TISSUE RESPONSE IN SHEEP VACCINATED WITH OIL-ADJUVANTED FOOT-AND-MOUTH DISEASE VACCINE

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SUMMARY

A preliminary study was conducted to determine tissue response in sheep vaccinated with oil-adjuvanted foot-and-mouth disease vaccine, according to route of inoculation and varying vaccine doses.

Based on field observations, veterinary inspection at slaughterhouses and histological studies, the following observations were made: (a) no signigicant differences were observed in the macroscopic or microscopic reactions of the oil-adjuvanted vaccines prepared as primary or double emulsions; (b) as previously demonstrated in cattle, this type of vaccine can be administered intramuscularly in the forward third part of the flat of the neck; (c) the intraperitoneal route was innocuous, and it is suggested that future studies be conducted to ascertain whether this route is applicable in the field, especially when double emulsion is utilized; (d) the immune response should be determined in sheep for the different routes and varying doses.

INTRODUCTION

In cooperation with the South American countries (Argentina, Brazil, Colombia, Paraguay and Uruguay), the Pan American Foot-and-Mouth Disease Center (PAFMDC) is engaged in field and laboratory research with oil-adjuvanted inactivated-virus vaccines (*5, 13*). To date, studies conducted with these vaccines in cattle have demonstrated that the foot-and-mouth disease (FMD) control programs using oil-adjuvanted vaccine can expect successful results in the future, based on the favorable immune response and the lower number of vaccinations required.

Serological studies conducted with serum samples taken at regular intervals from cattle, in the Montevideo milkshed area that have been vaccinated with this type of vaccine since March 1977 (*9*), have shown good antibody levels in both adult animals and cattle under two years old. The latter are vaccinated at six-month intervals until they reach the age of two, after which they are vaccinated only once a year.

According to Augé de Mello *et al.* (*1, 2*), cattle that have been systematically vaccinated with oil-adjuvanted vaccines during their first 2 years of age can, as adults, be vaccinated on a yearly basis. Under those conditions their expected percentage of protection was greater than 80% at 12 months after the fourth vaccination. The vaccination scheme has been more extensively studied in cattle because it is the most important animal species with respect to the disease. The vaccine is administered by intramuscular inoculation in upper part of the neck. Localized reactions at the site of inoculation sometimes appear when other routes are utilized. At meat inspection for exportation no rejection of carcasses or of tissue have occurred.

Oil-adjuvanted vaccines have been used in swine in Spain. The PAFMDC has likewise reported favorable results in pigs (*3, 4, 11*) with primary and double-emulsion FMD vaccines. The intensity of the reactions at the site of inoculation has varied according to whether the vaccination is subcutaneous (SC) or intramuscular (IM). No localized reactions were observed after intraperitoneal inoculation (IP) of double-emulsion vaccine.

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The U.S. Plum Island Animal Disease Center's Laboratory, and the PAFMDC (*6*), have published a preliminary study of experiments in which sheep were vaccinated with oil-adjuvanted FMD vaccine. It was shown that a primary-emulsion vaccine could induce an excellent immune reaction.

A preliminary test using oil-adjuvanted primary and double-emulsion vaccines was conducted in order to determine the acceptability of oil-adjuvanted vaccines for use in sheep, to observe eventual, general and local postvaccination reactions, and to ascertain the results of veterinary inspection at the slaughterhouse.

MATERIALS AND METHODS

Vaccines

The vaccines utilized in the study were prepared at the PAFMDC. Freunds incomplete adjuvant, composed of one part Arlacel A (mannide monooleate) and 9 parts mineral oil (Marcol 52), was mixed with an equal volume of inactivated FMD virus trivalent suspension to form a water-in-oil emulsion (*1*).

The oil-adjuvanted vaccine was prepared in primary and double emulsions, the latter being prepared from the same primary emulsion. The primary "water-in-oil" emulsion is transformed into double-emulsion (*3*) "water-in-oil-in-water" using an equal volume of the external aqueous phase with 2% Tween 80.

Animals

Thirty-six crossbred Corriedale sheep, 4-5 years old, weighing an average of 40 kg, were taken from a herd of 60 animals and used in the experiment.

Routes of inoculation and doses

The experiment is outlined in *Table 1*. The double-and primary-emulsion vaccines were used in 6 ml and 3 ml doses, respectively. The sheep were divided into three treatment groups of 12 animals. The sheep were sacrificed in groups of four for each treatment at 30, 60 and 90 days postinoculation (DPI). All the animals were examined before and after slaughter according to routine veterinary inspection procedures employed at the slaughterhouses (*7*).

Techniques

Tissue samples were taken from the inoculation area and from the regional lymphatic ganglia of all the sacrificed animals. These specimens were fixed immediately in a 10% formol solution for histological examination. The histological procedure was the routine processing of tissues embedded in paraffin and 4-5 μ sections, dyed according to the hematoxyline-eosine technique and Van-Gielson. Frozen sections were made from tissues fixed by formol and dyed with Sudan IV.

