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Crystal-Violet Vaccine

Experiments with crystal-violet vaccine
for the prevention of hog cholera

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For many years, research workers have endeavored to produce a vaccine from the blood of cholera infected pigs. Numerous chemical agents, heat, cold and desiccation have been used in attempts to destroy or attenuate the disease-producing properties of the blood and at the same time preserve its antigenic properties.

Investigators have been encouraged by the results of certain experiments, only to find that similar results could not be obtained in subsequent experiments carried out in exactly the same manner.

The need for an immunizing agent which would produce immunity against hog cholera without the danger connected with the use of live virus has long been recognized.

The use of anti-hog-cholera serum in conjunction with hog-cholera virus, known as the serum-virus method of immunization, or vaccination, has been of inestimable value in the prevention of hog cholera and this method of immunization is now employed extensively throughout the world. However, there have always been certain inherent drawbacks associated with this valuable prophylactic method.

The chief objection to the serum-virus method of immunization is the fact that a live and active virus is used in conjunction with the serum. When a live virus is injected into a susceptible hog, even with large doses of serum, a reaction always follows. This reaction, in the majority of cases, is invisible. However, experiments conducted by the Bureau have shown that a leucopenia regularly follows the simultaneous inoculation of serum and virulent blood, even in pigs which exhibited no fever and no visible symptoms. When the dose of serum was nearly double the amount required to prevent the development of visible symptoms, the leucopenia was still observed. In addition, there is the well established fact that the simultaneous inoculation is always followed by the appearance of the virus in the blood, where it circulates for two or three weeks even though there is no rise in temperature and no visible reactions.

In the case of perfectly normal and healthy swine, there is no danger from serum-virus immunization, but when secondary bacterial infections are present at the time of vaccination or are picked up shortly thereafter, the method may be fraught with danger, for the virus seems prone to activate such infections. Not only are bacterial infections, infestations with parasites, nutritional disturbances, and other delimiting conditions apt to be made more severe by the reaction following serum-virus vaccination, but the serum-virus reaction is itself likely to be intensified by the presence of any debilitating condition.

With a view of overcoming these objectionable features connected with the serum-virus method of immunization, officials and research workers in the Bureau of Animal Industry have had in mind for many years the development of a vaccine which would afford a safer and cheaper method of immunization. Much time and a great amount of work have been devoted to the preparation and testing of experi-

mental vaccines at the Bureau Experiment Station at Ames, Iowa, during the last fifteen or twenty years.

**Crystal-Violet Vaccine**

Crystal-violet vaccine is a modified virus made from the defibrinated blood of cholera pigs. It derives its name from the fact that crystal violet, an aniline dye derived from coal tar, is the principal attenuating agent used in its preparation. The late Doctor Marion Dorset, former chief of the Biochemic Division of the Bureau of Animal Industry, was the originator of the idea of using this dye in the production of hog-cholera vaccine.

The first lot of crystal-violet vaccine was prepared in November 1934, at the Bureau Experiment Station at Ames. The dye was first used alone and later in combination with several other chemical agents. Many experiments were conducted before a formula was discovered which could be used repeatedly with satisfactory results. The present formula was first used in May 1936, and since then many lots of vaccine have been prepared by the same method, which is briefly as follows:

To 800 cc. of selected defibrinated hog-cholera blood, 100 cc. of a three percent solution of disodium phosphate is slowly added while stirring. Next, 100 cc. of a 0.5 percent solution of crystal violet is added in the same manner. The mixture is placed in an incubator and left there for two weeks at a temperature of 37.5°C. Bacteriological examinations are made of the tissues of the pigs furnishing the virus and of the vaccine when it is removed from the incubator. After removal from the incubator the vaccine is kept in a refrigerator.

**Potency Tests**

Pigs weighing from forty to seventy pounds and of known susceptibility are used in potency tests. Two are injected with 3 cc., two with 5 cc. and two with 10 cc. of the vaccine and two are left untreated to serve as controls. In conducting these tests the vaccine is injected in the loose tissue of the thigh, beneath the subcutaneous tissue and fascia, but not in the muscular tissue. The test pigs are placed in clean, disinfected, outside pens, and kept under careful observation and away from exposure for three weeks. Previous tests have established the fact that an interval of from two to three weeks is required to establish immunity. Each pig is then given an injection of 1 cc. of virus of known virulence and carefully observed for another two weeks. Five weeks are thus required in carrying out the potency tests.

Numerous lots of vaccines have been prepared by the above-described method and several hundred pigs have been used in conducting the tests. The tests have demonstrated that the method used in the preparation of the vaccine had in all cases attenuated the virus to a point where it would no longer produce hog cholera in from 1 cc. to 10 cc. doses and that vaccine-treated pigs possess a high degree of immunity three weeks after vaccine administration.

**Farm Tests**

In carrying out these experiments, the nature of the new vaccine was carefully explained to each cooperating farmer. It was pointed out and emphasized that the treatment was in an experimental stage and its limitations as well as advantages were fully explained. The farmers were assured that the method was a safe one and that no ill effects would follow vaccination of their herds. They were also told that because of the slow development of immunity the vaccine should not be used in a neighborhood or community where hog cholera was known to be prevalent. Herds were therefore selected for treatment in communities where no cholera prevailed.

In the treatment of farm herds, every precaution was taken to prevent the introduction of disease. Equipment and clothing were used which had not come in contact with hog-cholera virus.

The herds were not kept under observation following vaccination, as this was not considered necessary in view of the absence of any symptoms of sickness in experimental and test pigs at the Bureau Station.

