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REPORT OF EXPERIMENTS
ON
IMMUNIZING
AGAINST HOG CHOLERA

BY L. L. LEWIS AND C. H. MCELROY

DEPARTMENT OF VETERINARY SCIENCE AND ZOOLOGY

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REPORT OF EXPERIMENTS ON IMMUNIZING AGAINST HOG CHOLERA

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INTRODUCTION

The experiments reported in this bulletin were begun early in 1916 and completed in March, 1918. The work is reported under four sections, as follows:

Section 1. Experiments to prove whether hogs given the simultaneous treatment will or will not be a source of infection for hogs that have not been vaccinated.

Section 2. To test the effect of desiccation on the virulence of hog cholera virus.

Section 3. To determine how long immunity will last in young hogs after they are immunized against cholera by the simultaneous method.

Section 4. To determine the length of time immunity will last in hogs when they receive only the serum treatment.

Conclusions

1. The results obtained by experiments under Section 1 indicate that hogs given the simultaneous treatment for cholera will not be a source of danger when put with hogs that have not been vaccinated.

2. Fresh hog cholera virus was found to be active after a period of 104 days drying at room temperature. Virus with preservative added appeared to be destroyed by drying much sooner than fresh virus. The general results obtained by experiments under Section 2 indicate that no immunity is produced by using hog cholera virus dried for such length of time that it will not produce the disease.

3. Results obtained in this series of experiments indicate that young pigs three weeks old, or older, may be successfully immunized by the simultaneous treatment. In order to produce what is probably a still higher degree of immunity, the pigs may be given $\frac{1}{2}$ cc. of virus in about two weeks after the simultaneous treatment is administered. Immunized pigs were tested for immunity at different lengths of time varying from 77 to 353 days. In each case they were able to withstand an injection of virus that proved fatal to non-immunized hogs.

4. Serum-alone treatment will not give hogs immunity for any considerable period of time. Pigs tested for immunity 17 days and 23 days after receiving the serum treatment were found to resist fatal doses of virus given hypodermically, but pigs tested 42 days after serum-alone treatment were found to be susceptible to virus inoculation, as all were sick and three died of cholera. In another test, made 52 days after serum was administered, five pigs were used. All were infected and three died. Apparently immunity from serum-alone treatment will not last longer than three to four weeks.

THE EXPERIMENTS

Section 1. Experiments to prove whether hogs given the simultaneous treatment will or will not be a source of infection for hogs that have not been vaccinated.

Plan of Work.—Two houses were used for the experiment, the plan being to put treated and non-treated hogs in each pen. The work was begun on January 25, 1916. On this date eight pigs were purchased. Four were given the simultaneous treatment and two placed in each pen. Two non-vaccinated pigs were placed in each pen. Temperatures were taken at intervals to determine the general condition of both vaccinated and non-vaccinated pigs.

Temperature records of pigs treated by the simultaneous method on January 25, 1916:

Date	No. 1	No. 2	No. 3	No. 4
February 1, 1916	104	102	101	102.8
February 2, 1916	101.2	101.8	103	104
February 4, 1916	104	103	103	103.4
February 5, 1916	104	103.2	102	105
February 7, 1916	102	101.2	103	103.8
February 10, 1916	103	101.4	102.6	103
February 15, 1916	103	103.2	103	104.2
February 18, 1916	103.2	101.6	102.8	104.2
February 19, 1916	101.6	101.4	102.2	102.6
February 22, 1916	103	102.8	103	103.4

Temperature record of non-vaccinated pigs in pens with simultaneously treated pigs:

Date	No. 5	No. 6	No. 7	No. 8
February 4, 1916	103.2	102	102.2	102.2
February 5, 1916	103	102	103.8	104.2
February 7, 1916	101.4	102	102.8	102.6
February 10, 1916	101.2	101.6	102.2	101.8
February 15, 1916	102.6	103	103.2	102.8
February 18, 1916	102	102	101.8	102.8
February 19, 1916	101.6	141.4	102.2	102.6
February 22, 1916	103	103.8	102.8	104

A review of the temperature records shows that pig No. 4, simultaneously treated, shows temperature of 104° on the eighth day after receiving the virus, and the temperature continued higher than for the other pigs until the eighteenth or twenty-fourth days after treatment. The pigs were kept together for twenty-eight days. No cholera developed.

