THE EFFECT OF MERTHIOLATE ON THE IMMUNOGENICITY OF FOOT-AND-MOUTH DISEASE VIRUS ANTIGENS

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BRIEF REPORT

Merthiolate is commonly used as a preservative in commercial inactivated foot-and-mouth disease (FMD) vaccines.

However, experiments with inactivated poliomyelitis vaccines have shown that degradation products of merthiolate can affect the virus antigen during storage of the vaccine (1). The effect of merthiolate can be neutralized by treatment with EDTA (trisodium salt of ethylene diamine tetra-acetic acid). Precipitation reactions in agar gel with FMD virus also show deleterious effects of merthiolate (2).

To test the effect of merthiolate in different concentrations on inactivated FMD virus antigens, inactivated FMD aluminum hydroxide gel vaccine without saponin, was prepared with O_1 type antigens produced in BHK cell cultures.

The vaccine was divided in 4 batches. No merthiolate was added to the first batch. Merthiolate was added to batches 2 and 3 at concentrations of 1/10,000 and 1/30,000, respectively. To the fourth batch merthiolate 1/10,000 and a solution of EDTA at a ratio of 10 mol EDTA per 1 mol of merthiolate was added. Groups of 6-8 cattle were inoculated with each of the batches after 1 and 3 months of storage at 4° C respectively.

These cattle were unvaccinated, 18-22 month old Hereford, with no specific circulating neutralizing antibodies. The farm where these cattle were bred and kept during the trial had been free of FMD for several years.

At 21 days after vaccination serum neutralization titers were determined in a tube test with BHK cell cultures using the variable serum and fixed virus method, with results expressed as the $\log_{10}$ of the reciprocal of the dilution protecting 50% of the cultures against 100 $TCID_{50}$ of type O_1 virus.

RESULTS

The results of this experiment are summarized in Table 1; they showed that subtype O_1 strain Campos was stable for as long as 3 months after the preparation of the vaccines.

In a second experiment, a trivalent inactivated aluminum hydroxide saponised vaccine was also divided into four batches and treated in the same way as the monovalent O vaccine. Groups of 7-9 cattle were vaccinated with the vaccines stored for 8 and 12 months. The sera were collected at the moment of vaccination and 25 days later, and tested against 3 virus types. The results, as shown in Table 2, demonstrate differences between the stability of the antigens in relation to the presence of merthiolate.

The immunogenicity of subtypes A_{24} strain Cruzeiro and C_3 strain Resende was unfavorably influenced by merthiolate, particularly after 12 months of storage of the vaccines. The addition of EDTA as used by Davisson (1) in polio vaccines resulted in loss of immunogenicity of the A and C antigens, but did not affect the O antigen.

Thus, concentrations of merthiolate in FMD vaccines higher than 1/30,000 are not recommendable. The addition of merthiolate should be done just prior to bottling of the vaccine.

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TABLE 1: *Mean serum neutralization titer of cattle 21 days post-vaccination with a monovalent type O_1 vaccine*

	Storage of vaccine	
	1 month	3 months
No merthiolate	1.0	1.4
Merthiolate 1/30,000	1.0	1.2
Merthiolate 1/10,000	1.3	1.4
Merthiolate 1/10,000 + EDTA	1.2	1.4

TABLE 2: *Mean serum neutralization titer of cattle 25 days after vaccination with a trivalent vaccine*

	O_1		A_{24}		C_3	
	Storage of vaccine in months					
	8	12	8	12	8	12
No merthiolate	1.9	1.6	1.8	1.6	1.4	1.1
Merthiolate 1/30,000	2.1	1.7	1.3	1.2	1.3	1.0
Merthiolate 1/10,000	2.1	1.6	1.2	0.9	1.4	0.7
Merthiolate 1/10,000 + EDTA	2.0	1.5	0.3	<0.4	1.0	<0.6

REFERENCES

1. DAVISSON, E.O.; POWELL, H.M.; MACFARLANE, J.O. The preservation of poliomyelitis vaccine with stabilized merthiolate. *J. Lab. Med. 47* (1): 8-19, 1956.

2. COWAN, K.M. Effect of merthiolate on agar gel diffusion precipitin reactions with foot-and-mouth disease virus. *J. Immun. 97* (5): 647-653, 1966.