RESULTS

No undesirable clinical manifestations such as death, loss of appetite or behavioral changes were observed during the test period. A painless swelling was observed at the site of inoculation in the sheep inoculated subcutaneously and intramuscularly. No weight loss, peritonitis, undesirable digestive effects or renal disorders were observed in animals inoculated intraperitoneally. Macroscopicly and microscopicly no differences between the primary and double emulsions were observed when injected subcutaneously or intramuscularly.

30 days postinoculation

At 30 DPI, the microscopic local lesions corresponded to a subacute inflammatory type with presence of a foreign body. Thus, they could be described as a lesion similar to a foreign body granulom.

1. Subcutaneous inoculation. The lesion from SC inoculation tended to spread to the deep muscle tissue and to the regional fatty tissue. When cut, the lesion appeared to have a pale yellow color and a more or less firm consistency. All the animals developed small yellowish-white pearllike nodules from 1 to 3 mm in diameter, containing a small drop of vaccine. Small nodules were observed inside the corresponding regional ganglion.

2. Intramuscular inoculation. Inoculation by the IM route caused the presence of an oily substance deposited as small droplets from 1 to 3 mm in diameter. Some droplets accumulated into larger clusters between the muscles.

3. Intraperitoneal inoculation. In animals inoculated intraperitoneally, the vaccine was observed to have spread along the great omentum and great mesenterium, producing bright, grayish-white blotches differing noticeably from the peritoneal fat. As in the other two routes of inoculation, droplets of free vaccine were observed.

TABLE 1. *Description of doses and inoculation routes.*

Objective of the test	No. of animals per test	Doses & routes of inoculation	Postinoculation sacrifice - days		
			30	60	90
I. Observe overall reaction to primary emulsion dose according to various inoculation routes.	3	Subctutaneous. Behind the ear	1	1	1
	3	Intramuscular. Forward third part of neck	1	1	1
	6	Intraperitoneal	2	2	2
II. Comparison study of primary and double-emulsion doses at four inoculation sites simultaneously on same animal.	12	Subcutaneous. Behind the ear 3 ml left side[a] 6 ml right side[b] / Subcutaneous. Axillary region 3 ml left side 6 ml right side	4	4	4
III. Comparison study of primary and double-emulsion doses at four inoculation sites simultaneously on same animal.	12	Intramuscular. Forward third part of neck 3 ml left side 6 ml right side / Intramuscular. Inside of leg 3 ml left side 6 ml right side	4	4	4
Totals	36		12	12	12

[a] Dose 3 ml of primary emulsion.
[b] Dose 6 ml of double emulsion.

4. Microscopically, an important subacute inflammatory reaction is observed for all the inoculation routes. The lesion is characterized by the presence of roundish vacuoles corresponding to droplets of vaccine. These vacuoles varied from 20-micra microdrops to 2-mm macroscopic drops.

The 3 ml or 6 ml intramuscular inoculations produced a partial replacement of the parenchyma by an inflammatory process of two characteristics: (a) an interfascicular diffusion that dissects and displaces the muscle fiber, and (b) or a focal process of different-sized nodules clustered around the vaccine. These granulomatous processes completely replaced the muscle fibers, producing muscular necrosis so that only isolated muscle fibers were observed (*Fig. 1*).

This process also extended to the interstitial fatty tissue. The cellular elements that constituted the diffuse infiltrate were cells of the lymphoplasmocytic type and polymorphnuclear leucocytes.

A variety of the focal inflammatory response could be established with relation to the size of the droplets. When small droplets were obserbed, the response was of the lymphoplasmocytic type or of epithelioid cells. The large granulomatous reactions were formed by a large central droplet surrounded by an overlying layer or epithelioid cells and giant foreign-body cells. Fibroblasts, lymphoplasmocytic and newly formed capillary elements were observed on the periphery.

60 days postinoculation

1. Macroscopically, the vaccine was found extending into the muscle tissue and onto the aponeuroses, as tiny yellowish-white droplets from 1 to 3 mm in diameter adhering strongly to the underlying layers.

The location of the point of vaccine inoculation could not be precisely determined, but in the inoculation area abundant yellowish-white fibrous tissue of a firm consistency was seen to have extended several centimeters into the muscle and between the muscle layers. A viscous liquid having the characteristics of the vaccine oozed out when the tissue was cut and pressed.

The regional ganglia showed enlargement of the cortical zone and presence of small 1 to 3 mm pearllike droplets similar to those observed in the muscle and subcutaneous tissues. The latter became somewhat edematous with an increase in lymphoid tissue, in the cortical and medullary zone. As in the results at 30 DPI, no differences were observed with relation to the route of inoculation.

2. Microscopically, no significant changes were noted with respect to the cell population observed at 30 DPI. But it is important to point out that there was greater fibroblastic reaction on the periphery of the granuloma in the central area of which there appeared necrosis of epithelioid cells. In some of the regional ganglia, droplets were found surrounded by numerous epithelioid cells; in other ganglia, there was a marked increase in the number of polynuclear eosinophils (*Fig. 2*).

