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Potential allergens in oil emulsion foot-and-mouth disease vaccines for pigs

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Reactions so far reported after the use of oil emulsion (OE) foot-and-mouth disease (FMD) vaccines in pigs have been infrequent and quick to resolve themselves. Although tentatively ascribed to anaphylaxis, these reactions have received little attention and their mechanism of causation has not been established conclusively.

In the past however, allergic vaccination reactions in cattle sometimes occurred with unexpected severity (Capstick *et al.* 1969; Beadle, 1971) and in these circumstances any delay in identifying the responsible allergens produces a loss of confidence in the vaccine concerned. Consequently screening to identify potential allergens seems a wise precaution even in vaccines which are at the time relatively free of untoward effects.

Anaphylactic-type reactions in pigs have been described after the use of water-in-oil atrophic rhinitis and pseudorabies vaccines (de Buysscher, Chong & Lukert, 1980) and substances identified as allergenic in pigs include lysozyme (Metzger, 1976), helminth extracts (Coop, 1969; Barrett, 1969; de Buysscher *et al.* 1980) and ovalbumin (Wells, Pass & Eyre, 1974).

In this paper we report an investigation into the allergenic potential of the main protein components of an OE FMD pig vaccine, namely bovine serum (BS) and baby hamster kidney (BHK) cell lysate. The latter may in some circumstances provoke reaginic antibodies and anaphylactic signs in multi-vaccinated cattle (Black & Pay, 1975; Black, 1977; Black & Francis, 1983).

The double emulsion (w/o/w) and single emulsion (w/o) vaccines used here were produced in BHK-21, Clone 13 deep-cell cultures (Telling *et al.* 1972) and contained O_1BFS 1860, A_{24} Cruzeiro and C_3 Pando FMD virus strain antigens blended by ultrasonication with Markol 52 (Esso), Arlacel A (ICI, America) and Tween 80 (Difco) as described by Basarab (1978).

The vaccines were injected intramuscularly (i.m.) in 2 ml doses into six 12-week old Large White pigs at days 0, 35 and 56 and blood samples were taken at weekly intervals for 9 weeks. Ten days after the third vaccination the animals were challenged by injecting them intravenously (i.v.) with 1 ml of BHK cell lysate containing 5 mg protein (Black, 1977) and, half an hour later, with 1 ml of BS. Three unvaccinated (control) pigs of the same breed and similar age were also injected with BS i.v. Any untoward clinical signs which developed after the injections were noted.

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Table. 1. *Clinical responses to i.v. injections of BHK cell lysate and BS in vaccinated and unvaccinated pigs*

Sensitization regimen	Pig no.	Challenge: substances injected at day 66	
		BHK cell lysate	BS
Three w/o/w vaccinations at 0, 35 and 56 days	A	No ill effects	Collapse, gasping apnoea, red ears (destroyed)
	B	No ill effects	Slight tremors only
	C	No ill effects	Collapse, gasping, red ears
Three w/o vaccinations at 0, 35 and 56 days	D	No ill effects	Sways on feet. Gasping respiration
	E	No ill effects	Collapse, convulsions, apnoea (destroyed)
	F	No ill effects	Collapse, convulsions, red ears (destroyed)
Unvaccinated controls	C1	Not done	Slight tremors and fast respiration
	C2	Not done	Slight tremors and fast respiration
	C3	Not done	Slight tremors and fast respiration

Contrary to results obtained previously in cattle (Black & Pay, 1975) the BHK cell lysate challenge caused no reactions. On the other hand BS provoked severe reactions in 4 of the 6 pigs (Table 1). The signs, which developed within a few minutes of injection, were loud fast breathing, dark red blotches on the skin where the ears had been held, staggering, collapse, loss of consciousness and apnoea. The last-mentioned persisted for about a minute, after which respiration was resumed, consciousness returned and recovery ensued. The severity of the reactions, graded subjectively as mild to very severe, is shown in Fig. 1 together with the serological results. None of the unvaccinated control pigs showed any clinical response after the i.v. BS injections other than a transient increase in respiratory rate.

To establish the allergic nature of the reactions the serum samples were examined for reaginic antibodies. The blood samples, taken weekly (days 0–63) and again at day 66 (immediately before challenge) were separated and the sera stored at −20 °C until used for passive cutaneous anaphylaxis (PCA) tests. The method used was similar to that described for cattle sera (Black *et al.* 1975). Briefly, six 8-week-old pigs were anaesthetized with azaperone (Suicalm) and metomidate (Hypnodil, Jenssen Laboratories, Belgium) and, after clipping, the skin over the chest wall and flanks was marked out into 50 blocks, approximately 3 × 3 cm. The test sera, coded and randomized to avoid bias, were then injected intradermally (i.d.), 0·1 ml to a block, all 66 sera being used in each of the six animals. After 72 h 8 ml of 2% Evans blue in 0·15 M saline were given i.v. followed at 30 min intervals by various sequences of the substances to be tested (BHK cell lysate, BS and bovine plasma albumin (BPA)) as shown in Table 2. The positive reactions (purple stains at the antiserum injection sites) were measured 25 min

