

CATEDRA DE PARASITOLOGIA
(Prof. Dr. M. CORDERO DEL CAMPILLO)

**FIELD AND LABORATORY EXPERIENCIES WITH AN
ANTIAPHTHOUS VACCINE FOR PIGS, INACTIVATED
WITH EEI AND WITH DEAE-DEXTRAN AS AN
ADJUVANT***

By *M. Cordero del Campillo*

P. Cármenes Díez

A. Martínez Fernández

Foot-and-Mouth disease (FMD) is of utmost importance in Spain, both from the epizootiological and zootechnical point of view. First of all, the fact is that no eradication programme will be successful, unless, in one way or another, there is a possibility to diminish FMD incidence in swine, since the large receptive and unprotected population supposes a constant infection nidus for all the remaining receptive species, more or less protected. This is most obvious when considering that, as far as pigs is concerned, the classical scheme of epizootical waves has become —precisely within the areas of major pig density— into an enzooty, that maintains its seriousness the whole year through, with a slight incidence decrease during summer time. The seriousness of the situation can be assessed by considering that, in hundreds of farms, not a single fattening cycle comes to an end without the presence of the disease, which exceptionally ends up affecting almost three times the same animals.

The economical aspect of the problem can be easily derived from the foregoing, specially when considering that the attack of the disease implies

* Paper presented to the Session of the Research Group of the Standing Technical Committee. European Commission for the Control of Foot-and-Mouth Disease. FAO. Federal Research Institute for Animal Virus Diseases. Tübingen (Germany). 20 - 22 October, 1971.

a decline in the growth rate, what means a delay of the end of the fattening period varying from two to four weeks (depending on the breed, age, weight handling, lodging, etc.). In other words, a decreased efficiency with which food is converted.

From all the above stated, one may conclude the tremendous importance that any agent preventing pig-FMD could have in Spain.

The difficulties in preventing the disease basically turn up during the fattening period, that is to say, from the weaning (7-8 weeks) until they reach a weight of some 90-100 kg, what normally implies a period of almost 4 months. In the remaining phases of the economic life of a pig, FMD can be prevented with relative easiness.

Protection of pigs against FMD by means of an inactivated vaccine has been revised by MUSSGAY and WITTMANN (1968). The conclusion was that up to now there was no vaccine available warranting the protection of pigs on the field during a sufficient period and liable to be used without hazards. Most experimental works performed so far were based upon the use of aluminum hydroxide or saponine as adjuvants, without succeeding in substantially increasing its strength by augmenting the antigen dose or by using different inactivators.

Even though a series of promising experiences had already been published, the authors were doubtful on whether an immunity of pigs, similar to that of bovine, could be achieved. Moreover, they based this assumption on the fact that pigs, even having undergone the disease, did not develop a lasting immunity (BAUER *et al.*, 1969).

During the last years it has been proved that vaccines with incomplete Freund adjuvant can stimulate a high rated and lasting immunity in pigs (McKERCHER and GIORDANO, 1967, and ANDERSON *et al.* (1971), to quote the first and the last). On the other hand, WITTMANN *et al.* (1970) published a series of papers wherein they stated that the use of DEAE-Dextran as an adjuvant can provide vaccines achieving an almost complete protection to pigs up to 12 months after vaccination.

We thus find ourselves in the presence of three basic vaccine types, against FMD in pigs:

- 1) Bovine vaccine, the improvement of which is attempted in certain cases by increasing antigen and saponine concentration.
- 2) Vaccines with incomplete Freund adjuvant.
- 3) Vaccines with DEAE-Dextran.

The first named are of an utmost limited efficiency, specially when considering that, in order to achieve an adequate protection level, setting out from 20-25 kg piglets, two up to three vaccine applications being needed, with the result that the animal is rather unprotected during a period of time representing approximately 1/3 of the fattening period.

Regarding those named under item 2), which do provide a high and lasting protection level, no solution has been found so far in order to avoid the serious damages produced at the point of inoculation, specially when considering conditions prevailing on the field, where the injection is far less meticulous as compared with that being performed on lab level.

As far as the third type of vaccine is concerned, it is the only one where at present immediate solutions can be found.

Setting out from the works of WITTMANN *et al. (ibid)* on monovalent vaccines, inactivated with EEI and DEAE-Dextran as an adjuvant, a series of experiences with bivalent vaccines have been performed, the results of which are listed hereafter.

