutination or gel precipitin tests. In the light of the marked discrepancies among the various serotyping systems (Baxi, Blobel & Scharman, 1970; Brogden & Packer, 1979), this finding is not surprising and is in accordance with the findings of Heddleston, Watko & Rebers (1964). Moreover, strains which possess virtually identical phenol extractable antigens do not give cross-protection. It is evident that the antigen or antigens of *P. multocida* Type A strains which are responsible for the induction of immunity are different from those which are detected by *in vitro* serological tests.

Various cell components (Srivastava, Foster, Dawe, Brown & Davis, 1970; Baba, 1977) as well as numerous antigenic preparations and extracts have been found to possess immunizing properties. The latter include a lipopolysaccharide-protein antigenic complex (Rebers, Heddleston & Rhoades, 1967; Genfield, Rebers & Heddleston, 1976), a free endotoxin which is capable of producing an immunity in chickens (Heddleston & Rebers, 1975), and a Westphal-type lipopolysaccharide (Rebers & Heddleston, 1974; Brogden & Rebers, 1978). The strain specificity of

these antigens has not been elucidated, however, but in the light of our experience with the phenol extract antigen, prospects that positive results will be obtained are poor.

Attempts to overcome the narrow spectrum of immunity by employing a polyvalent vaccine were disappointing, since known immunogenic strains gave poor results in this system. The only exception was Strain A.14 (A:3). Production of polyvalent vaccines should thus be avoided and vaccines should contain, rather, a limited number of proven antigenic strains. The strains used should be representative of the immunotypes present in a particular territory as assessed by passive or active mouse protection tests (Tereszczuk, 1965).

Live *Pasteurella* vaccines have been used with considerable success both in turkeys (Bierer & Scott, 1969) and in mice and rabbits (Linde, 1976; Wei & Carter, 1978). Our results in this respect were erratic and the problem requires further investigation. However, even if a good immunity could be induced with a live vaccine, there is no guarantee that the problem of strain specific immunity would be solved.

Finally, it should be remembered that pneumonic pasteurellosis is commonly a complex disease syndrome (Carter, 1973; Perreau, 1976; Sharp, Gilmour, Thompson & Rushton, 1978) and, although serum antibodies offer spectacular protection against systemic infection to *P. multocida*, they may not be equally effective in the lung (Collins, 1976). In fact, in the case of *P. haemolytica*, cellular immunity may play an important role in affording protection (Wells, Evans, Burrells, Sharp, Gilmour, Thompson & Rushton, 1979) and there is evidence that similar mechanisms may be operative in the case of *P. multocida* immunity (Maheswaran & Thies, 1979).

In conclusion, it may be stated that immunity to *P. multocida* Type A organisms is generally highly strain-specific and that the antigen responsible for inducing this immunity as yet eludes definition and serological detection.

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