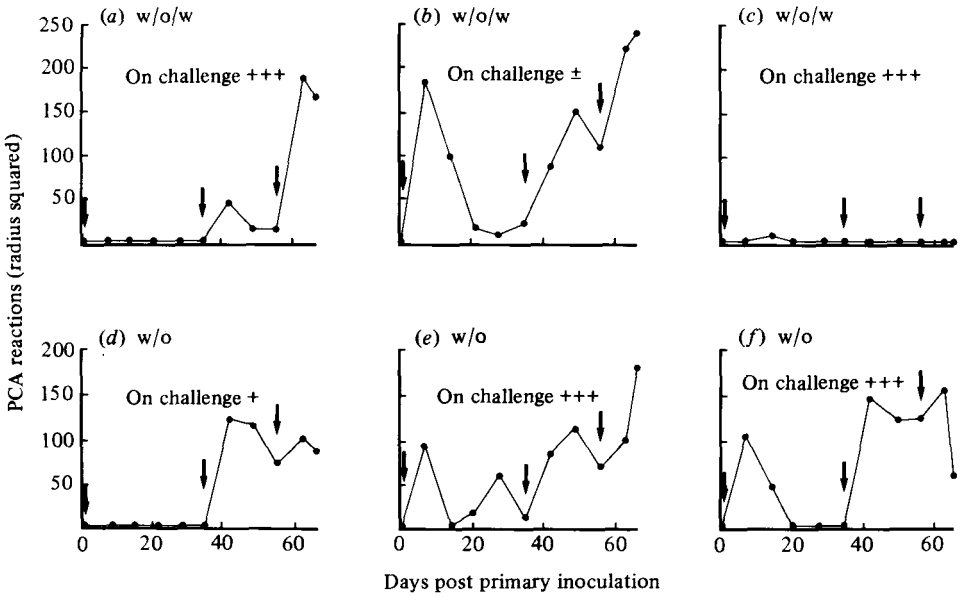

Fig. 1. Development of anti-bovine serum reagins in the sera of pigs after vaccination. The graphs show the results of PCA tests performed after injections of double emulsion (w/o/w) and single emulsion (w/o) vaccines. ±, Mild; + + +, very severe.

after each i.v. injection and the mean diameters of the corresponding reactions on different pigs were calculated.

The results of the PCA tests confirmed those previously obtained from the challenge tests. No antibodies against BHK cell lysate were demonstrated but reagins against BS were present in the sera of all six vaccinated pigs. The squared radii of the anti-BS reactions are shown in Fig. 1 and the results obtained using both human and cattle sera suggest that these are a linear function of the reaginic antibody titres (Stanworth, 1973; Black, 1977). Peak titres of anti-BS reagins were found in sera taken at 7 days after vaccination, indicating that serum samples for testing purposes are best taken at about that interval after any observed reactions. It is worth noting, however, that bleedings were not carried out between the 7th and 14th days after vaccination and hence sera taken about 10 days after reactions may contain higher antibody titres, as they do in cattle

Table 2. *PCA test substances injected i.v. at 72 h after the i.d. serum injections*

First PCA test (four pigs)	Second PCA test (two pigs)
(i) 8 ml 2% Evans blue	(i) 8 ml 2% Evans blue
(ii) 2 ml BS	(ii) 2 ml BHK cell lysate (0·5% protein)
	(iii) 2 ml 0·5% BPA*
	(iv) 2 ml BS*

* Injected at 30 minute intervals

BHK, baby hamster kidney; BPA, bovine plasma albumen; BS, bovine serum.

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(Black, 1977). The rate of decline in the level of reagins in the pig appeared to be similar to that in cattle in which a serum half-life of approximately 3 days has been calculated (Black, 1977). The relatively short half-life of these antibodies in the serum indicates that delayed sampling may give inconclusive results because the serum antibodies were often either waning or had subsided to undemonstrable levels by 21 days after vaccination.

The severity of the clinical reactions after challenge did not correlate with the titres of reaginic antibodies in the sera (see Fig. 1). This agrees with the results obtained in other species and has been discussed elsewhere (Stanworth, 1973; Black, 1977). Nevertheless, PCA reactions to BPA were observed only in sera which contained high titres of anti BS reagins.

In conclusion, the results suggest that BS, but not BHK cell remnants, in OE vaccines are potentially allergenic in pigs. Furthermore, BPA is probably one of the potentially allergenic components although other serum proteins may also be implicated. Finally, while no problem of allergy has yet been reported using commercial O/E vaccines in pigs, the results reported here may enable manufacturers to eliminate potential allergens from their vaccines.

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