

RESPONSE OF SHEEP AND CATTLE TO COMBINED POLYVALENT *PASTEURELLA HAEMOLYTICA* VACCINES

C. M. CAMERON and FAITH J. BESTER, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

CAMERON, C. M. & BESTER, FAITH, J., 1986. Response of sheep and cattle to combined polyvalent *Pasteurella haemolytica* vaccines. *Onderstepoort Journal of Veterinary Research*, 53, 1-7 (1986).

The antibody response to various combined polyvalent *Pasteurella haemolytica* vaccines was studied in sheep and cattle.

In sheep, certain oil adjuvant vaccines gave rise to a better antibody response to *P. haemolytica* than an Al(OH)₃-adsorbed vaccine. This finding, however, was not consistent for all serotypes, and with respect to *P. multocida*, oil adjuvants had no advantage. Furthermore, it was found that the removal of all the culture supernatant fluid during the production process had no deleterious effect on the antigenicity of the product.

In cattle, good responses were obtained with both alum-precipitated and Al(OH)₃-adsorbed vaccine where all culture supernatant fluid was not removed during the production process. No advantage was gained with oil emulsion vaccines.

The degree of immunity afforded to mice and the antibody response to different serotypes of *P. haemolytica* varied considerably. Further detailed studies with respect to specific serotypes of *P. haemolytica* are therefore required.

INTRODUCTION

Pasteurella haemolytica is a recognized pathogen in the bovine respiratory disease complex and usually occurs concomitantly with infectious bovine rhinotracheitis virus (IBR) and parainfluenza Type 3 virus (PI3). *P. haemolytica* Type A1 is by far the most frequent serotype encountered in this syndrome and may even be the primary pathogen (Gibbs, Allan, Wiseman & Selman, 1984).

P. haemolytica is likewise an important pathogen of sheep and goats. In the sheep respiratory syndrome it may be associated with various viruses and *Mycoplasma* infections (Davies, Dungworth, Humphreys & Johnson, 1977; Sharp, Gilmour, Thompson & Rushton, 1978; Davies, Herceg, Jones & Thurley, 1981; Davies, Herceg & Thurley, 1982; Lehmkuhl & Cutlip, 1982; Buddle, Herceg & Davies, 1984). However, it has also been proven to be an independent primary pathogen (Gilmour, Angus & Sharp, 1980; Gilmour, Angus, Donachie & Frazer, 1982). Various serotypes may be involved (Thomson, Frazer & Gilmour, 1977; Frank, 1982; Gilmour, 1982), but in South Africa the majority of cases are caused by Serotypes 1, 2 and 6 (M. M. Menton & F. J. Bester, personal communication, 1984). *P. haemolytica* is also frequently responsible for mastitis (blue udder) in sheep (Gilmour, 1978). In this instance, the most common serotypes are 2, 7 and 9 (E. M. van Tonder & M. M. Menton, personal communication, 1984).

From the foregoing it is evident that, when compiling vaccines, cognisance must be taken of the multiplicity of *P. haemolytica* serotypes (Gilmour, 1980) and other pathogens, including *P. multocida* serotypes that are involved. In previous publications (Cameron & Bester, 1984a, 1984b) accent was placed on the response of sheep and cattle to the *P. multocida* component of polyvalent vaccines, whereas in this study the response to the *P. haemolytica* components were those primarily followed.

MATERIALS AND METHODS

Bacterial strains

All the strains used in this study have already been described (Cameron & Bester, 1984a, 1984b; Cameron, Bester & Du Toit, 1984).

Preparation of vaccines

The basic procedures were similar to the methods previously described (Cameron & Bester, 1984a, 1984b;

Cameron *et al.*, 1984). To fulfil certain objectives the following formulations were compiled for the various experiments.

Vaccines for sheep

For the 1st experiment, which was designed to study the response of sheep to polyvalent *P. haemolytica* vaccines containing various adjuvants, the following vaccines were composed:

P. haemolytica serotypes 1 (Strain 9479); 2 (Strain J28), 6 (Biberstein); 7 (Strain 4024/1) and 9 (Biberstein) were cultivated separately and inactivated by the addition of 0,3 % formalin.

Vaccine A: All the strains were concentrated to a packed cell volume of 2,5 % by centrifugation, but not all the supernatant culture fluid was removed. Equal volumes of the strains were mixed to give a final p.c.v. of 0,5 % per strain. The mixed cultures were then precipitated by the addition of 10 ml of an 11 % solution of potassium alum to give a final concentration of 1,0 %.

Vaccine B: Vaccine B was prepared exactly like Vaccine A except that the final mixture was adsorbed by the addition of 12,5 ml/100 ml of a 50 % Al(OH)₃ packed gel to give a final concentration of approximately 6,0 % (Cameron & Bester, 1984b).

Vaccine C: The bacterial cells were concentrated as for Vaccines A and B, but all the supernatant fluid was removed and the bacteria resuspended in 0,15 M phosphate buffered saline pH 7,2 containing 0,01 % merthiolate. After mixing, the suspension was adsorbed with Al(OH)₃ as outlined above.