Second Experiment.—On March 3 the pens were disinfected by spraying and were well cleansed. Four pigs were given the simultaneous treatment and two placed in each pen. Four untreated pigs were placed with them, two in each pen. These pigs were kept under the same conditions as pigs in the previous test.

Temperature record of pigs treated by the simultaneous method on March 3, 1916:

Date	No. 12	No. 13	No. 14	No. 15
March 9, 1916	103	102.6	102.8	103.2
March 11, 1916	101.4	102	102	102
March 15, 1916	101.1	102.6	101	102
March 17, 1916	104.6	102.8	103.6	103
March 20, 1916	102.8	102.1	102	101.6
March 23, 1916	102.6	103.1	102.6	101.8
March 24, 1916	103.1	102	102.4	101.8

Temperature record of untreated pigs exposed to simultaneously treated pigs:

Date	No. 16	No. 17	No. 18	No. 19
March 9, 1916	103.8	105.2	104.2	102.2
March 11, 1916	102.6	104.8	101.6	101.4
March 15, 1916	103	102	102	102.1
March 17, 1916	103.2	102.4	102.6	103
March 20, 1916	103.8	102.1	102.4	103
March 23, 1916	103.6	102.8	102.8	103.1
March 24, 1916	103	102.8	103.2	101.6

The observations in this experiment were continued for twenty-eight days, and the temperature records do not show any probable infection in the untreated pigs, or any temperature reaction in those that were vaccinated.

Third Experiment.—On March 31 four pigs (Nos. 20, 21, 22 and 23) were given the simultaneous treatment and placed two in each pen, and had placed with them four untreated pigs, two in each pen. The pens were thoroughly sprayed, as were the pigs, at the time they were placed in the pens. The untreated pigs were off feed on the second day of the experiment, and on April 5 pig No. 21 (non-vaccinated) was found dead. The untreated pigs continued sick, and all were dead by April 14. Postmortem examination showed these pigs died from hog cholera. They were undoubtedly infected at the time the test was started, as the temperatures were found high on the second day of the test. If temperatures had been taken before they were placed in the pens, this fact would have been detected, although they were eating and apparently healthy at that time.

Fourth Experiment.—On April 22 the pens were disinfected and vaccinated and non-vaccinated pigs were placed together.

Pen No. 1.—Pigs 29 and 30, treated with serum and virus. Pigs 25 and 26, not treated.

Pen No. 2.—Pigs 31 and 32 treated with serum and virus. Pigs 27 and 28 untreated.

Temperature records of pigs treated by the simultaneous method on April 22, 1916:

Date	No. 29	No. 30	No. 31	No. 32
April 26, 1916	102.8	103	101.6	102.4
April 25, 1916	102.6	103.4	102.6	103.2
May 2, 1916	103	101.8	104	102.6
May 4, 1916	103.6	102.4	102.6	103.4
May 9, 1916	102.8	101.8	102.4	102.8

Temperature records of untreated pigs:

Date	No. 25	No. 26	No. 27	No. 28
April 26, 1916	102.8	102.4	103	103.2
April 29, 1916	103	102.8	104	103.6
May 2, 1916	101.8	100.2	102.2	102.4
May 4, 1916	102.6	102.8	102.4	103.2
May 9, 1916	102.8	103	102.8	103.2

These pigs were kept under observation until May 12, or twenty days from the time they were exposed to simultaneous treated pigs. The temperature readings do not show any infection.

Fifth Experiment.—On May 18, 1916, the pens were thoroughly disinfected and four simultaneously treated pigs placed in them with untreated pigs as follows:

Pen No. 1.—Pigs numbered 45 and 46 were treated. Pigs numbered 49 and 50 were untreated.

Pen No. 2.—Pigs numbered 47 and 48 were treated. Pigs numbered 51 and 52 were untreated.

In Experiment 3 the non-treated pigs were lost because they were undoubtedly infected at the time they were purchased.

