HISTOLOGICAL EXAMINATION OF PIGS VACCINATED WITH OIL ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE

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SUMMARY

The gross and microscopic tissue reactions induced by water-in-oil primary (PE) and water-in-oil-in-water double emulsion (DE) types of foot-and-mouth disease vaccine administered by the subcutaneous, intramuscular or intraperitoneal route were evaluated. Oil droplets in the subcutaneous and intramuscular inoculation sites of the PE vaccine were larger than those in the sites of the DE vaccine. Intraperitoneal inoculation of the PE vaccine caused granulomas in the peritoneal cavity; the DE vaccine did not. Of the 2 preparations and 3 routes of inoculation tested, the intraperitoneal inoculation of the DE vaccine caused no aesthetically objectionable tissue reaction.

INTRODUCTION

Water-in-oil foot-and-mouth disease (FMD) vaccines, primary emulsion (PE) type, induce a high degree of protection in pigs (*1, 5, 6, 7, 8, 9,*). However, the aesthetically objectionable extensive tissue reaction at the site of inoculation and in the regional lymph nodes prevents large-scale application of this vaccine (*8*). Gross and microscopic examinations of swine to PE FMD vaccine inoculated by intramuscular (IM) and subcutaneous (SC) routes have already been reported (*10*).

Water-in-oil-in-water double emulsion (DE) vaccines as described by Herbert (*4*) inoculated intraperitoneally into pigs produced no significant gross lesion. The animals had a high degree of protection for at least 4 months after the first vaccination (*2, 3*).

The present paper describes the histological examination of samples collected from pigs vaccinated with PE or DE FMD vaccine inoculated by SC, IM or intraperitoneal (IP) routes.

MATERIALS AND METHODS

Vaccines

Primary and DE vaccines were prepared as described (*2, 3*). Briefly, the PE was prepared using mineral oil[3] containing 10% of mannide monooleate[4] emulsified with equal parts of a trivalent antigen suspension. The DE (*4*) consisted of the PE vaccine re-emulsified with an equal volume of phosphate buffer solution (PBS), pH 7.4, containing 2% polyoxethylene 20 sorbitan monooleate[5].

Pigs

Mixed Landrace pigs recently weaned and of approximately 20 kg were used. Pigs were vaccinated with PE or with DE FMD vaccine using the SC, IM (masseter m.), or IP route. In experiment 1, the pigs were killed 30 days post inoculation (DPI) for gross and histologic examination; the pig number, type and amount of vaccine, route of inoculation and average of largest droplet size are shown in Table 1. The PE and DE vaccines were evaluated via the SC route in the same pigs by injecting one preparation into each ear.

In experiment 2, four groups of 10 pigs each were vaccinated either SC with PE vaccine or SC,

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[3]Marcol 52 - Exxon Corporation, U.S.A.

[4]Arlacel A - ICI American Inc. Atlas Chemical Division.

[5]Tween 80 - ICI American Inc. Atlas Chemical Division.

TABLE 1. *Summation of the type of vaccine, route of inoculation and average size of the largest oil droplets at the site of inoculation and in the regional lymph node for the animals in experiment 1*

Animal	Type of vaccine	Route	Dose (ml)	Tissue	Average largest droplet size (μ) PE	DE
533	PE	SC[a]	1.5	Site	610	
				LN	450	
533	DE	SC[b]	3	Site		130
				LN		270
535	PE	SC[a]	1.5	Site	500	
				LN	150	
535	DE	SC[b]	3	Site		160
				LN		180
538	PE	IM	1.5	Site	120	
				LN	70	
539	DE	IM	1.5	Site	180	
				LN	130	
536	PE	IP	1.5	Composit	300	
534	DE	IP	3	Composit		40

[a] Left ear
[b] Right ear

PE Primary emulsion
DE Double emulsion
IM Intramuscular
IP Intraperitoneal
SC Subcutaneous
LN Regional lymph node
Site Site of inoculation

IM, or IP with DE vaccine, respectively. Resistence to FMD was challenged by contact exposure to 4 non-vaccinated pigs inoculated interplantarly with $10^{4.6}$ mouse $LD_{50\%}$ of O_1 Campos virus at 110 DPI and killed 140 DPI. Site of inoculation in all pigs was examined for gross lesions, and tissues from two pigs randomly selected from each group were collected for gross histologic examination; Table 2 shows the pig number, type and amount of vaccine, route of inoculation and average of largest droplet size. Serological and challenge inoculation procedures have been reported (*2*).