MATERIALS AND METHODS

The vaccines has been prepared according to WITTMANN *et al. (ibid.)* with types 0₁ Spain and C on tissue culture (continuous cell line from bull calf testis), polyethylene-glycol concentrated, inactivated with ethyl-ethyleneimine and DEAE-Dextran adjuvated (100 mg/ml.)

Vaccine dose has been always 10 ml, applying 5 ml on each side of the neck, by deep intramuscular route.

Animals. Pigs submitted to current fattening conditions prevailing in Spain (different cross-breeds between Large White, Landrace and pigs of the country) with weights ranging between 15 and 80 kg, even though 90 % of the pigs used weighted about 20 kg. A total of 197 pigs under laboratory conditions and 1,416 on the field were vaccinated.

RESULTS

Innocuity

Local reactions.—A tumefaction at the site of inoculation was observed in vaccinated pig, with heat and pain. This reaction was evident already after 6-8 hours, reached its maximum 20 hours postinjection and was almost inappreciable at 36 hours.

The occurrence of abscesses and necrosis was very rare, representing

only 0.2 % of the vaccinated pigs. Several lymph nodes draining the point of inoculation, as well as muscular specimens around that site have been histopathologically studied. (Dr. A. Escudero, Dept. de Patología Morfológica y Funcional). Samples were taken 4 weeks after vaccination, from young pigs and fattened ones. No alterations whatsoever could be observed in the muscular mass. Most part of the lymph nodes showed a normal macroscopic aspect and microscopically a secondary immunological reaction process was found. In some cases, ganglionic involution could be observed as a result of the aggressive process set in motion by the adjuvant. All pigs used in these tests have already been slaughtered and in no case any kind of change could be observed. In fact, they passed veterinary inspection without condemnation.

General reaction.—The intensity and the way of reaction varied from one animal group to another, even though, in spite of all that, there was a certain uniformity.

The most frequent reaction that usually affected 80 % of the pigs turned out to be anorexia and fever. It shows up 2-3 hours after vaccination, lasting usually between 24 and 36 hours. Temperatures taken after 48 hours were normal. As far as the loss of appetite is concerned, generally speaking it may be stated that they lost a complete meal, and only a part of the following one, to express it using the pig-keepers expression.

In some cases, in certain groups of animals, immediately after vaccination, some of them exhibited sialorrhoea, vomiting and cyanosis. This reaction, although spectacular to some extent, was transitory and without any consequence for the animal, since after 20-30 minutes they were normal again. We could observe this reaction occurring mostly during intensive heat hours and when animals had eaten recently.

The possible consequences of these reactions on the animal growth rate were studied on 27 genetically homogeneous pigs (Prof. Dr. E. Zorita, Depart. de Nutrición Animal). They got weaned after two weeks and kept together from that moment on until the completion of the test. They underwent no stress whatsoever (castration, stable change, other vaccinations etc.). They were fed *ad libitum*. Vaccination was effected when between 5-6 weeks old. Weighing was performed once they got vaccinated, and afterwards it was repeated each week. The test was completed 4 weeks after vaccination. Although there was a slight difference during the first week, favouring the controls, from the third week onwards there were no substantial difference in weight gain.

Immediate protection

According to WITTMANN *et al.* (*ibid.*), the specific antibody levels are noticeable starting from the fourth day after vaccination, a fact that is rather unusual with an inactivated vaccine. Since the aforementioned authors admit there is no direct correlation between the neutralizing antibody titer and protection, we compared the following ones:

- 1) A cattle vaccine (aluminum hydroxide plus saponine)
- 2) A similar one, with higher antigene and saponine concentration.
- 3) The experimental vaccine we are speaking about (EEI-DEAE-Dextran).
- 4) A DEAE-Dextran solution (100 mg/ml).

Vaccinate pigs were challenged by contact infection, one week afterwards. Observations were made not only of the number of completely and partially protected animals, but also of the moment the first apthha appeared, as well as the seriousness of the disease. Results are summarize in table 1.