Temperature record of pigs treated by the simultaneous method on May 18, 1916:

Date	No. 45	No. 46	No. 47	No. 48
May 27, 1916	104	103.8	103.6	103.6
May 31, 1916	103	102.8	101.4	103
June 2, 1916	103.6	102.8	102.8	103.4
June 5, 1916	102	102.8	102	103.2
June 7, 1916	102.6	103	102	103
June 8, 1916	103.2	102.4	103.2	102.8
June 9, 1916	103.6	102.6	102.6	103.2

Temperature record of non-vaccinated pigs:

Date	No. 49	No. 50	No. 51	No. 52
May 27, 1916	102.6	101.8	103.4	104
May 31, 1916	102.6	103.4	103	104.8
June 2, 1916	102.6	103.6	104	103.8
June 5, 1916	103.2	102.4	102.8	103
June 7, 1916	103.4	103.2	105	104
June 8, 1916	102	102.8	102.6	103
June 9, 1916	103.2	102.4	102.8	103.2

An inspection of the temperature records of both vaccinated and non-vaccinated pigs show that there is not a sufficient rise of temperature in consecutive days to indicate an infection. Pig No. 51 on June 7 had a high temperature, but was normal on the 8th and 9th.

Sixth Experiment.—On June 26, 1916, pigs Nos. 39 and 40 were given simultaneous treatment for hog cholera and placed in a clean pen. On June 28 three non-vaccinated pigs were placed in the pen, and the five pigs were kept together until August 9. At no time during the exposure was any evidence of sickness seen, and all five of the pigs gained considerably in weight.

Seventh Experiment.—On October 18, 1916, pigs Nos. 195 and 196 were given the simultaneous treatment and placed in pen No. 1 with untreated pigs numbered 197 and 198. These pigs were kept under observation until November 8. Temperatures taken during this period showed no infection in the untreated pigs.

Discussion.—The uniform results obtained in six of the experiments under this section of the work indicate that hogs given the double treatment to prevent cholera will not be a source of infection for non-vaccinated hogs. In giving the double treatment it is very easy to infect pens by dropping virus from the syringe, or possibly in some cases by the virus oozing through the needle puncture in the skin. In the experiments these sources of infection were guarded against.

Section 2.—To test the effect of desiccation on the virulence of hog cholera virus.

Virus was obtained through an Iowa laboratory on January 9, 1916. A portion of this material was immediately placed in a thin layer in shallow dishes where it would dry quickly. These dishes were kept in diffused light of the room from that date.

On March 1 two pigs were inoculated, each with approximately the amount of virus represented by 1 cc. of the fresh blood. This dried virus was scraped from the dish and placed in 5 cc. of sterile salt solution and injected subcutaneously. These pigs received numbers 9 and 10, respectively. One other pig, No. 11, received the same amount of treated virus that was received from a commercial serum company on February 11, and had been drying under the same conditions until March 1, when used.

Temperature of pigs on test:

Date	No. 9	No. 10	No. 11
March 5, 1916	104.8	105.2	103.6
March 9, 1916	106.6	107	104.8
March 10, 1916	105	102.2	105
March 11, 1916	105	105.6	101.6
March 13, 1916	108.2	107.6	106.4
March 15, 1916	103.6	102.2	102
March 16, 1916	105.5	104	103
March 17, 1916	104.6	106.8	104.4
March 18, 1916	105.6	106.4	102.8
March 20, 1916	105.8	105.4	102.6
March 22, 1916	102.4	104.4	102.8
March 23, 1916	102.8	103.6	
March 24, 1916	104.2	104.4	
March 27, 1916	dead		
March 31, 1916		dead	

Second test of virus from Iowa laboratory, April 22, dried since January 9.

Purchased pig No. 33 and gave a hypodermic injection of the equivalent of 1 cc. of fresh virus. Pig was then placed in a clean pen.

Temperature record:

Date	No. 33
April 29, 1916	105.1
May 2, 1916	104
May 4, 1916	103.8
May 9, 1916	106
May 10, 1916	105
May 12, 1916	104.2
May 16, 1916	104.6
May 18, 1916	dead

Virus received from the Iowa laboratory proved virulent on two tests made 52 and 104 days, respectively, after the material was placed under experimental conditions. The virus received from the commercial plant was what is known as treated virus, i. e., had preservative added. Evidently much of the original strength is lost on account of the action of the preservative.

A third test of the Iowa virus was made on June 22, 1916.

One pig was inoculated on June 22 with the equivalent of 2½ cc. of the original virus dissolved in physiological salt solution. This pig did not show any evidence of cholera, and on September 4 was given, subcutaneously, ½ cc. of fresh virus to see if the inoculation on June 22 with dried virus gave any immunity. This pig was kept under observation for twenty-eight days and showed no effect from injection of virus on September 4.

