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TECHNICAL TRANSLATION

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FOOT AND MOUTH DISEASE VACCINE PREPARED FROM VIRUS  
CULTIVATED IN CALF KIDNEY CELL SUSPENSION

by

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13. ABSTRACT  
**Five series of foot and mouth disease vaccine were prepared in calf kidney cell suspensions. Three were highly active, protecting all vaccinated cattle from controlled infection. One series was satisfactory, protecting three out of four animals, with no evidence of generalised disease. One series was less effective, with one example of generalised disease. Culturing in calf kidney cell suspensions allows one to obtain large quantities of foot and mouth disease virus more easily than the monolayer culture method.**

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| Foot and Mouth Disease Virus<br>Vaccine<br>Suspension Culture |        |    |        |    |        |    |

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## FOOT AND MOUTH DISEASE VACCINE PREPARED FROM VIRUS CULTIVATED IN CALF KIDNEY CELL SUSPENSION

In 1964 to 1965, we carried out 30 experiments in order to determine the optimal conditions of culturing foot and mouth disease virus in suspensions of trypsinized cells from kidneys of large cattle.

The purpose of the research was to obtain foot and mouth disease virus in a suspension of kidney epithelial cells and to prepare an experimental series of vaccines from virus-containing material.

### Materials and Methods

**Preparation of Cell Suspensions.** Kidney tissue with 2-quarterly growth was treated with 0.25% solution of Difko trypsin (1:250) at room temperature. The cell suspension was passed through a two-layered gauze filter, and then was centrifuged for 5 minutes at 1,000 rpm. The trypsin was washed from the cells two or three times with Hanks' solution. The trypsinized calf kidney cells were suspended in 0.5% hydrolysate of lactalbumin and neutralized with Hanks' buffer solution without serum at pH 7.6. The original concentration of cells was 1 to 3 million in 1 ml of suspension.

**Virus.** Three strains of foot and mouth disease virus were used for the experiment (two type O and one type A), which were adapted to monolayer tissue culture of large cattle kidneys (five to nine passages).

**Culturing of Virus.** The cell suspension was introduced into a flask of 1.5 l capacity in a quantity of 250 ml. The virus culture fluid was added to the suspension. The infecting dose was 0.02 TCD<sub>50</sub> in the cell. The flask was mixed in a hot room (37°), and the virus was cultured without aeration and stirring.

Virus was collected after 28 hours, titrated in mice-suction boxes of five-seven day growths or in monolayer cultures of kidney tissue from large cattle, and grown in test tubes. The titre was determined by the method of Rid and Myench and conveyed in  $DL_{50}$  in mice and  $TCD_{50}$  in tissue culture (mL).

Preparation of Inactivated Vaccine. The vaccine was prepared by the method used in Italy (Zaval'i), and also by a modified method.

After 28 hours of culturing the flask with the virus suspension was stirred in a cold chamber at  $-40^{\circ}$  for two days, after which the virus was thawed at room temperature. This procedure was repeated three times.

Then the virus was centrifuged at 3,000 rpm for 10 minutes. The supercharged culture fluid--virus was used for preparation of vaccine according to the following prescription: 50% virus, 45% hydrate of aluminum oxide, 0.05% formalin, 4% glycine buffer (120 g glycine + 163 g sodium chloride in 4 l of distilled water), 1% alkali (30 g NaOH in 1 l of distilled water); pH of vaccine is 8--8.2.

According to the Italian method, immediately after centrifugation, the virus is mixed with formalin (0.05%), kept at a temperature of  $27^{\circ}$  for 72 hours, and then the remaining ingredients are added.

The difference in the modified method is that the formalin is added together with all of the ingredients, and only after this, the vaccine is inactivated at a temperature of  $27^{\circ}$  for 24 hours.

## Results

From the hoof and mouth disease virus, grown in suspension of calf kidney cells, 32 experimental series of vaccines were prepared (the volume of a series was 200-800 mL). For guinea pigs and white mice, all the series of vaccines prepared were harmless.

Five series of vaccines were tested in large cattle.

In order to determine a virulence, each series of vaccine was injected into three animals subcutaneously at a dose of 20 mL. The period of observation was 10 days. Fifteen animals were used for this purpose. All of them remained clinically healthy.

The activity of each series of vaccines was studied in six animals: in two--the vaccine was injected subcutaneously at a dose of 5 mZ, in two--in 10 mZ, and two remained as controls.

On the twentieth day after injection of the vaccine, four immunized and two un-immunized control animals were artificially infected. An homologous strain of aphthous virus of foot and mouth disease was used for this, in dilutions of 1:500. Virus was rubbed with a toothbrush on mucous membrane of the tongue (Table).

Results of Tests of Immunogenic Properties of Foot and Mouth Disease Vaccine in Large Cattle

| Type of Virus and Number of Passages in Tissue Culture | Virus Titre In Mice-Suction Boxes (Neg DL <sub>50</sub> in 1 ml) | Number of Series of Vaccine | Method of Preparation of Vaccine | Reaction (On Infection of Animals) |             |             |
|--|--|-----------------------------|----------------------------------|------------------------------------|-------------|-------------|
|  |  |                             |                                  | Vaccinated                         |             | Control     |
|  |  |                             |                                  | 5 mZ                               | 10 mZ       |             |
| A-9  | 5,2  | 9                           | I                                | ○ ○ ○ ○ ○ ○                        | ○ ○ ○ ○ ○ ○ | ● ● ● ● ● ● |
| A-9  | 5,2  | 10                          | M                                | ○ ○ ○ ○ ○ ○                        | ○ ○ ○ ○ ○ ○ | ● ● ● ● ● ● |
| O-7  | 6  | 15                          | M                                | ○ ○ ○ ○ ○ ○                        | ○ ○ ○ ○ ○ ○ | ● ● ● ● ● ● |
| O-7  | 6  | 17                          | M                                | ○ ○ ○ ○ ○ ○                        | ○ ○ ○ ○ ○ ○ | ● ● ● ● ● ● |
| O-8  | 3,3  | 31                          | M                                | ○ ○ ○ ○ ○ ○                        | ○ ○ ○ ○ ○ ○ | ● ● ● ● ● ● |

Key. Method of Preparation of Vaccine: I--Italian (Zaval'i); M--Modified Method. Reaction in Animals Upon Infection: G--Generalization of Process; P--Primary Aphthas; O--Absence of Reaction.

From the Table it can be seen that the vaccine series No. 10, 17, and 31 were highly active. They protected all the animals from the disease after controlled infection. In the unvaccinated animals, a generalized form of foot and mouth disease was observed.

Results

1. The foot and mouth disease vaccine series No. 10, 17, and 31 from virus grown in suspension of calf kidney cells, during tests in large cattle demonstrated high activity, protecting all animals from disease after controlled infection.



2. Vaccine series No. 9 possessed satisfactory immune properties-- from four animals, receiving the vaccine, after controlled infection one was stricken without clinical signs of generalization of the process. Vaccine series No. 16 was less active (after controlled infection of four immunized animals, one was stricken with foot and mouth disease with generalization of the process).

3. Culturing in calf kidney cell suspensions allows one to obtain foot and mouth disease virus in large quantities and with less outlay of work than the monolayer culture method.

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