Vaccine D: Vaccine D was prepared exactly as for Vaccine B but the cells of each strain were concentrated to 5,0 % p.c.v. to give a concentration of 1,0 % p.c.v. after mixing with and the addition of Al(OH)₃. This suspension was then emulsified with an equal volume of oil composed of 85 parts Marcol 52⁽¹⁾ and 15 parts Arlacel A⁽²⁾ by means of a Waring blender (Wells, Gilmour, Burrels & Thompson, 1979).

Vaccine E: The cells were prepared as for Vaccine A to a concentration of 7,5 %. The 5 strains were mixed to give a concentration of 1,5 % packed cells per strain. This suspension was emulsified in oil⁽³⁾ so that the final product contained 0,5 % packed cells per strain.

⁽¹⁾ Esso, P.O. Box 78011, Sandton, 2146

⁽²⁾ ICI, P.O. Box 3784, Alrode, 1451

⁽³⁾ ICI Formula "C"

Vaccine F: Vaccine F was prepared like Vaccine E except that the formula recommended by Bokhout, Van Gaalen & Van der Heijden (1981) for the oil emulsion was used. The cell densities were adjusted in such a way that the final product contained 0,5 % packed cells per strain.

In the 2nd experiment in sheep, 3 vaccines containing both *P. haemolytica* and *P. multocida* strains but different adjuvants were compared. The vaccines were prepared as follows: *P. haemolytica* Type 1 (9479), Type 2 (J28) and Type 6 (Biberstein). *P. multocida* Type A (8473); and Type D (4) were grown and inactivated, as outlined above, centrifuged and resuspended in PBS (0,15 M; pH 7,2 plus 0,01 % merthiolate) to a concentration of 2,5 p.c.v. The 5 strains were mixed to give a concentration of 0,5 % p.c.v. per strain.

The following vaccines were prepared from this suspension:

Vaccine J: An aliquot of the above suspension was further concentrated by centrifugation to give a concentration of 7,5 % p.c.v. This suspension was then emulsified with oil and emulsifiers as recommended by a commercial company⁽¹⁾ so that the final product contained 2,5 % p.c.v. or 0,5 % p.c.v. per strain.

Vaccine K: Al(OH)₃ to a final concentration of 6,0 % packed gel was added to an aliquot of the 2,5 % p.c.v. suspension and stirred for 30 min at room temperature. The final product therefore contained 0,5 % packed cells per strain.

Vaccine L: To simulate the procedure of Wells *et al.* (1979), 200 ml of vaccine K was allowed to settle out for 48 h at 4 °C. The supernatant fluid (140 ml) was siphoned off and the remaining 60 ml of sediment emulsified as for Vaccine J.

A commercial vaccine designated Vaccine "O"⁽¹⁾ was also included in the experiment. This product is similar to one recently described (Wells, Robinson, Gilmour, Donachie & Sharp, 1984).

For the 3rd experiment, vaccines were composed of *P. haemolytica* Type 2 (J28), Type 7 (4024/1) and Type 9 (Biberstein) and two strains of *Staphylococcus aureus* [Strains 24276 (68V5) and 1141]. The *P. haemolytica* component was prepared as outlined above in such a way that the mixture contained a final concentration of 1,0 % p.c.v. per strain. This suspension was mixed with an equal volume of *S. aureus* bacterin toxoid (Cameron, Fuls & Botha, 1979) and adsorbed with Al(OH)₃ (Vaccine M). For the preparation of Vaccine N, 200 ml of Vaccine M was allowed to settle out and the residual 60 ml of sediment was emulsified with oil, as outlined for Vaccine L.

Vaccines for cattle

The *Pasteurella* component was prepared from the following strains:

- P. multocida* Type A (Strain 9539)
- P. multocida* Type D (Strain D4)
- P. multocida* Type E (Strain 7630)
- P. haemolytica* Type I (Strain 9479)

They were grown and inactivated as for sheep vaccines and adjusted to the required densities by centrifugation and removal of the appropriate volume of supernatant culture fluid. The sedimented cells were resuspended in the residual culture fluid.

The IBR and PI3 components were prepared by growing the viruses on BHK cells according to conventional techniques and inactivated by the addition of 0,2 % for-

malin. The virus suspensions had titres of 10^{7.5} and 10^{7.0} respectively before inactivation. The 2 virus components were mixed in equal proportions.

The following vaccines were composed from the above:

Vaccine P: The inactivated *Pasteurella* strains were mixed in the following proportion and precipitated with potassium alum at a final concentration of 1,0 %.

		pcv %	Volume (ml)	Final pcv %
<i>P. multocida</i>	Type A	2,5	200	0,5
<i>P. multocida</i>	Type D	2,5	200	0,5
<i>P. multocida</i>	Type E	2,5	50	0,125
<i>P. haemolytica</i>	Type I	5,0	200	1,0

The proportions used were based on previous findings (Cameron & Bester, 1984a). The dosage for cattle was 2,0 ml subcutaneously and 0,1 ml for mice.

Vaccine Q: The composition of Vaccine Q was exactly the same as for Vaccine P, except that an equal volume of the combined virus components was mixed with the *Pasteurella* component before sedimentation with potassium alum.

Vaccine R: Vaccine R was prepared exactly the same as for Vaccine Q except that it was adsorbed with Al(OH)₃ at a final concentration of 6,0 % packed gel.