On September 10, virus (without antiseptic) was received and divided into two lots. Lot 1 of this virus was dried at room temperature. Lot 2 was dried at incubation temperature, 35° C. On October 18 a second lot of virus was received and handled as was Lot 1. In each case the virus was placed (1 cc. to 4 cc.) in shallow glass dishes and protected from the light.

Test of Attenuated Virus.—On November 9, pigs Nos. 189 and 190 were inoculated with attenuated virus. Pig 189 received the equivalent of ½ cc. of fresh virus that had been drying at room temperature since September 10. Pig 190 received the same amount as pig 189 of virus that had been drying at 35° C. since October 18. These pigs showed no effect from the inoculation for a period of thirteen days. On November 22 the pen was infected by putting fresh virus in the feed.

The following temperatures show the course of the infection:

Date	No. 189	No. 190
November 27, 1916	104.6	105.2
November 28, 1916	104.5	106.6
November 30, 1916	106.8	106.6
December 2, 1916	dead	106.8
December 4, 1916		dead

On November 1, pigs 191, 192, 193 and 194 were inoculated with virus that had been drying at 35° C. since September 10. Each pig received the equivalent of $\frac{2}{3}$ cc. of fresh virus, except pig 192, where 1 cc. was used. These pigs showed no temperature reaction from the infection of attenuated virus.

On November 22, pigs 192 and 194 received $\frac{1}{2}$ cc. of fresh virus hypodermically. The two pigs receiving fresh virus were left in the pen with pigs 191 and 193.

The following temperature record will show the course of the infection in this pen:

Date	No. 191	No. 192	No. 193	No. 194
November 27, 1916	103	104.6	X	104.2
November 28, 1916	102.5	107.5	103.1	107.7
December 2, 1916	104.8	108	104.6	dead
December 4, 1916	105.6	104	105.4	
December 5, 1916	106.2	106	108	

An inspection of the above temperatures shows that on November 28 the two pigs receiving virus had very high temperatures and that on December 2 the other two were coming down with cholera by exposure. The hogs alive on December 5 developed a lingering type of cholera. No. 192 died on December 11; 191 and 193 died on December 14.

Pigs Nos. 199 and 200 were inoculated with virus that had been dried for thirty-three days at 35° C. Each pig received the equivalent of $\frac{1}{2}$ cc. of fresh virus. No symptoms of cholera developed. On November 22, pig No. 200 received $\frac{1}{2}$ cc. of fresh virus and pig No. 199 was placed in an infected pen with sick pigs. Pig 200 died on December 3, and pig No. 199 died on December 18.

Discussion.—A brief summary of the facts in this series of tests is as follows:

The virulence of the Iowa virus, received on January 9, 1916, was proven by inoculation when it was received. The first test was made after the virus had been drying for fifty-two days. Results fatal. The second test was made after the virus had been drying for 104 days. Results fatal. A third test was made on one pig after the virus had been drying for 165 days. Results not fatal. This pig was subsequently inoculated with virus, but failed to come down with cholera. Possibly was immunized by the dried virus.

In a test of virus from a southern laboratory that had been drying for fifty-nine days at room temperature, and another lot that had been dried twenty-one days at 35° C., both lots proved virulent by killing inoculated pigs. A test of four pigs made with virus that had been drying for fifty-one days at 35° C. showed that it was not virulent, and a subsequent test of these pigs with active virus showed they were not protected by the inoculation with dried virus. In another test of commercial virus with preservative added, virulence was lost after a period of twenty days drying at room temperature.

The few experiments reported here, together with considerable unpublished data, indicate variable results will be obtained by using virus that has been dried either at incubator or room temperatures.

Section 3.—To determine how long immunity will last in young hogs after they are immunized against cholera by the simultaneous method.

A number of experiments were carried out in this test, using pigs of different ages. In each experiment the pigs were immunized in the experimental pens where they were kept under observation for ten to fifteen days. After this period of observation they were sprayed with a disinfecting solution and removed to the College farm and turned in large enclosures. After varying lengths of time they were returned to the experimental pens and exposed in different ways to hog cholera infection.