With these results at hand, we wanted to prove the possibilities of this vaccine on the field, in order to control a FMD outbreak. The initial test took place at 17 farms totalling 1,416 pigs in all. At 10 of those farms there had been FMD from 1 to 6 weeks before the admission of 801 new animals, that got vaccinated just on their discharge from the lorry. At the remaining 7 farms, at the time of vaccination different stages of FMD were present. In the first group, no case of FMD occurred with the vaccinated animals. In only one farm where there were 220 animals left as controls (unvaccinated), 70 % of which suffered FMD and, although the disease was not very severe, there were 8 casualties. In the second group, the percentage or animals that caught the disease after vaccination was never beyond 3-4 % and, in most cases, it did not exceed from 1 %. Animals suffering the disease show it clinically not latter than the third day after vaccination.

It can be assured that, when vaccinating a group of animals where there is no one ill, and when this yard is slightly separated from the initial focus, vaccination proves 100 % efficient. When in the group there are already some animals suffering the disease, the efficiency of the vaccine depends on the percentage of sick animals. Whenever the percentage is low ($\neq 10$ %) the percentage of vaccinated animals suffering the disease is likewise reduced (10 - 20 %). With the increase of the number of animals that have caught the disease within the group, the possibilities of controlling

FMD are scarcer. Thus, it does not appear venturesome to state that vaccination can suppress the development of the disease in an animal even after having been in contact, during 24 hours, with a sick animal.

Duration of the protection

Most papers published up to now have been devoted to the study of the duration of immunity with monovalent vaccines. In order to check whether or not protection lasts similarly with bivalent vaccine, 110 piglets 8 - 10 weeks old, weighing an average of 19 kg were used. Those animals got deparasitized and vaccinated against classical hog cholera, before starting the proper test, which was initiated 2 weeks after hog cholera vaccination. During the 3 week period, where the animals were being prepared, 2 of them died from pasteurellosis. To perform the test, pigs were distributed into 4 groups of 20 animals, and 2 more of 14 each. In the 20 animal groups, 15 got vaccinated, whereas 5 were left as controls. In the other two groups, 10 got vaccinated, 4 being left as controls.

During the two weeks following vaccination (December 20, 1970, up to January 5, 1971), extreme low temperatures were prevailing in the area (up to -20° C), with the result that during this most critical immunization period the animals did not find themselves under favorable conditions.

Experimental infections were brought about by means of contact. Two of the controls of either group got infected by means of intensive friction applied on the tongue and buccal cavity with a swab saturated with a 2 % bovine lingual epithelium suspension, diluted with isotonic phosphate buffer. Results are summarized in table II.

DISCUSSION

Vaccination at neck level does not imply so many disadvantages as the one performed at thigh level (spectacular lameness, swelling of the whole limb etc.), so that we consider it being the election point for that vaccine. On the other hand, intramuscular injection is easier to perform as compared with subcutaneous one, and with this vaccine it can be achieved without any difficulty and with similar or even better results.

The vaccine has proved to be entirely innocuous, allowing to apply it under field conditions without any hazard.

Bivalent vaccines prepared according to methods described by WITTMANN *et al.* (*ibid.*) are just as effective as are the monovalent ones.

The spectacular rapid protection provided by that vaccine offers vast possibilities of action where there are FMD focuses, allowing to suppress them from the very start.

A lasting immunity along the usual fattening period of pigs can be considered enough for mass protection. As demonstrated, after 8 weeks animals are 100 % protected against type C (most frequent in Spain among pigs) and as high as 86 % against type O. Even though after 12 weeks percentages of protected animals are diminished (70 % against C and 60 % against O), the high percentage of partially protected animals must be taken into account, specially considering that such animals probably would behave as being immune on the field. At any rate, the favourable anamnestic response demonstrated by ANDERSON *et al.* (1971), likewise offers possibilities in case revaccinations should be necessary, either as a result of the fattening period being exceedingly prolonged, or because the convenience of revaccination is considered to face a serious epizooty threatening pig vaccinated several months earlier.

RESUMEN

En condiciones de laboratorio (197 cerdos) y de campo (1.416 cerdos), se ha estudiado experimentalmente el valor de una vacuna contra la glosopeda, preparada a partir de cultivos hísticos (línea celular continua procedente de testículo bovino), concentrada por medio de polietilenglicol, inactivada mediante etil-etilen-imina (EEI) y ayudada con DEAE-dextrano. Se aplicó una dosis de 10 ml., dividida en dos de 5 ml., inyectadas a cada lado del cuello, por vía intramuscular.