Collection of samples for histologic examination

In both experiments, the sites of inoculation and regional lymph nodes were collected from pigs inoculated SC or IM. In experiment 1, all lesions, several lymph nodes in the peritoneal cavity, and spleen were collected from pigs inoculated IP. In experiment 2, sections of mesenteric lymph node, spleen, pancreas, kidney and liver were collected from pigs inoculated IP. Tissues were fixed in buffered 10% formalin; a cross section was trimmed to include any grossly visible lesions, embedded in paraffin, sectioned at 6 μ and stained with Mayer's hematoxylin eosin-phlyoxine B.

Lesions in tissue sections were photographed and the 5 largest clear spaces (oil droplets) in each photograph were measured. If more than 1

TABLE 2. *Summation of the type of vaccine, route of inoculation and average size of the largest oil droplets at the site of inoculation and in the regional lymph node for animals selected in experiment 2 for histological evaluation*

Animal	Type of vaccine	Route	Dose (ml)	Tissue	Average largest droplet size (μ) PE	DE
1	PE	SC	1.5	Site	NA	
				LN	80	
2	PE	SC	1.5	Site	430	
				LN	350	
11	DE	SC	3	Site		134
				LN		39
12	DE	SC	3	Site		90
				LN		40
28	DE	IM	3	Site		220
				LN		68
29	DE	IM	3	Site		120
				LN		46
37	DE	IP	3	Composit		0
38	DE	IP	3	Composit		0

PE Primary emulsion
DE Double emulsion
IM Intramuscular
IP Intraperitoneal
SC Subcutaneous
LN Regional lymph node
NA Note available
Site Site of inoculation

slide or photograph was made of a specimen, the average largest oil droplet size was based on all photographs for the specimen.

RESULTS

Experiment 1

Preliminary tests of primary and double emulsion oil vaccines for pigs

Results are summarized in Table 1. Histologic findings in pig 533 at the sites of SC inoculation of 1.5 ml of PE or 3 ml of DE vaccine and in the lymph nodes draining the respective sites were similar except for the size of the oil droplets. The average size of the largest oil droplets associated with inoculation of the PE vaccine was about 4 times larger than the average largest droplet associated with the DE vaccine. The DE inoculation site was composed of granulomas separated by connective tissue septa. Small granulomas had an oil droplet in the center surrounded by giant cells, then macrophages and then a layer of fibroblasts. Larger granulomas had a lymphocytic area between the macrophages and connective tissue. Lymph nodules in nodes draining the PE and DE inoculation sites had numerous mitotic figures. The subcapsular and paratrabecular areas of the nodes had a marked granulomatous reaction - macrophages and giant cells - and numerous oil droplets. Large oil droplets in the node draining the PE site were surrounded by giant cells and a few neutrophilic leukocytes.

Histologic findings at the sites of SC inoculation of 1.5 ml of PE and 3 ml of DE vaccine in pig 535 differed from the preceeding pig. The sites in pig 535 were surrounded by dense connective tissue in which were embedded small granulomas; the center of both sites contained necrotic granulomatous tissue and oil droplets. The site of DE vaccine inoculation also contained 2 small rosettes. The average size of the largest oil droplets in PE site was about 3 times larger than in the DE site. The histologic appearance of the lymph nodes draining the sites were similar to those in pig 533.

The IM inoculation site of 1.5 ml of PE vaccine in pig 538 histologically consisted of numerous small granulomas many of which contained a small oil droplet. In the regional lymph node from this pig, the reticular cells in the subcapsular and paratrabecular areas were hyperplastic and these areas contained numerous scattered granulomas and small oil droplets. The IM inoculation site of 1.5 ml of PE vaccine in pig 539 consisted of a connective tissue encapsulated granulomatous mass and oil droplets. The 5 largest oil droplets in the inoculation site averaged 180 μ. The regional lymph nodes from this pig contained granulomas; 3 of the granulomas had neutrophilic leukocytes around oil droplets and several granulomas had necrotic centers.

Pig 536 inoculated IP with 1.5 ml of PE vaccine had well developed granulomas on the capsule of the spleen and mesentery of the small intestine and colon. Histologically the granulomas on the spleen were typical of those on other serosal surfaces. From the outside in, the granulomas had a connective tissue capsule; a layer of epithelioid cells in which there were occasional small polykaryocytes and oil droplets; a layer of inflammatory cells, mostly neutrophilic leukocytes, many of which were necrotic; and a center composed of bluish-purple (necrotic) epithelioid cells and oil droplets (*Fig. 1*). The average size of the largest oil droplets in granulomas in the peritoneal cavity was 300 μ.

