STUDIES ON CERTAIN FILTRABLE VIRUSES
IX. ANTIGENIC PROPERTIES OF UNDESICCATED ENTIRE EMBRYO FOWL POX VIRUS IN BUFFERED GLYCERINE

H. S. BRYAN AND ROBERT GRAHAM
University of Illinois, Urbana

In a previous report the results of immunization of susceptible chickens with fowl-pox vaccine prepared from the entire chicken embryo, desiccated and suspended in various diluents was reported by Kerlin and Graham (1). Brandly and Dunlap (2) previously demonstrated the immunizing properties of fowl-pox-virus material prepared from the pox skin lesions and also from the chorioallantoic membranes of inoculated embryos showing typical fowl-pox lesions.

These three forms of the virus, after desiccation, are stored in sterile vials at –4°C. Immediately prior to field use the triturated virus material is suspended in distilled water, physiological saline, mineral oil or other suitable diluent.

Since the viability and immunizing properties of desiccated entire embryo virus suspended in various diluents have been demonstrated under laboratory and field conditions, experimental inquiry into the viability as well as the immunizing properties of the undesiccated entire embryo virus was inaugurated. It is the purpose of this paper to report the results of this study.

PREPARATION OF THE ENTIRE EMBRYO VIRUS

A desiccated, chorioallantoic virus stored at –4C° was the source of the virus used in this study. A 1:100 tryptone broth suspension of the chorioallantoic fowl-pox virus was inoculated onto the chorioallantoic membrane of ten to twelve day old developing chicken embryos by a modification of Burnet's technique (3). After five days further incubation at 37°C, the shells were swabbed with colloidal iodine and the chorioallantoic membrane of each egg was carefully examined for characteristic fowl-pox lesions. Membranes showing characteristic lesions together with the entire embryos were harvested aseptically by removing the contents of the eggs into sterile Petri dishes. The harvested entire embryos were immediately pooled, transferred to an electric tissue grinder and homogenized to the consistency of thick cream. The homogenized embryos were placed in sterile flasks and either used immediately in vaccine preparation or stored at –4°C, until needed.

PREPARATION OF THE BUFFERED GLYCERINE DILUENT

The only diluent employed in this experiment was a sterile 50 percent suspension of buffered glycerine in distilled water. The diluent was prepared by suspending 375 cc. of glycerine (C.P.) and 1.62 grams anhydrous potassium dihydrogen phosphate in 375 cc. of distilled water.

Sodium hydroxide was used to adjust to pH 7.6. The diluent was sterilized for 45 minutes under 15 pounds steam pressure.

PREPARATION OF THE VACCINE

The undesiccated, entire-embryo, fowl-pox-virus material was added directly to the diluent and suspended by gentle shaking. The virus material was readily suspended and miscible. For field use the vaccine consisted of a 5 percent suspension of the virus material in the buffered glycerine diluent. The prepared vaccine was stored at –4°C. and dispensed in screw capped vaccine bottles. One lot of vaccine was prepared for this investigation. The viability of the vaccine was checked monthly by the feather follicle method of inoculation of susceptible chickens. Viability tests proved the vaccine capable of producing take reactions during a four-month storage period at –4°C.

INOCULATION OF FLOCKS WITH THE VACCINE

A healthy flock of 2475 chickens was vaccinated by the three-stick inoculation method into the patagium of the wing in June 1945. Observations of over 10 per cent of the birds seven days after inoculation revealed positive take reactions in 97.9 percent. No ill effects were noted in the birds after inoculation with the vaccine. Ninety days after vaccinating, ten chickens from this flock were artificially exposed to viable fowl-pox virus. None of the ten showed a reaction to this exposure.

The experimental vaccine was supplied to several veterinarians and flock owners throughout the state of Illinois for experimental field use on farm flocks, with directions for application and interpretation of results. A total of 17 flocks including 23,282 birds, of which 7,087 were turkeys, were vaccinated by the stick method by eleven different field vaccinators. Take reactions reported in the various flocks varied from 25.4 percent to 100 percent (Table 1).

It was estimated that 18,899 of the 23,282 birds vaccinated developed take reactions, and that 4,383 birds did not develop takes. The overall estimate of the number of birds vaccinated in the field, either by veter-

TABLE 1.—FIELD RESULTS OF BIRDS VACCINATED WITH UNDESICCATED ENTIRE EMBRYO FOWL POX VACCINE

Group Numbers	Birds vaccinated	Percent takes reported
1 (3 flocks)	3800*	100.0
2 (1 flock)	1014	100.0
3 (1 flock)	2700	95.0
4 (1 flock)	4087*	95.0
5 (1 flock)	353	95.1
6 (1 flock)	235	99.0
7 (5 flocks)	8600*	67.5
8 (1 flock)	653	60.0
9 (1 flock)	342	61.0
10 (1 flock)	766	25.4
11 (1 flock)	732	66.0
	23,282	

* Turkeys included in groups 1, 4, and 7.

inarians or flock owners, and developing positive take reactions was 81.17 percent.

DISCUSSION

The undesiccated, entire-embryo, fowl-pox virus when suspended in buffered glycerine induced a high percentage of take reactions in susceptible chickens in the laboratory and in a flock of 2475 chickens when inoculations were made and takes were read by experienced handlers of fowl-pox vaccine. The vaccination of farm flocks by fowl-pox vaccinators, which in some cases had had less experince, resulted in a slightly lower percentage of take reactions. The possibility that certain of the farm flocks had been exposed to fowl-pox before the time of vaccination was not determined. However, it seems reasonable to expect that such a condition could have occurred in flocks where take reactions were low. The possibility of faulty technique having been used in inoculating birds or in reading take reactions could not be excluded. The results of farm flock vaccinations included in this report, suggest that proper care of the vaccine in the field, proper vaccinating technique, and a flock history are all essential in the establishment of a satisfactory fowl-pox vaccinating program.

SUMMARY

Results of the immunizing properties of an experimental fowl-pox vaccine, prepared from undesiccated, entire-embryo, fowl-pox-virus material suspended in a buffered glycerine diluent, is reported. A 5 percent suspension of undesiccated virus material was readily miscible and remained in suspension well in the diluent. The vaccine proved potent during a four-month storage period when held at –4°C. A flock of 2475 chickens inoculated by the three-stick method with the vaccine resulted in 97.9 percent of the birds showing positive take reactions. Field vaccination of farm flocks by veterinarians and flock owners resulted in take reactions in 81.17 percent of 23,282 birds, which included 7,087 turkeys. Factors essential in operating a satisfactory fowl-pox vaccinating program such as proper care of the dispensed vaccine, proper vaccinating technique, and the importance of a flock history are also discussed.

REFERENCES

1. KERLIN, D. L. AND GRAHAM, R.: Studies on Certain Filtrable Viruses VIII. Viability of Chicken Embryo Fowl Pox Virus. Am. J. of Vet. Res., 6. (1945): 204-207.
2. BRANDLY, C. A., AND DUNLAP, G. L.: Studies on Certain Filtrable Viruses II. Immunization Against Fowl Pox with Fowl- and Pigeon-pox Viruses Cultivated in Vivo and in Vitro. J.A.V.M.A., 95, (1939): 340-349.
3. BURNET, F. M.: The Use of the Developing Egg in Virus Research. Med. Res. Council (Gt. Brit.), Spec. Rept. Ser. No. 220, 1936.