Vaccine S: Vaccine S was prepared by mixing the following components.

		pcv %	Volume (ml)
<i>P. multocida</i>	Type A	7,5	100
<i>P. multocida</i>	Type D	7,5	100
<i>P. multocida</i>	Type E	7,5	25
<i>P. haemolytica</i>	Type I	15,0	100
IBR	—	—	175
PI3	—	—	175

The above mixture was then emulsified in oil as outlined for Vaccine J (sheep).

The dosage for Vaccines Q, R and S was 5,0 and 0,2 ml subcutaneously for cattle and mice respectively.

A commercial Vaccine⁽²⁾ (T) was included in the experiment for comparison.

Immunization of experimental animals

Sheep: In all the experiments groups of 8 Merino wethers (6–12 months old) were used for each vaccine. They were pre-bled and given 2 injections of 2,0 ml vaccine subcutaneously 4 weeks apart and bled 2 and 4 weeks after the 2nd injection. For the vaccines containing also *S. aureus*, a 5,0 ml dose was used. In some instances they were given a 3rd injection and bled more frequently. The details are shown in the tables under Results.

Cattle: Groups of 8 6–12-month old Bonsmara cattle were used for the respective vaccines. They were initially given 2 injections 4 weeks apart and bled 2 weeks, 4 weeks and 3 months after the 2nd injection. They were subsequently given a 3rd booster injection of vaccine 3 months after the 2nd injection and bled 2 weeks and 6 weeks later. The details of the assays that were done are indicated in Fig. 1.

Mice: The potency of the *P. haemolytica* components of the various vaccines were assayed in mice as described previously (Cameron *et al.*, 1984).

Rabbits: The potency of the *S. aureus* component of polyvalent vaccines was assayed in rabbits as described previously (Cameron *et al.*, 1979).

Serological and immunological assays

The indirect haemagglutination antibody titres (IHA) for *P. haemolytica* were determined essentially as

⁽¹⁾ "Ovovac", Hoechst UK Ltd, Milton Keynes, Bucks, England

⁽²⁾ Rhivax 3 + P, Pitman-Moore, New Jersey, USA

TABLE 1 Antibody response in sheep to polyvalent *P. haemolytica* vaccines containing different adjuvants

Vaccine composition and adjuvant	H.A. reciprocal titres. Means of 4 assays					Means of all serotypes
	Serotype 1	Serotype 2	Serotype 6	Serotype 7	Serotype 9	
A. Culture supernatant fluid not removed 1.0% alum	8,0	32,9	10,5	6,1	13,6	14,2
B. Culture supernatant fluid not removed 6% Al(OH) ₃	12,9	82,3	13,9	7,9	4,8	24,3
C. Cells resuspended in PBS 6,0% Al(OH) ₃	20,6	75,4	17,3	3,4	3,9	24,1
D. Combined Al(OH) ₃ and oil emulsion (Wells <i>et al.</i> , 1979)	31,4	50,8	45,6	26,3	53,3	41,4
E. Oil emulsion ICI formula "C"	23,2	130,3	30,5	7,4	15,7	41,4
F. Oil emulsion (Bokhout <i>et al.</i> , 1981)	10,21	85,8	15,4	5,0	5,2	24,3

TABLE 2 Protection of mice to *P. haemolytica* infection by combined *P. haemolytica*: *P. multocida* vaccines with different adjuvants

Vaccine	<i>P. haemolytica</i>								<i>P. multocida</i>			
	Number of surviving mice after challenge/10						Protection all 3 serotypes		Protection Types 1 and 6 only		Protection logs	
	Challenge strain and dilution						Total survivors/60	Percentage protection	Total survivors/40	Percentage protection	Type D (D4)	Type A (8473)
	Type 1 (9479)		Type 2 (J28)		Type 6 (Biberstein)							
	Undiluted	1/5	Undiluted	1/5	Undiluted	1/5						
Vaccine J Oil adjuvant ICI formula "C"	5	7	3	6	5	9	35	41,6	26	50,0	2,2	3,1
Vaccine K Al(OH) ₃	4	6	2	6	6	5	29	31,7	21	37,5	2,0	3,1
Vaccine L Al(OH) ₃ plus oil (Wells <i>et al.</i> , 1979)	5	6	5	5	6	8	35	41,7	25	47,5	1,2	1,9
Ovivac	7	9	3	7	3	5	34	40,0	24	45,0	0,1	0,0
Non-immunized controls	0	3	1	3	2	1	10	0,0	6	0,0	0,0	0,0

described by Biberstein, Gills & Knight (1960), whereas the antibody titres to IBR and PI3 viruses were determined by standard plaque-reduction assays.

The passive mouse protection tests for *P. multocida* were conducted and interpreted as described by Cameron, Engelbrecht & Vermeulen (1978).

RESULTS

Sheep

The antibody response of sheep to polyvalent *P. haemolytica* vaccines is shown in Table 1. The figures are the mean of 4 assays done on sera collected 2 and 4 weeks after the 2nd and booster injection of vaccine. The response to the individual serotypes was rather erratic but when the mean of all serotypes is taken into account, it would appear that the Al(OH)₃ adjuvanted vaccines were slightly better than the alum precipitated vaccine. In contrast to what has been established in cattle (Cameron & Bester, 1984a), removal of the supernatant culture fluid has no effect.