Pig 534 inoculated IP with 3 ml of the DE vaccine had one small granulomatous area containing small oil droplets on the colonic mesentery. The average size of the largest oil droplets was 40 μ.

Experiment 2

Comparison of primary and double oil emulsion vaccines for pigs

The 1.5 ml of PE vaccine inoculated SC in pig 1 was apparently deposited in the lymph node for this was the only site of reaction. Histologically, the regional node contained a large necrotic area, granulomatous nodules some of which had a necrotic center, and moderate subcapsular and paratrabecular reticular cell hyperplasia. The largest average oil droplet size in the lymph node was 80 μ.

The inoculation site in pig 2 which received 1.5 ml of PE vaccine subcutaneously had histologically the following structure from outside in: a connective tissue capsule, granulomatous tissue, and a necrotic center which contained large oil droplets. The average size of the largest oil droplets was 430 μ (*Fig. 2*). The tissue section from the regional lymph node had granulomatous nodules in the cortex. One granulomatous nodule contained a large oil droplet and the others small droplets. The average size of the largest oil droplets was 350 μ.

Sites of inoculation in pigs 11 and 12 which received 3 ml of DE vaccine SC had numerous granulomatous nodules separated by connective tissue. The granulomatous nodules contained small oil droplets (*Fig. 3*). The average sizes of the largest oil droplets were 134 and 90 μ, respectively. The regional lymph node in pig 11 had subcapsular and paratrabecular reticular cell hyperplasia, small oil droplets and a subcapsular granulomatous nodule. The average size of the largest oil droplets was 39 μ. In pig 12, the regional lymph node had subcapsular and paratrabecular reticular cell hyperplasia; 2 small oil droplets were in the section.

At the site of IM inoculation of 3 ml of DE vaccine, pig 28 had a large granulomatous area containing numerous oil droplets in the connective tissue adjacent to the muscle. The perimysium was widened by connective tissue and diffuse and nodular granulomatous tissue in which were numerous small oil droplets (*Fig. 4*). The average size of the largest droplets was 220 μ. The site of IM inoculation of 3 ml of DE vaccine in pig 29 had the perimysium widened by connective tissue

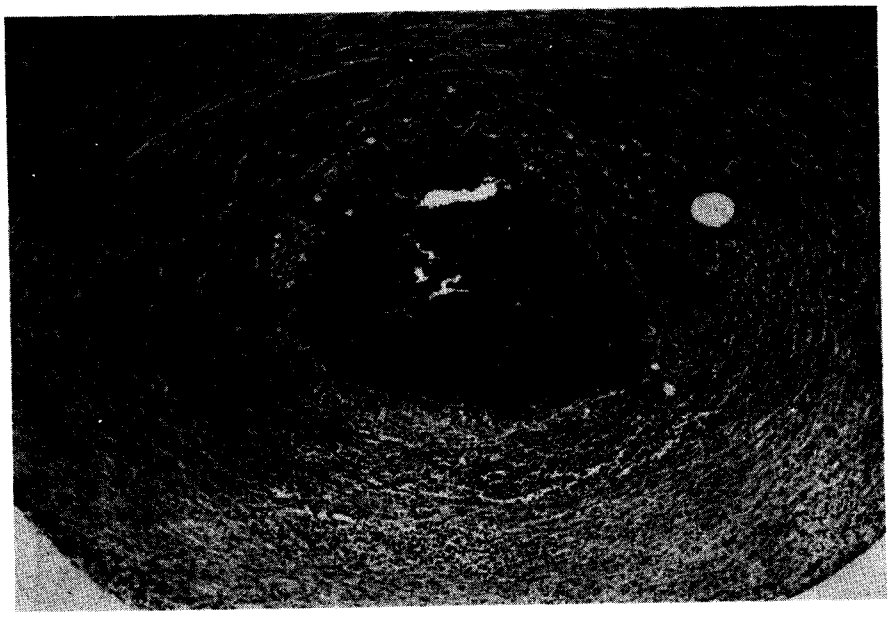

FIGURE 1. *Granuloma on the capsule of the spleen from pig 536 inoculated intraperitoneally with 1.5 ml of PE vaccine. H + E x 70.*