The farmers were informed that the
vaccine appeared to protect the treated animals through the usual fattening period, but no guarantee was given as to the duration of immunity. Retreatment with vaccine or serum-virus treatment at the end of eight months was advised for animals to be kept for breeding stock.

The treatment was administered free of charge with the one *proviso* that when the pigs were ready for market a certain number of animals from each herd, usually four, would be delivered to the Bureau Station for an immunity test. These hogs were purchased at the prevailing market price or immune hogs of equivalent value were given in exchange.

News of the new treatment spread rapidly throughout the surrounding country and farmers made applications for the treatment in large numbers. The first farm herd was treated on November 5, 1935, near the Ames Station. Since that time, 248 herds containing 13,281 pigs have been treated with vaccine made by the improved formula. In order to ascertain whether the vaccine possessed or would regain virulence during the animal passage, 196 untreated controls were left in 78 of these herds, and no sickness developed in these pigs.

**Administration**

The vaccine was administered by subcutaneous injection on the inner side of the thigh. Pigs weighing under 75 pounds received a 5 cc. dose while those weighing over 75 pounds were given a 10 cc. dose. In a limited number of herds, a portion of the herd was given two treatments administered two weeks apart. Ear marks were used to identify the various groups.

Herd owners were instructed to report in case sickness of any kind developed. In only three of the 248 herds treated did any disease develop which required an investigation. Sickness developed two weeks after treatment in one herd and two months after treatment in the other two herds. In these herds lesions were found that could be regarded as suspicious of cholera and they were treated with serum and virus. However, two pigs were purchased by the Bureau from two of these farms and left in the herds without any treatment except the original vaccine treatment. All of these pigs remained normal.

The treatment of 248 herds consisting of 13,281 pigs with sickness developing in only three herds during the fattening period, would seem to furnish evidence that the crystal-violet vaccine is a safe product to use.

In some recent farm experiments herds which are sometimes referred to by practicing veterinarians as problem herds have been selected for treatment with crystal-violet vaccine. These are herds which quite regularly show visible reactions following the administration of serum and virus. In one such herd, apparently well when treated, a loss of 75 percent of the hogs was sustained after the administration of serum and virus. No visible reactions occurred when crystal-violet vaccine was administered to these herds.

**Immunity Tests**

As previously stated, when the vaccine-treated hogs were ready for market a number of hogs were selected from as many herds as possible for the purpose of testing their immunity.

These hogs were brought to the Experiment Station and injected with 2 cc. each of tested virus. In a few cases pen exposure was substituted for virus injection.

Removing hogs from farm herds and subjecting them to changes in feed, care and housing conditions, in addition to an injection of hog-cholera virus, constitute a severe test. Furthermore, many of these tests were conducted during severe winter weather. Had it been possible to expose these hogs on farms under their normal surroundings it is probable that the results would have been somewhat better. Any symptom of sickness following exposure was recorded as a reaction regardless of the cause or nature of the sickness.

The interval between vaccine treatment and exposure varied from two to twelve months, the majority of the hogs being exposed at from five to eight months.

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In all, 841 hogs from 222 farm herds treated with vaccine prepared by the above-described method have been subjected to immunity tests. A number of these hogs were treated by procedures which would not be considered good practice in the light of present knowledge. Approximately 83 percent either remained normal or showed only a slight reaction, 12 percent showed severe reactions and 5 percent died.

Included in the foregoing tests were market hogs that had been treated with vaccine before weaning. As will be discussed later in detail, it was found that unweaned pigs, particularly those farrowed by immune mothers, were not well protected by the vaccine. Confining the summary to pigs vaccinated when 10 weeks of age or over and tested by virus injection within the following 8 months, 89 percent were found adequately protected and only 1 percent died.

The fact that the hogs in the group which exhibited severe reactions recovered and a number of those in the group which succumbed died from causes other than hog cholera would indicate that considerably more than 83 percent of all farm hogs that were subjected to immunity tests had received some protection from the vaccine treatment.

Duration of Immunity

While the duration of immunity following treatment with crystal-violet vaccine has not, as yet, been definitely established, the vaccine seems to protect swine against cholera quite well through the fattening period, provided they have not been treated when under eight or ten weeks of age.

Vaccine Treatment of Pigs Nursing Non-Immune and Immune Sows

The observation was made in the course of earlier experiments with crystal-violet vaccine that there was a distinct interference in the antigenic action of the vaccine when anti-hog-cholera serum is given at the same time as or shortly after the vaccine. The passive immunity from the serum evidently interfered with the usual antigenic action of the vaccine.

With a view to determining whether a similar interference might occur when the vaccine was given to pigs nursing immune sows, an experiment was carried out at the Bureau Station in which a group of little pigs nursing non-immune sows and another group nursing immune sows were treated with crystal-violet vaccine and later exposed by virus injection with a very striking difference in results. The protection afforded the first group was 100 percent and in the second group only 61.5 percent. This would indicate that the natal immunity in pigs nursing immune sows interferes with the antigenic action of the vaccine in a similar manner that was noted in the experiments where anti-hog-cholera serum was given in conjunction with the vaccine or shortly thereafter.

Tests in Cooperation with Practicing Veterinarians

In the spring of 1940 arrangements were made for the distribution of the vaccine through Government channels to veterinarians in various parts of the country in order that the new product could be tried out in the hands of practitioners and results compared with those obtained in farm tests around Ames. Vaccine has been distributed to veterinarians in Iowa and a number of other states. This project is being continued as well as other experimental work with various phases of crystal-violet vaccine.

PALOMINO

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separate room with running water, electric lights, and with a thick layer of straw on the floor. Every morning, each stallion is walked, trotted, and galloped for ten miles, and when lathery is allowed to roll in fine beach sand which helps maintain

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