90 days postinoculation

The disappearance of lesions was evident at all the inoculation sites; only slight traces of yellowish-white fibrous tissue, corresponding to scarred areas, were observed. These areas were firm when cut. Small droplets from 1 to 3 mm in diameter were noted in the muscle layers and adjacent fatty tissue; when cut, they contained an oily substance that resembled the vaccine. There was no evidence of necrosis or formation of abscesses. Histologically, the cell composition was similar to those described above but with a distinct formation of conjunctive tissue between the reaction areas.

The histological aspects of the IP inoculation route evolved in a manner identical to that observed in the other routes of inoculation; encapsulated vaccine was likewise observed in the retrorenal zone.

After 90 DPI, no part of the inspected carcasses was rejected because of IM inoculation for the different vaccines and doses tested.

The histopathological characteristics of the lesions observed indicate an early immune response represented by macrophages, plasmocytes, lymphocytes and polymorphnuclear cells found at the site of inoculation at 60 and 90 DPI.

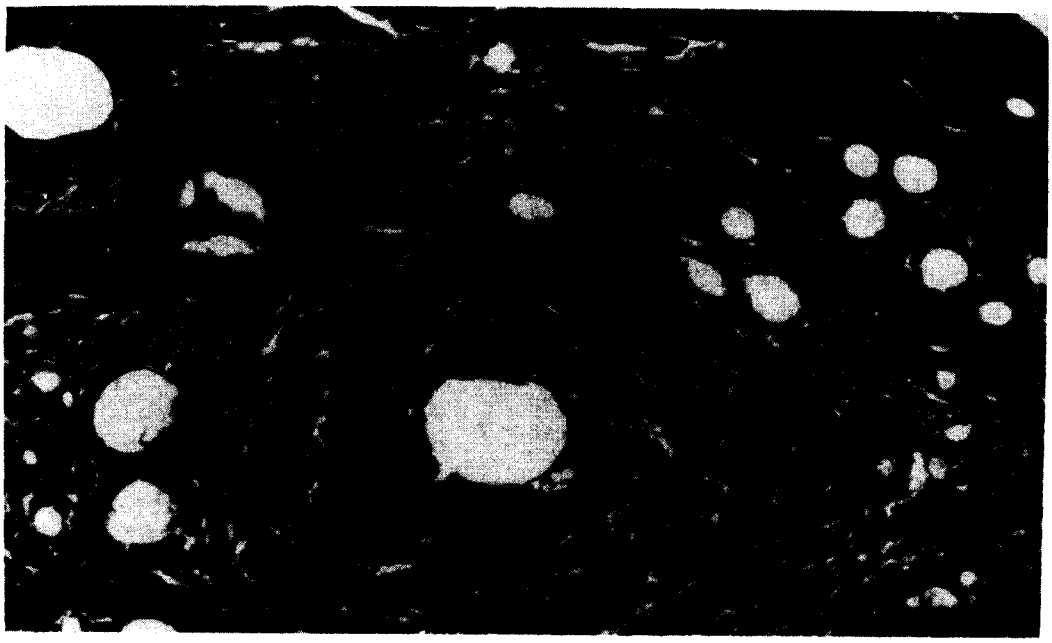

FIGURE 1. *Microscopic lesions observed in sheep tissues 30 days after intramuscular inoculation with oil-adjuvanted foot-and-mouth disease vaccine.*

FIGURE 2. *Microscopic lesions observed in sheep tissues 60 days after intramuscular inoculation with oil-adjuvanted foot-and-mouth disease vaccine.*

DISCUSSION

From the standpoint of veterinary inspection at slaughterhouses, it is important to establish a differential diagnosis, supported by histological studies, capable of distinguishing the vaccine reactions from granulomatous changes produced by other diseases that might be confused with the use of this type of vaccines (*10*). In Uruguay, undesirable reactions have not been observed in cattle in a field experiment involving dairy cattle (*8*).

Previous studies involving oil-adjuvanted vaccines in pigs have indicated that localized lesions could persist for a year after SC or IM inoculations (*12*). However, recent studies on pigs at the PAFMDC (*3, 4, 11*) revealed no undesirable local reaction after IP inoculation of double-emulsion vaccine, even when the animal was slaughtered one month after inoculation.

The sheep in this experiment were inoculated intraperitoneally, but only with primary-emulsion vaccine. No differences in local reactions in the animals were observed for either SC or IM inoculations of primary or double-emulsion vaccines. The local lesions were rather extensive at 30 DPI, but regressed progressively at 60 and 90 DPI.

Studies on swine (*3*) suggest that vaccine doses smaller than the ones used in this study might induce an adequate level of protection. Therefore, subsequent studies should be carried out to determine what the practical minimum vaccine dose and inoculation routes should be used for sheep under field conditions.

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