It is also clear that 2 of the oil emulsion vaccines (D and E) elicited a better antibody response than the alum or Al(OH)₃ adjuvant vaccines.

The results of the following experiments in which the immunogenicity of an oil adjuvant vaccine, an Al(OH)₃ adjuvant vaccine and an Al(OH)₃ plus oil vaccine are compared are given in Tables 2 and 3.

From the results shown in Table 2 it appears that the experimental oil emulsion vaccines afforded a slightly better protection to mice than the Al(OH)₃ adjuvant vaccine. However, this difference was not apparent with respect to protection against *P. multocida*.

The same vaccines were also compared in sheep. As Table 3 shows, the vaccines containing oil elicited a better antibody response to *P. haemolytica* than the Al(OH)₃ adjuvant product. The oil adjuvant vaccines were also superior to the commercial product. The results regarding *P. multocida* were generally good but inconsistent, and the superiority of the oil adjuvant vaccines in respect of these organisms could not be established. This observation confirms previous findings (Cameron & Bester, 1983).

In the following experiments, the response to combined *P. haemolytica*:*S. aureus* vaccines was investigated.

The results given in Table 4 show that mice can be adequately immunized against *P. haemolytica* Types 7 and 9 and to a lesser extent against Type 2 with vaccines containing also *S. aureus* bacterin toxoid. In this experiment the oil emulsion vaccine had no apparent advantage and both experimental vaccines compared favourably with the commercial product.

The same vaccines (M, N and O) were also tested in sheep, but the antibody response induced by all 3 of

RESPONSE OF SHEEP AND CATTLE TO COMBINED POLYVALENT *PASTEURELLA HAEMOLYTICA* VACCINES

TABLE 3 Antibody response in sheep to *P. haemolytica* polyvalent vaccines also containing *P. multocida*

Adjuvant	<i>P. haemolytica</i> Reciprocal haemagglutination titres						<i>P. multocida</i> Passive protection logs	
	Serotype 1 (9479)		Serotype 2 (J28)		Serotype 6 (Biberstein)		Serotype D (D4)	Serotype A (8473)
	A	B	A	B	A	B	C	C
Oil adjuvant ICI formula "C"	16,5	20,0	5,4	5,4	173,7	91,4	2,4	4,4
A1(OH) ₃	6,0	2,7	3,7	3,5	10,2	18,0	0,4	3,8
A1(OH) ₃ plus oil (Wells <i>et al.</i> , 1979)	37,0	40,5	2,5	8,7	100,0	118,0	1,4	3,4
Ovovac ⁽¹⁾	2,5	2,0	8,0	6,0	16,3	20,2	0,0	0,0

A = 2 weeks after 2nd injection B = 4 weeks after 2nd injection C = 2 weeks after booster injection

TABLE 4 Protection of mice by polyvalent *P. haemolytica* vaccine also containing *S. aureus*

Vaccine	Number of surviving mice after challenge/10						Total surviving mice/60	Percentage protec- tion minus controls
	Challenge strain and dilution							
	Type 2 (J28)		Type 7 (4024/1)		Type 9 (Biberstein)			
	Undiluted	1/5	1/25	1/125	1/25	1/125		
Vaccine M Cells resuspended in buf-A1(OH) ₃	2	6	10	8	7	8	41	50,0
Vaccine N Combined A1(OH) ₃ ICI formula "C"	2	5	7	7	7	7	35	40,0
Vaccine O Ovovac ⁽¹⁾	3	7	6	9	5	9	39	46,6
Controls	3	1	2	0	4	1	11	0,0

TABLE 5 Immunization of mice and rabbits with a combined *P. haemolytica*: *S. aureus* commercial A1(OH)₃ adsorbed vaccine (Onderstepoort production batch 3)

<i>P. haemolytica</i>				<i>S. aureus</i>												
Percentage protection minus control				Mean surface area of lesions cm ²												
				Immunized						Non-immunized						
Challenge strain and dilution				Challenge strain and dilutions						Challenge strain and dilutions						
Type 2 (J28)		Type 7 (4024)		1141			24276 (68)V5			1141			24276 (68)V5			
1/5	1/25	1/5	1/25	Undil.	1/2	1/4	Undil.	1/2	1/4	Undil.	1/2	1/4	Undil.	1/2	1/4	
50	30	50	30	17,8	18,1	2,6	3,6	3,5	3,1	41,4	28,3	35,7	13,1	8,5	9,5	

them to *P. haemolytica* Types 2, 7 and 9 were too poor to merit recording.

Table 5 gives the results obtained in mice and rabbits with a production batch of a combined *P. haemolytica*: *S. aureus* A1(OH)₃-adsorbed vaccine. As in the previous experiment, the results obtained in mice with respect to the *P. haemolytica* components were good. Likewise, the immunity to *S. aureus* induced in rabbits was in accordance with previous findings (Cameron *et al.*, 1979).

Cattle

The results of the comprehensive assays that were done on the cattle sera as well as the active immunity tests for *P. haemolytica* Type 1 are given in Fig. 1.