La inocuidad (estimada valorando la reacción local y la general), el efecto sobre la tasa de crecimiento, la protección inmediata y la duradera, así como la comparación con dos vacunas absorbato con hidróxido de aluminio, han resultado muy satisfactorias. La protección inmediata, realmente espectacular, sugiere la posibilidad de controlar la glosopeda en el campo, mediante la vacunación en masa de los efectivos. El nivel de protección es suficiente para cubrir el periodo de ceba comprendido desde el destete hasta el envío de los cerdos al matadero, (tipo «baconer»). Si han de mantenerse los animales durante periodos más prolongados se sugiere la conveniencia de re-vacunar.

RESUME

Dans des conditions de laboratoire (197 cochons) et de champ (1.416 cochons), on a étudié d'une manière expérimentale la valeur d'un vaccin contre fièvre aphteuse préparé à partir de cultures histiques (ligne cellulaire continue provenant d'un testicule bovin), concentré au moyen de polyéthylenglycol, inactivé avec de l'éthyléthylèneimine (EEI) et adjuvé avec du DEAE-dextrane. On appliqua seulement une dose de 10 ml. distribuée en deux de 5 ml. chacune d'elles, injectées à chaque côté du cou, par voie intramusculaire.

L'innocuité (calculée en titrant la réaction locale et la générale), l'effet sur le taux de croisement, la protection immédiate et la permanente, ainsi que la comparaison avec deux vaccins-adsorbats avec de l'hydroxide d'aluminium, ont été très satisfaisantes. La protection immédiate, vraiment spectaculaire, suppose la possibilité de contrôler fièvre aphteuse dans le champ au moyen de la vaccination de cochons par groupes. Le niveau de protection est suffisant pour couvrir la période d'engraissement comprise entre le sevrage et l'envoi des cochons à l'abattoir (type «baconer»). Si les cochons doivent vivre pendant des périodes plus prolongées, on conseille la convenance de vacciner de nouveau.

SUMMARY

A vaccine against foot-and-mouth disease, prepared from tissue culture (a continuous cell line from bull calf testis), concentrated by means of polyethylene glycol, inactivated with ethyl-ethylene-imine (EEI) and with DEAE-Dextran as an adjuvant, has been studied under laboratory (197 pigs) and field conditions (1,416 pigs). A single 10 ml dose, divided into two 5 ml injected on each side of the neck, by intramuscular route has been used.

Innecuity (local and general reaction), effect on growth rate, protection, both immediate and lasting immunity and comparison tests with two aluminum hydroxide vaccines (a normal and a concentrated one, saponine added) proved very satisfactory. A spectacular immediate protection suggest the possibility of controlling FMD outbreaks in the field by mass vaccination. The level of protection is enough to cover the fattening period of piglets up to the time to send them to the abattoir. Should the animals be kept for longer, revaccination is suggested.

ADDENDUM

After completion of the above mentioned experiments, a wider field test has been concluded in the Spanish province of Lérida (Cataluña), results of which are here summarized.

The test was initiated on June 9th, 1971, in 56 pig farms with 19,851 fattening pigs. Of them, 8,310 were vaccinated and 6,027 left as controls. Other present animals were not controlled. In 31 pig-farms there was no FMD case at the time of vaccination. Present animals were divided into 5,010 vaccinated and 4,347 controls (Total 9,357).

In 25 pig-farms there were different stages of FMD. A total of 3,300 pigs got vaccinated and 1,680 left as controls (Total 4,980).

RESULTS & DISCUSSION

Innocuity.—Six animals died as a result of vaccination. Anorexia was present in 80 % of vaccinated pigs for a maximum period of 24-36 hours.

Most vaccinated animals have already been sent to the abattoir and passed Veterinary Inspection without condemnation.

Protection. No case of FMD has been detected in healthy vaccinated animals, on days immediately following the injection.

Immediate protection has been observed in animals from FMD contaminated farms. Of those symptomless vaccinated pigs, no more than 4 % developed the disease and, in certain cases, only about 1 % got infected.

When FMD appeared after the vaccination, nearly 100 % of vaccinated animals were protected. Controls were affected and in one farms severe cases with casualties were observed.