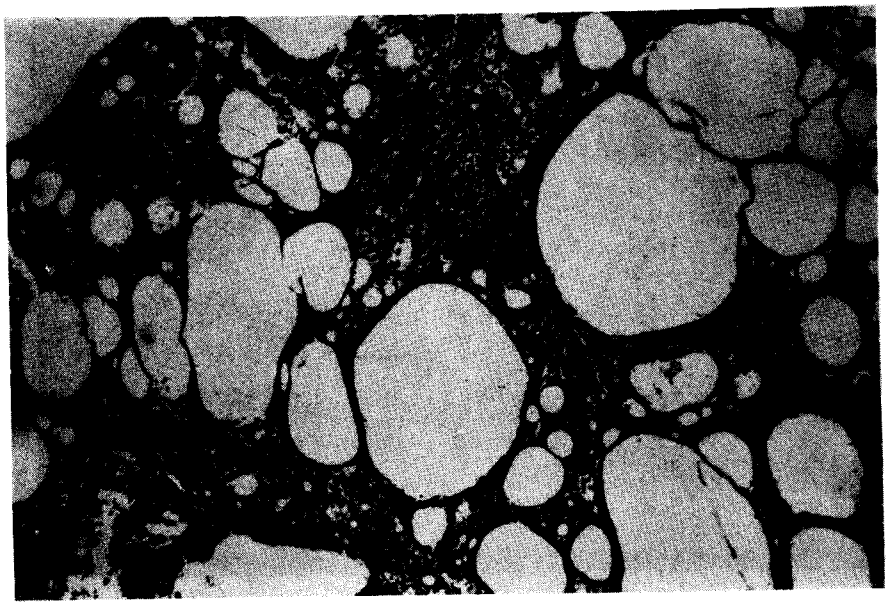

FIGURE 2. *Large oil droplets at the site of subcutaneous inoculation of 1.5 ml of PE vaccine in pig 2. H + E x 58.*

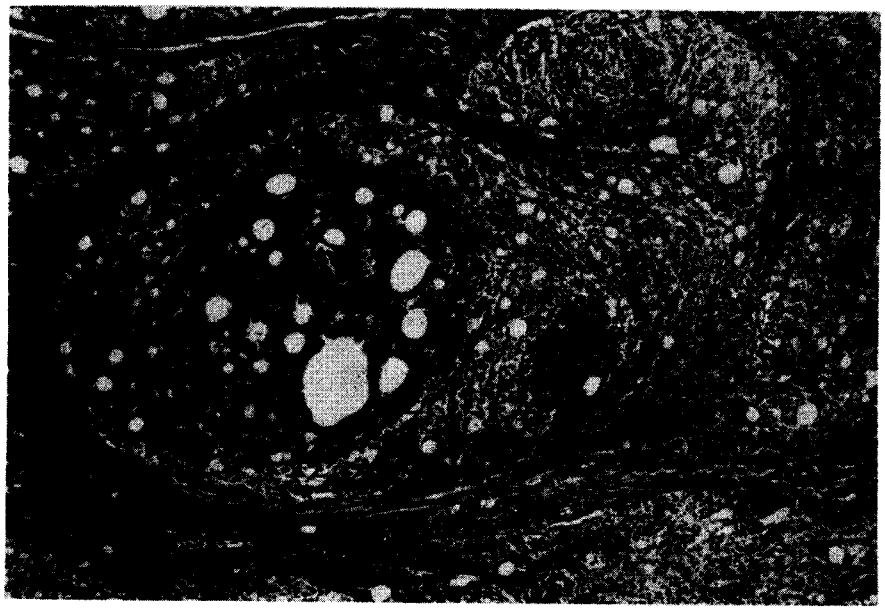

FIGURE 3. *Granulomatous nodules and small oil droplets at the site of subcutaneous inoculation of 3 ml DE vaccine in pig 11. H + E x 70.*

FIGURE 4. *Granulomatous reaction and small oil droplets at the site of intramuscular inoculation of 3 ml DE vaccine in pig 28. H + E x 80.*

and diffuse and nodular granulomatous tissue which contained numerous oil droplets. The center of one large granulomatous area was necrotic. The average size of the largest oil droplets was 120 μ. The regional lymph node in both pigs had subcapsular and paratrabecular reticular cell hyperplasia, scattered granulomatous nodules, and oil droplets (*Fig. 5*). The average size of the largest oil droplets in the regional lymph node of the 2 pigs were 60 and 46 μ, respectively.