Experiment No. 1. For this test six shoats were given the simultaneous treatment on February 9, 1916. The pigs were numbered and treated as follows:

	Weight	Serum Received	Virus Received
No. 176	50 lbs.	30 cc.	1 cc.
No. 177	52 lbs.	30 cc.	1 cc.
No. 178	61 lbs.	40 cc.	1 cc.
No. 179	55 lbs.	30 cc.	1 cc.
No. 180	50 lbs.	30 cc.	1 cc.
No. 181	50 lbs.	30 cc.	1 cc.

These pigs were brought to the experimental pens for the purpose of exposing them to hog cholera infection as follows:

- On June 8—Nos. 177 and 179
- On August 3—Nos. 176 and 180
- On November 10—Nos. 178 and 181.

Those tested for immunity on June 8 received 2.5 cc. each of fresh, untreated virus in feed. The remaining four pigs received virus hypodermically, 1 cc. each. In all cases the pigs were kept under observation for twenty days after exposure to infection. No symptoms of cholera developed.

Experiment No. 2. On February 13, 1917, six shoats were given the simultaneous treatment. They were numbered and treated as follows:

	Weight	Serum Given	Virus Given
No. 182	20 lbs.	20 cc.	1 cc.
No. 183	22 lbs.	20 cc.	1 cc.
No. 184	27 lbs.	20 cc.	1 cc.
No. 185	21 lbs.	20 cc.	1 cc.
No. 186	26 lbs.	20 cc.	1 cc.
No. 187	31 lbs.	30 cc.	1 cc.

These pigs were brought to the experiment pens on the following dates for the purpose of testing out their immunity:

- On June 8—Nos. 183 and 184
- On August 3—Nos. 182 and 186
- On November 10—Nos. 185 and 187.

Those tested for immunity on June 8 received 2½ cc. of virus in feed. The remaining four pigs each received 1 cc. of fresh virus hypodermically. These pigs were kept under observation in each case for a period of twenty days. No symptoms of cholera developed.

Experiment No. 3. On March 3, 1917, a sow and four pigs were purchased. They were numbered and treated as follows:

	Serum Given	Virus Given	
No. 1	15 cc.	¼ cc.	3 weeks old
No. 2	15 cc.	¼ cc.	3 weeks old
No. 3	15 cc.	¼ cc.	3 weeks old
No. 4	¼ cc.	3 weeks old
No. b 200	60 cc.	1.5 cc.	weight 285 lbs.

Pig No. 4 was given virus alone as a check on the virus, which produced a typical case of cholera, the pig dying on March 12. The sow and three remaining pigs were kept under observation until March 22. The pigs were 18 days old when immunized. Only the three pigs were to be used in testing for immunity. These pigs were tested for immunity as follows:

June 8—Pig No. 1 tested.

August 3—Pig No. 3 tested.

November 10—Pig No. 2 tested.

The test of June 8 was made by placing 2.5 cc. of virus in feed, the two remaining tests by giving hypodermically 1 cc. of fresh cholera virus. No cholera symptoms developed during the twenty-day observation period.

Experiment No. 4. A large sow with nine pigs were purchased on March 22 for this test. One pig was given virus alone. The remaining eight pigs each received $\frac{1}{2}$ cc. of virus and 15 cc. of serum. The sow received 1 cc. of virus and 60 cc. of serum. This lot of pigs was 3 weeks old at time of immunizing. On April 10 the sow and eight pigs were in good condition and were removed from the experimental pens to the farm. On April 16 the check pig died from cholera.

These pigs were tested for immunity as follows:

June 8—Two pigs each received 2.5 cc. of virus in feed.

August 3—Two pigs each received 1 cc. virus hypodermically.

November 10—Two pigs each received 1 cc. virus hypodermically.

March 11 (1918)—Two pigs each received 1 cc. of virus hypodermically.

In each case, after the virus was used, the pigs were kept under observation for a sufficient length of time to determine if any infection resulted. In no case were the pigs off feed during this period.

Experiment No. 5. On June 22 a sow and seven pigs were purchased and given the simultaneous treatment. Each pig received 15 cc. of serum and $\frac{1}{2}$ cc. of virus. The sow received 60 cc. of serum and 1 cc. of virus. Only the pigs were to be used in the experiment. On July 6 each pig received an additional injection of $\frac{1}{2}$ cc. of fresh cholera virus hypodermically. After a period of observation of nineteen days the pigs were sent to the farm to be returned later for immunity test.