Animals inoculated with	Fig Nr.	24 h.	48 h.	72 h.	96 h.	120 h.	144 h.	168 h.	Summary
Standard cattle vaccine (Bivalent OC) Dose 10 ml/subc.	1	—	—	—	—	—	—	—	66 % with FMD 66 % NI
	2	—	—	—	—	—	—	—	
	3	—	++ + +	—	—	—	—	—	
	4	—	++ + +	—	—	—	—	—	
	5	—	++ + +	—	—	—	—	—	
	6	—	++ + +	—	—	—	—	—	
Concentrated vaccine (Bivalent OC) Dose 10 ml/subc.	7	—	—	—	+	+	—	—	100 % with FMD 33 % NI. 66 % PI.
	8	—	—	—	—	—	—	—	
	9	—	++ + +	—	—	—	—	—	
	10	—	—	—	—	—	—	—	
	11	—	—	—	—	—	—	—	
	12	—	++	—	—	—	—	++ + +	
EEI-DEAE-Dextrane vaccine (Bivalent OC) 100 mg DEAE-dext./ml. Dose 10 ml/intram.	13	—	—	—	—	—	—	—	
	14	—	—	—	—	—	—	—	
	15	—	—	—	—	—	—	—	
	16	—	—	—	—	—	—	—	
	17	—	—	—	—	—	—	—	
	18	—	—	—	—	—	—	—	

Animales inoculated with	Pig Nr.	24 h.	48 h.	72 h.	96 h.	120 h.	144 h.	168 h.	Summary
Solution of DEAE dextrane (100 mg./ml.) Dose 10 ml.	19	—	++ +	++ + +					100 % with FMD 66 % NI. 33 % PI.
	20	—	—	++ + +					
	21	—	++ + +	++ + +					
	22	—	++ + +	++ + +					
	23	—	—	++					
	24	—	+						
Controls	25	—	++ + +						100 % with FMD 83 % NI. 16 % PI.
	26	—	++ + +						
	27	—	++ + +						
	28	—	++ + +						
	29	—	++ + +						
	30	—	++ + +						

TABLE I

Notes: Each cross stands for one aphthous limb.
 NI. Non immune
 PI. Partially immune

Type 0

Weeks	Nr. of animals	I		PI		NI	
4	15 V	12	80 %	2	14 %	1	7 %
	5 T					5	100 %
8	15 V	13	86 %	1	7 %	1	7 %
	5 T					5	100 %
12	10 V	6	60 %	2	20 %	2	20 %
	4 T					4	100 %

Type C

Weeks	Nr. of animals	I		PI		HI	
4	15 V	15	100 %	—	—	—	—
	5 T					5	100 %
8	15 V	100 %	—	—	—	—	—
	5 T					5	100 %
12	10 V	7	70 %	2	20 %	1	10 %
	4 T					4	100 %

V. Vaccinated

T. Controls.

I. Immune

PI. Partially immune (apthae in 1 or 2 feet)

NI. Non immune (apthae in 3 or 4 feet).

TABLE II

LITERATURE

ANDERSON, E. C., MASTERS, R. C. and MOWAT, G. H. (1971). Immune Response of Pigs To Inactivated Foot and Mouth Disease Vaccines. Response to Emulsion Vaccines. *Res. vet. Sci.*, **12**, 342-350.

_____, _____, _____ (1971). Immune Response of Pigs to Inactivated Foot and Mouth Disease Vaccines. Response to DEAE-Dextran and Saponin Adjuvated Vaccines. *Res. vet. Sci.* **12**, 351-357.

BAUER, K., LEIPPRAND, H. et WITTMANN, G. (1969) Recherches sur l'immunité conférée au porc par une atteinte de Fièvre aphteuse de type 0. *Bull. Off. int. Epiz.* **71**, 519-538.

MCKERCHER, P. D. and GIORDANO, A. R. (1967) Foot and Mouth Disease in Swine. I. The Immune Response of Swine to Chemically-treated and Non-treated Foot and Mouth Diseases Virus. *Arch. ges. Virusforsch.* **20**, 39-53.

MUSSGAY, M. und WITTMANN, G. (1968) Über den gegenwärtigen Stand der Forschung zur Entwicklung von MKS-Impfstoffen für Schweine. *Berl. Münch. tierärztl. Wschr.* **81**, 124-129.

WITTMANN, G., BAUER, K. und MUSSGAY, m. (1970) Versuche zur Schutzimpfung von Schweinen mit Vakzinen aus inaktiviertem Maul —und Klauen— seuche. (MKS)-Virus. II. Versuche mit Aethylaethylenimin (EEI)-inaktiviertem Virus und Diatylaminoethyl-Dextran (DEAE-D) als Adjuvants. *Arch. ges. Virusforsch.*, **29**, 139-158.