Pigs 37 and 38 inoculated IP with 3 ml of DE vaccine, had a few small connective tissue villous-like projections from the capsule of the spleen. A section of messenteric lymph node from pig 38 had reticular cell hyperplasia.

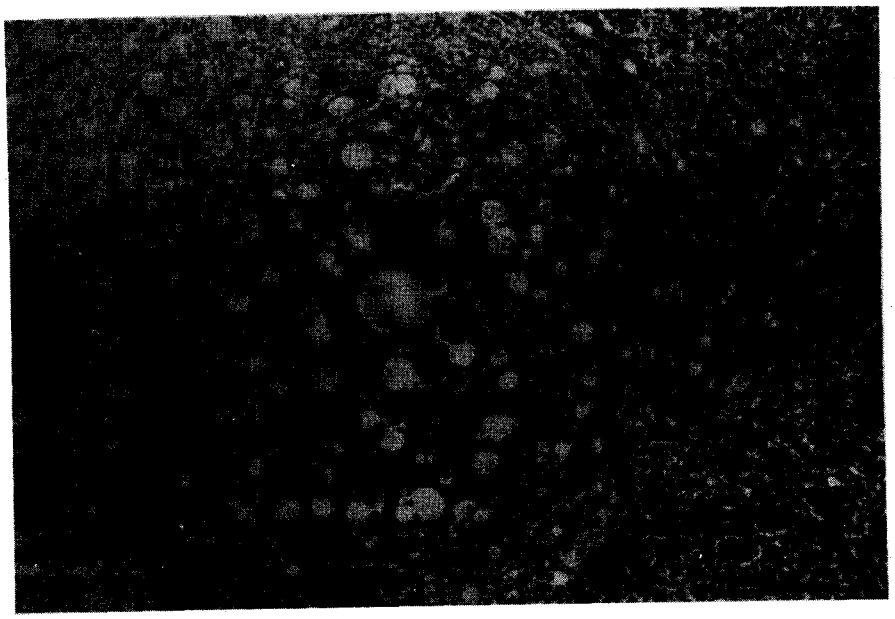

FIGURE 5. *Granulomatous reaction and small oil droplets in the regional lymph node of pig 28 inoculated intramuscularly with 3 ml of DE vaccine. H + E x 158.*

DISCUSSION

In the preliminary experiment, the average size of the largest oil droplets at the sites of PE vaccine inoculation was larger than at the sites of DE vaccine inoculation. The necrosis at the sites of SC inoculation of PE and DE vaccine in pig 535 could have resulted from either the large oil droplets in the SC areas or an unfavorable reaction of the animal. The granulomatous reaction at the sites of IM inoculation of the PE vaccine while desirable for processing the antigen was aesthetically undesirable in meat producing animals and could possibly be confused with a granuloma resulting from infection. The most pronounced difference in the reaction to PE and DE vaccine occurred with the IP route of inoculation. The large granulomas in the peritoneal cavity of the pigs inoculated with PE vaccine was aesthetically undesirable and could be confused with infection. In contrast, the DE vaccine caused one small granuloma in 1 or 2 pigs inoculated IP.

The aesthetically more desirable tissue reaction of the DE vaccine in the preliminary experiment resulted in more extensive testing which

consisted of a larger number of pigs, comparison of serum microneutralization titers, protection efficacy and histologic examination of the sites of inoculation. As previously reported, the serum neutralization titers and protection results for the PE and DE vaccines and the 3 routes of inoculation were similar. Most of the vaccinated animals developed vesicular lesions but many were mild; the animals did not appear to suffer and moved about easily. Any economic loss would have been minimal (*2*). Necrosis was again present at the sites of subcutaneous inoculation of PE but not DE vaccine. Since there was a marked difference in average largest oil droplet size between the 2 vaccines, the suspicion was strengthened that large oil droplets in the SC location could cause necrosis. In experiment 2, the essential absence of detectable gross or microscopic reaction in the IP inoculated pigs was similar to those observed in the preliminary experiment.

Of the 2 preparations and 3 routes of inoculation tested, the IP inoculation of DE vaccine was the most desirable combination for it resulted in protection and produced no significant detectable gross or histologic lesion. Additional limited studies of this method of vaccination have confirmed these results (*3*). Thus, the DE vaccine administered IP appears to be a desirable method for immunizing pigs to FMD.

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