November 10 two of the above pigs were tested by giving each $2\frac{1}{2}$ cc. of virus in feed.

March 11, 1918, the remaining five pigs were brought to the experimental pens and tested by giving each 1 cc. of fresh cholera virus hypodermically.

These pigs were kept under observation the usual time, but no symptoms of cholera developed.

Experiment No. 6. August 3, 1917, four pigs, 22 days old, were given the simultaneous treatment, using $\frac{1}{2}$ cc. of virus and 12 cc. of serum. These pigs were given an additional injection of 1 cc. of fresh virus on August 15. No ill effects from either treatment followed. Two of these pigs were tested for immunity on March 11, 1918, by giving each 1 cc. of fresh virus hypodermically. No symptoms of cholera developed. The remaining two pigs were lost by accident while on the farm.

Discussion.—The problem in this series of tests was to determine whether it was a practical procedure to vaccinate sucking pigs and small shoats. For this work sucking pigs 3 weeks old, and large pigs of 20 to 50 pounds, were used. In Experiments 5 and 6 the pigs were given an additional injection of virus; in Experiment 5 they received ½ cc. in fourteen days after the first treatment; in Experiment 6 the pigs received 1 cc. of virus twelve days after the first treatment.

The 20 and 50-pound shoats were tested for immunity at varying lengths of time, varying from 114 days up to 273 days. In every case the simultaneous treatment gave protection.

In Experiments 3, 4, 5 and 6 the pigs used were in most cases about 3 weeks old. These pigs did not show any ill effects from the simultaneous treatment or from subsequent injection of virus as in Experiments 5 and 6. The pigs used were tested for immunity at intervals varying from 77 to 353 days. The experiment running the greatest length of time was with pigs receiving their immunizing treatment on March 22, 1917. Two of these pigs were tested for immunity on March 11, 1918. All of the pigs in the above four experiments were protected against hog cholera by the simultaneous treatment administered while they were about 3 weeks old. Virus was used on non-immunized pigs in all of the tests for immunity, and in the test on March 11, 1918, two pigs were inoculated. Both died of cholera on March 24.

While the number of pigs used in this phase of the work was not very large (thirty-one head), the results seem to indicate that immunity obtained in very young pigs by using hog cholera serum and virus can be relied upon for at least one year.

Section 4.—The object of this phase of the experiment is to determine the length of time the immunity will last in hogs when they receive only the serum treatment. In order to start this work, eighteen shoats were purchased and were given the serum-alone treatment on January 10. Two or three of these pigs were to be brought in at various intervals of time and receive ½ cc. of virus in order to test out the immunity received by the single treatment on the 10th. The following shows the weight of the shoats and amount of serum injected:

No.	Weight	Serum Given
No. 482	40 lbs.	20 cc.
No. 414	80 lbs.	35 cc.
No. 481	110 lbs.	40 cc.
No. 464	100 lbs.	40 cc.
No. 468	50 lbs.	20 cc.
No. 469	75 lbs.	30 cc.
No. 473	75 lbs.	30 cc.
No. 472	60 lbs.	25 cc.
No. 483	65 lbs.	30 cc.
No. 467	50 lbs.	20 cc.
No. 475	65 lbs.	35 cc.
No. 466	35 lbs.	20 cc.
No. 465	40 lbs.	25 cc.
No. 471	30 lbs.	20 cc.
No. 470	35 lbs.	20 cc.
No. 490	35 lbs.	35 cc.
No. 491	100 lbs.	30 cc.
No. 492	100 lbs.	00

These vaccinated hogs were kept on the College farm entirely away from any infected pens or premises, and were brought to the experimental pens as needed.

On January 27 four hogs were brought to the pens, inoculated with ½ cc. of fresh cholera virus, thoroughly sprayed and put in a clean pen.

Temperature record and notes:

Date	No. 481	No. 469	No. 491	No. 490
February 2	101.6	102.6	103	102.6
February 4	103.4	102.8	104.6	103
February 5	103.2	103.4	104.4	103.6
February 7	101.8	102.6	102.4	103.8
February 10	102	101.4	102.8	103
February 15	102.2	101.4	102.6	103.2
February 18	102.6	102.8	103	103.2
February 19	101.8	103.2	103.2	102.6
February 22	102	104	103.6	101.8

Very little temperature reaction was noted except in pig 491. On February 4 the pig was very sluggish and not eating, but appeared to be normal again on the 7th.