Vaccine P afforded the best protection to *P. haemolytica* Type 1 in mice, but the antibody response to this organism in cattle was poor. The passive protection against *P. multocida* was also satisfactory but generally poorer than for Vaccines Q and R. It should be noted that the animals in this group developed antibody titres

against PI3 virus, even though the vaccine (P) did not contain it. This observation therefore largely invalidates the findings with respect to PI3 antibody titres in the other groups.

Overall there was very little if any difference in the response to Vaccines Q, R and S. In this group, Vaccine Q afforded the best protection to *P. haemolytica* Type 1 in mice and the antibody response to this organism was also marginally better than Vaccines R and S. Vaccine S (oil adjuvant) did not effectively protect mice against *P. haemolytica*. Both the Q and R vaccines were distinctly superior to Vaccine T (commercial) with respect to all the bacterial components. Vaccine T, however, elicited the best response to IBR virus.

DISCUSSION

The most striking aspect of this study was the discrepancies that were revealed with respect to the antibody response in sheep and cattle in respect of the *P. haemolytica* and *P. multocida* components and the different vaccine formulations.

⁽¹⁾ Hoechst UK Ltd, Milton Keynes, Bucks, England

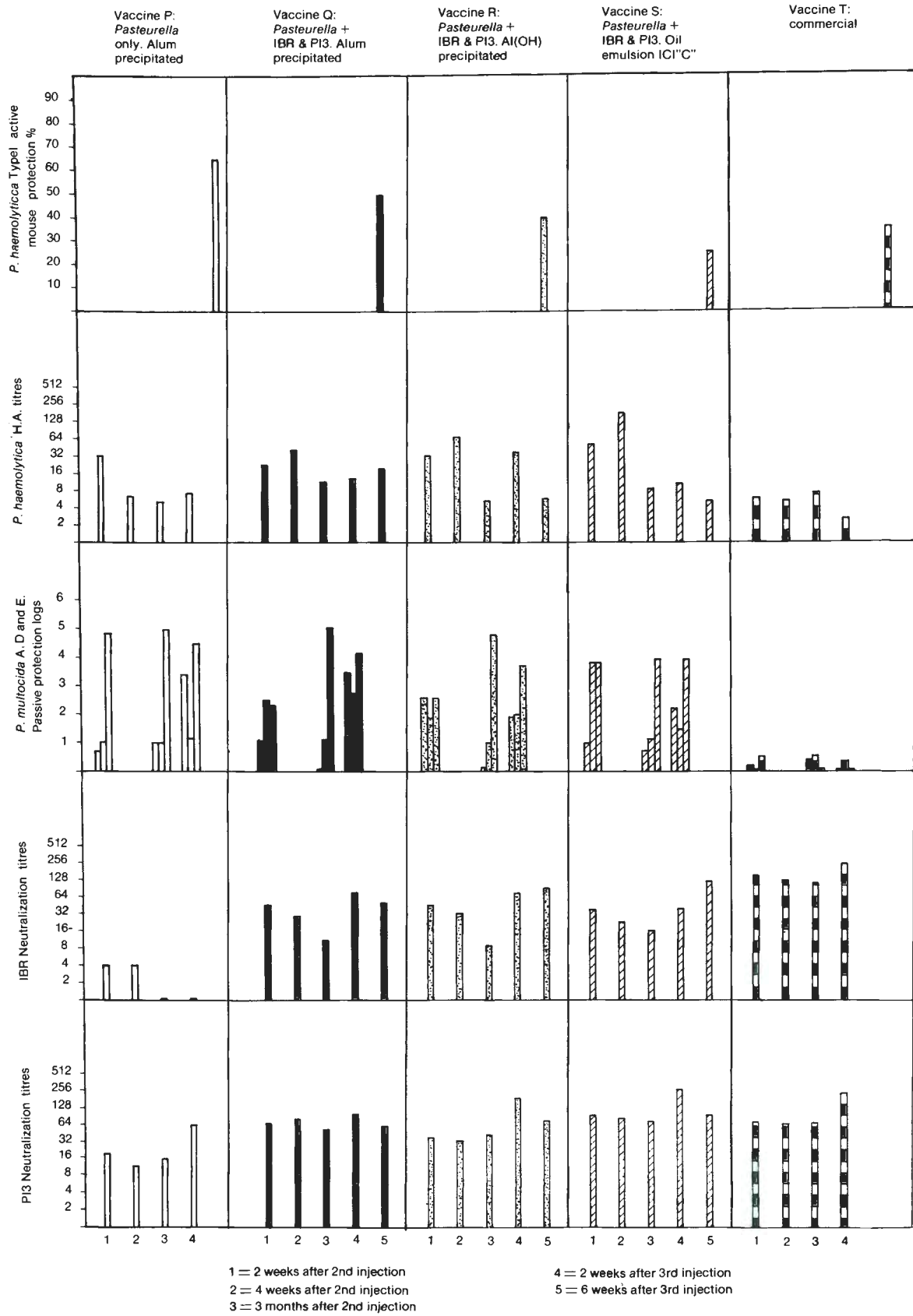


FIG. 1 Comparison of different polyvalent vaccines in mice and cattle

REFERENCES

- In sheep, the best response to *P. haemolytica* was obtained with oil emulsion vaccine, whereas in the case of the *P. multocida* component the response was similar for the oil emulsion vaccine and Al(OH)₃ adsorbed vaccines. In cattle, on the other hand, oil emulsion vaccines did not improve the response to *P. haemolytica*, provided that the culture supernatant fluid was not removed in the process of preparing Al(OH)₃-adsorbed products. This observation is in accordance with previous findings (Cameron & Bester, 1984a). *P. haemolytica* oil emulsion vaccines, however, should not be used without further critical assessment, since there is the possibility that they may in fact depress the immune response (Wilkie, Caoili & Friend, 1976).
- A disquietening observation during these studies was that, although various *P. haemolytica* vaccines afforded a satisfactory degree of immunity in mice, the antibody response in cattle and particularly in sheep as measured by an indirect haemagglutination test was generally poor. This was specially true for *P. haemolytica* Type 2, which is a notoriously poor antigen (Gilmour, Sharp, Donachie, Burrels & Frazer, 1980). The response to other serotypes was also distinctly variable. Since immunity to *P. haemolytica* is serotype specific (Biberstein & Thompson, 1965; Gilmour, Martin, Sharp, Thompson & Wells, 1979; Shewen & Wilkie, 1983), in-depth studies regarding the immunogenicity of specific serotypes are necessary. These should include the evaluation of live mutants (Kucera, Wong & Feldner, 1983) as well as a study of sub-cellular components (Squire, Smiley & Croskell, 1984).
- There is no direct correlation between serum antibody titres as determined by IHA tests and immunity, and such titres may in fact be misleading (Smith, Dawson, Wells & Burrels, 1976; Burrels, Wells & Dawson, 1978). Greater emphasis should therefore be placed on the concentration of the various classes of antibodies that are present in the secretions of the respiratory tract (Wells, Dawson, Smith & Smith, 1977; Wilkie & Markham, 1979; Walker, Corstvet, Lesley & Panciera, 1980; Scicchitano, Husband & Cripps, 1984). Furthermore, investigations by numerous authors indicate that cellular immune mechanisms may be at least as important for protection against *P. haemolytica* as humoral mechanisms (Walker, Corstvet & Panciera, 1980; Davies & Penwarden, 1981; Shewen & Wilkie, 1982; Markham, Ramnaraine & Muscoplat, 1982; Newman, Corstvet & Panciera, 1982). In this regard the role of the neutrophil cytotoxin that is produced by *P. haemolytica* is also of consequence (Berggren, Baluyut, Simonson, Bemrick & Maheswaran, 1981; Baluyut, Simonson, Bemrick & Maheswaran, 1981; Sutherland, Gray & Wells, 1983). It is therefore quite possible that sheep and cattle may be effectively immune to respiratory infection despite a low level of serum antibodies. This can only be conclusively established, however, by respiratory challenge of vaccinated animals (Gilmour *et al.*, 1982; Gilmour, Martin, Sharp, Thompson, Wells & Donachie, 1983).
- Similar questions arise with respect to the effectiveness of mammary protection in sheep. It has been shown that *P. haemolytica* antibodies are transferred to the colostrum (Gilmour *et al.*, 1980), but the protective value of this process is unclear (Wells, Evans, Burrels, Sharp, Gilmour, Thompson & Rushton, 1979; Cowan & McBeath, 1983; Smith, 1982) and can only be assessed by controlled laboratory and field trials.
- ACKNOWLEDGEMENTS
- We wish to thank Dr B. H. J. Barnard and the personnel of the Section of Virology, Veterinary Research Institute, Onderstepoort, for undertaking the serum-virus neutralization tests.
- BALUYUT, C. S., SIMONSON, R. R., BEMRICK, W. J. & MAHESWARAN, S. K., 1981. Interaction of *Pasteurella haemolytica* with bovine neutrophils: Identification and partial characterization of a cytotoxin. *American Journal of Veterinary Research*, 42, 1920-1926.
- BERGGREN, K. A., BALUYUT, C. S., SIMONSON, R. R., BEMRICK, W. J. & MAHESWARAN, S. K., 1981. Cytotoxic effects of *Pasteurella haemolytica* on bovine neutrophils. *American Journal of Veterinary Research*, 42, 1383-1388.
- BIBERSTEIN, E. L., GILLS, MARJORIE & KNIGHT, H., 1960. Serological types of *Pasteurella haemolytica*. *Cornell Veterinarian*, 50, 283-300.
- BIBERSTEIN, E. L. & THOMPSON, D. A., 1965. Type specificity of immunity to *Pasteurella haemolytica* infection in mice. *Journal of Comparative Pathology*, 75, 331-337.
- BOKHOUT, B. A., VAN GAALLEN, C. & VAN DER HEIJDEN, P. J., 1981. A selected water-in-oil emulsion: Composition and usefulness as an immunological adjuvant. *Veterinary Immunology and Immunopathology*, 2, 491-500.
- BUDDLE, B. M., HERCEG, M. & DAVIES, D. H., 1984. Experimental infection of sheep with *Mycoplasma ovipneumoniae* and *Pasteurella haemolytica*. *Veterinary Microbiology*, 9, 543-548.
- BURRELS, C., WELLS, P. W. & DAWSON, A. McL., 1978/1979. The quantitative estimation of antibody to *Pasteurella haemolytica* in sheep sera using a micro enzyme-linked immunosorbent assay (ELISA). *Veterinary Microbiology*, 3, 291-301.