On February 9 three hogs were brought to the pens and each inoculated with $\frac{1}{2}$ cc. of fresh virus.

The temperature records of these pigs were as follows:

Date	No. 482	No. 470	No. 414
February 16	103.8	102.4	103.2
February 18	102.4	102.2	102.4
February 19	102.4	101.6	102.8
February 22	103.8	102.6	103.2
February 23	102.8	101.6	102.8
February 25	102.2	101.2	102.6
February 26	101.4	102	101.2
February 28	103.4	102.4	102.6
March 1	103	102.6	102.8

An inspection of the above temperatures shows no reaction in any of the pigs following the injection of the virus.

On February 19 two pigs were brought to the experimental pens and placed in infected pens with sick pigs. They were kept in infected pens for a period of thirty-eight days. At no time were the pigs off feed. It had been forty days since these pigs received serum until they were exposed to infection.

March 3 two pigs were brought to the experimental pens and were given $\frac{1}{2}$ cc. of fresh virus. On March 4 three more pigs were inoculated with virus and placed in pens with those inoculated on the 3d.

Temperature record:

Date	No. 465	No. 466	No. 467	No. 464	No. 468
March 7	103.8	104.6	104.8	105	104.8
March 9	104.8	107	105.4	106.6	106
March 10	103.4	106.2	103	105	104.6
March 11	105.4	104.8	104	107	107
March 13	105.1	106.8	103.2	107	105.4
March 15	103.6	105.2	104.6	106.4	105.6
March 16	102.8	106.2	X	106.6	106.2
March 17	103.4	105.4	103.2	106	106.2
March 18	103.2	106.6	103.2	107	105.6
March 20	101.8	106.8	103	106.2	105.2
March 22	102.2	103.8	102.8	104	102.4
March 24	X	103.3	X	103	104.2
March 25	105	104.4	X	103.6	104.6
March 26		dead		dead	

Notes.—Pigs 465 and 467 appeared normal on March 28 when they were taken out of the experiment. On April 5 pig No. 468 died. Postmortem showed hog cholera lesions and pneumonia complications.

An inspection of the temperature records shows that all of the pigs reacted to the inoculation of virus. The temperatures of 465 and 467 reached normal conditions after about thirteen days. Postmortem showed typical lesions in 466 and 464, while pneumonia complications were very pronounced in No. 468.

On March 15 two pigs were brought to the experimental pens and each given $\frac{1}{2}$ cc. of virus and placed in clean pens for observation. March 19 both pigs were off feed.

Date	No. 472	No. 473
March 21	105	102.6
March 22	106	101
March 24	106	101
March 25	106	102
March 27	dead	

Postmortem showed cholera lesions were developed in pig No. 472. Pig No. 473 died on March 25, but no cholera lesions were found on postmortem.

On March 31 two pigs were brought to the pens. One of the pigs, No. 492, failed to receive any serum on January 10, and at this time was given serum-alone treatment. The other pig, No. 471, had received serum on January 10. Both pigs were placed in a very dirty, badly infected pen. They were fed shelled corn on the floor in order to insure their picking up all the infection possible. These pigs were kept under observation until April 20, when they were taken out of the experiment. During the twenty days these pigs were not off feed and had gained in weight at the end of the observation period.

Two pigs were given the serum-alone treatment on April 24, 1916. At this time the pigs weighed about 80 pounds each. On June 26 these pigs received $\frac{1}{4}$ cc. of virus subcutaneously. The following notes indicate the result of the inoculation:

Date	No. 35	No. 36
June 30	normal	normal
July 3	108	107
July 5	107.8	107
July 7	107.5	107
July 8	107.8	dead
July 9	105.2	
July 10	dead	

Postmortem lesions showed that both had well developed cases of cholera, complicated in No. 36 with pleurisy. Virus was administered sixty-three days after the serum treatment.