- CAMERON, C. M., ENGELBRECHT, MARIA M. & VERMEULEN, ALETTA, S. M., 1978. The serological and immunological relationship of type Strains A and D of *Pasteurella multocida* to field isolates from sheep. *Onderstepoort Journal of Veterinary Research*, 45, 214-220.
- CAMERON, C. M., FULS, W. J. P. & BOTHA, WILNA, F., 1979. Composition and evaluation of the efficacy of a *Staphylococcus aureus* vaccine. *Onderstepoort Journal of Veterinary Research*, 46, 1-8.
- CAMERON, C. M. & BESTER, FAITH J., 1983. The inefficacy of polyvalent *Pasteurella multocida* vaccines for sheep. *Onderstepoort Journal of Veterinary Research*, 50, 101-104.
- CAMERON, C. M., BESTER, FAITH J. & DU TOIT, D. J., 1984. Factors affecting the immunogenicity of *Pasteurella haemolytica* in mice. *Onderstepoort Journal of Veterinary Research*, 51, 97-102.
- CAMERON, C. M. & BESTER, FAITH J., 1984a. Preliminary report on the formulation of a polyvalent *Pasteurella* vaccine for cattle. Proceedings XIIIth World Congress on Diseases of Cattle, Durban, South Africa, Vol 1. 298-304.
- CAMERON, C. M. & BESTER, FAITH J., 1984b. Formulation of an effective *Pasteurella multocida* vaccine for sheep. *Onderstepoort Journal of Veterinary Research*, 51, 189-191.
- COWAN, S. & McBEATH, D. G., 1983. Passive protection of lambs against septicaemic pasteurellosis. *Veterinary Record*, 111, 185-186.
- DAVIES, D. H., DUNGWORTH, D. L., HUMPHREYS, S. & JOHNSON, A. J., 1977. Concurrent infection of lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. *New Zealand Veterinary Journal*, 25, 263-265.
- DAVIES, D. H., HERCEG, M., JONES, B. A. H. & THURLEY, D. C., 1981. The pathogenesis of sequential infection with parainfluenza virus type 3 and *Pasteurella haemolytica* in sheep. *Veterinary Microbiology*, 6, 173-182.
- DAVIES, D. H. & PENSWARDEN, R. A., 1981. The phagocytic cell response of the ovine lung to *Pasteurella haemolytica*. *Veterinary Microbiology*, 6, 183-189.
- DAVIES, D. H., HERCEG, M. & THURLEY, D. C., 1982. Experimental infection of lambs with an adenovirus followed by *Pasteurella haemolytica*. *Veterinary Microbiology*, 7, 369-381.
- FRANK, G. M., 1982. Serotypes of *Pasteurella haemolytica* in sheep in the midwestern United States. *American Journal of Veterinary Research*, 43, 2035-2037.
- GIBBS, H. A., ALLAN, E. M., WISEMAN, A. & SELMAN, I. E., 1984. Experimental production of bovine pneumonic pasteurellosis. *Research in Veterinary Science*, 37, 154-166.
- GILMOUR, N. J. L., 1978. Pasteurellosis in sheep. *Veterinary Record*, 102, 100-102.
- GILMOUR, N. J. L., MARTIN, W. B., SHARP, J. M., THOMPSON, D. A. & WELLS, P. W., 1979. The development of vaccines against pneumonic pasteurellosis in sheep. *Veterinary Record*, 104, 15.
- GILMOUR, N. J. L., 1980. *Pasteurella haemolytica* infections in sheep. *Veterinary Quarterly*, 2, 191-198.
- GILMOUR, N. J. L., ANGUS, K. W., DONACHIE, W. & FRAZER, J., 1982. Vaccination against experimental pneumonic pasteurellosis. *Veterinary Record*, 110, 450.
- GILMOUR, N. J. L., ANGUS, K. W. & SHARP, J. M., 1980. Experimental pulmonary infections of sheep caused by *Pasteurella haemolytica* biotype T. *Veterinary Record*, 106, 507-508.

- GILMOUR, N. J. L., SHARP, J. M., DONACHIE, W., BURRELS, C. & FRAZER, J., 1980. Serum antibody response of ewes and their lambs to *Pasteurella haemolytica*. *Veterinary Record*, 107, 505-507.
- GILMOUR, N. J. L., ANGUS, K. W., DONACHIE, W. & FRAZER, J., 1982. Experimental pneumonic pasteurellosis in sheep and cattle. *Veterinary Record*, 110, 406-407.
- GILMOUR, N. J. L., 1982. Ovine pasteurellosis vaccines. *Veterinary Record*, 111, 354.
- GILMOUR, N. J. L., MARTIN, W. B., SHARP, J. M., THOMPSON, D. A., WELLS, P. W. & DONACHIE, W., 1983. Experimental immunization of lambs against pneumonic pasteurellosis. *Research in Veterinary Science*, 35, 80-86.
- KRUCERA, C. J., WONG, J. C. S. & FELDNER, T. J., 1983. Challenge exposure of cattle vaccinated with a chemically altered strain of *Pasteurella haemolytica*. *American Journal of Veterinary Research*, 44, 1848-1852.
- LEMHKUHL, H. D. & CUTLIP, R. C., 1982. Characterization of parainfluenza type 3 virus isolated from the lung of a lamb with pneumonia. *American Journal of Veterinary Research*, 43, 626-628.
- MARKHAM, R. J. F., RAMNARAIN, M. L. R. & MUSCOPLAT, C. C., 1982. Cytotoxic effect of *Pasteurella haemolytica* on bovine polymorphonuclear leukocytes and impaired production of chemotactic factor by *Pasteurella haemolytica* infected alveolar macrophages. *American Journal of Veterinary Research*, 43, 258-285.
- NEWMAN, P. R., CORSTVET, R. E. & PANCIERA, R. J., 1982. Distribution of *Pasteurella haemolytica* and *Pasteurella multocida* in the bovine lung following vaccination and challenge exposure as an indicator of lung resistance. *American Journal of Veterinary Research*, 43, 417-422.
- SCICCHITANO, R., HUSBAND, A. J. & CRIPPS, A. W., 1984. Immunoglobulin-containing cells and the origin of immunoglobulins in the respiratory tract of sheep. *Immunology*, 52, 529-537.
- SHARP, J. M., GILMOUR, N. J. L., THOMPSON, D. A. & RUSHTON, B., 1978. Experimental infection of specific pathogen-free lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. *Journal of Comparative Pathology*, 88, 237-243.
- SHEWEN, PATRICIA E. & WILKIE, B. N., 1982. Cytotoxin of *Pasteurella haemolytica* acting on bovine leucocytes. *Infection and Immunity*, 35, 91-94.
- SHEWEN, PATRICIA E. & WILKIE, B. N., 1983. *Pasteurella haemolytica* cytotoxin: Production by recognized serotypes and neutralization by type-specific rabbit antisera. *American Journal of Veterinary Research*, 44, 715-719.
- SMITH, W. D., DAWSON, A. M., WELLS, P. W. & BURRELS, C., 1976. Immunoglobulins in the serum and nasal secretions of lambs following vaccination and aerosol challenge with parainfluenza 3 virus. *Research in Veterinary Science*, 21, 341-348.
- SMITH, G. R., 1982. Difficulty in protecting lambs against *Pasteurella haemolytica*. *Zoological Society of London, Regents Park, London*, 50, 128-129.
- SQUIRE, P. G., SMILEY, D. W. & CROSKELL, R. B., 1984. Identification and extraction of *Pasteurella haemolytica* membrane proteins. *Infection and Immunity*, 45, 667-673.
- SUTHERLAND, A. D., GRAY, E. & WELLS, P. W., 1983. Cytotoxic effect of *Pasteurella haemolytica* on ovine bronchoalveolar macrophages in vitro. *Veterinary Microbiology*, 8, 3-15.
- THOMPSON, D. A., FRAZER, J. & GILMOUR, N. J. L., 1977. Serotypes of *Pasteurella haemolytica* in ovine pasteurellosis. *Research in Veterinary Science*, 22, 130-131.
- WALKER, R. D., CORSTVET, R. E., LESLEY, B. A. & PANCIERA, R. J., 1980. Study of bovine pulmonary response to *Pasteurella haemolytica*: Specificity of immunoglobulins isolated from the bovine lung. *American Journal of Veterinary Research*, 41, 1015-1023.
- WALKER, R. D., CORSTVET, R. E. & PANCIERA, R. J., 1980. Study of bovine pulmonary response to *Pasteurella haemolytica*: Pulmonary macrophage response. *American Journal of Veterinary Research*, 41, 1008-1014.
- WELLS, P. W., DAWSON, A. M., SMITH, W. D. & SMITH, B. S. W., 1977. The transfer of circulating IgG₁ and IgG₂ to the nasal secretions of sheep. *Research in Veterinary Science*, 22, 201-204.
- WELLS, P. W., EVANS, HELEN B., BURRELS, C., SHARP, J. M., GILMOUR, N. J. L., THOMPSON, D. A. & RUSHTON, B., 1979. Inability of passively acquired antibody to protect lambs against experimental pasteurellosis. *Infection and Immunity*, 26, 25-29.
- WELLS, P. W., GILMOUR, N. J. L., BURRELS, C. & THOMPSON, D. A., 1979. A serological comparison of *Pasteurella haemolytica* vaccines containing different adjuvants. *Research in Veterinary Science*, 27, 248-250.
- WELLS, P. W., ROBINSON, J. T., GILMOUR, N. J. L., DONACHIE, W. & SHARP, J. M., 1984. Development of a combined clostridial and *Pasteurella haemolytica* vaccine for sheep. *Veterinary Record*, 114, 266-269.
- WILKIE, B. N., COAILI, F. & FRIEND, S., 1976. Nonspecific immunosuppression induced in mice with killed *Pasteurella haemolytica* in Freund's Complete Adjuvant. *International Archives of Allergy and Applied Immunology*, 50, 745-750.
- WILKIE, B. N. & MARKHAM, R. J. F., 1979. Sequential titration of bovine lung and serum antibodies after parenteral or pulmonary inoculation with *Pasteurella haemolytica*. *American Journal of Veterinary Research*, 40, 1690-1693.