A review of the results of this test shows that the administration of $\frac{1}{2}$ cc. of virus did not produce marked symptoms of cholera either sixteen or thirty days after vaccination. Exposure of vaccinated pigs in infected pens (two pigs), begun on the fortieth day after vaccination, was without results. Pigs inoculated on the fifty-third and fifty-fourth days following vaccination were susceptible to cholera. All pigs of this lot showed marked infection, except No. 467, and three died from cholera. The very lingering type of cholera seen in No. 468 showed that this pig probably either had considerable natural resistance or else was protected to some degree by the serum given on January 10. Two pigs were inoculated on the sixty-sixth day following vaccination, but only one showed any result from the infection.

Of the pigs brought in on the eighty-second day, only one had received the serum. This pig was exposed to severe infection, but had showed no infection after twenty days observation.

The virus used in this work was in each case fresh, potent virus, and it is quite likely that more of the pigs would have survived exposure to infection in pens than survived the infection of virus, and it is quite likely that more of the pigs would have survived exposure to infection in pens than survived the infection of virus in $\frac{1}{2}$ cc. doses subcutaneously.

Experiment No. 3. On June 28 sixteen pigs were treated with serum-alone. The serum used was from two laboratories. These will be designated as No. 1 and No. 2.

The following table will indicate the treatment for these pigs:

No.	Weight	Serum Given	Source
No. 45	50 lbs.	30 cc.	No. 1
No. 46	60 lbs.	30 cc.	No. 1
No. 47	55 lbs.	30 cc.	No. 1
No. 48	65 lbs.	30 cc.	No. 1
No. 49	55 lbs.	40 cc.	No. 1
No. 51	80 lbs.	40 cc.	No. 1
No. 53	60 lbs.	30 cc.	No. 2
No. 56	75 lbs.	40 cc.	No. 2
No. 59	70 lbs.	60 cc.	No. 2
No. 60	65 lbs.	60 cc.	No. 2
No. 61	55 lbs.	30 cc.	No. 2
No. 62	50 lbs.	20 cc.	No. 2
No. 63	60 lbs.	30 cc.	No. 2
No. 64	60 lbs.	30 cc.	No. 2
No. 65	65 lbs.	39 cc.	No. 2
No. 60	65 lbs.	30 cc.	No. 2

Hogs numbered 59 and 60 received larger doses than other hogs of the same weight, the object being to see if immunity could be influenced in any appreciable degree by size of dose.

On August 9, or forty-two days after giving serum-alone treatment, pigs 48, 49, 61 and 66 were brought to the experimental pens. Two of the above pigs were immunized with serum No. 1, and two with serum No. 2. Each of these pigs received subcutaneously $\frac{1}{2}$ cc. of fresh virus. Result: Pig 61 died on September 3, Pig 48 died on September 11, and pig 66 died on September 1. Pig No. 49 recovered from a light infection with cholera.

A second lot of pigs was brought to the pens on August 21. Two were vaccinated with serum No. 1 and two with serum No. 2. Pigs 47 and 65 were given serum hypodermically. Pigs 45 and 53 received virus in feed and water. Result: Both pigs that received virus subcutaneously died of cholera, No. 65 on September 4 and No. 47 on September 9. Both pigs receiving virus in feed survived, although both of them showed light infection from the eighth to the twelfth day after exposure.

On August 25 a third lot of pigs was secured. Two of these pigs, Nos. 56 and 46, were inoculated subcutaneously with $\frac{1}{2}$ cc. of fresh virus, and Nos. 51 and 64 received fresh virus in feed and water. Results: The two pigs receiving virus subcutaneously died of cholera on September 15. The two receiving virus in feed were slightly affected, but soon recovered normal condition.

On September 10 the remaining hogs were brought to the experimental pens. They were treated as follows:

No. 62, placed in an infected pen with sick hogs.

Nos. 59, 60 and 63 were each given $\frac{1}{2}$ cc. of preserved or treated virus.

These hogs were all normal on September 25, when the hogs were removed from the experiment.

Discussion.—All of the hogs in the experiment received the serum-alone treatment, but when brought to the experimental pens they were infected by two methods, one was to inject the virus subcutaneously, the other was to scatter virus in the pen and troughs. Four that were infected forty-two days after serum treatment were all susceptible to cholera, and three of them died. All were inoculated subcutaneously with $\frac{1}{2}$ cc. of virus. The second lot of pigs received virus fifty-four days after the serum-alone treatment. All were sick, the two receiving virus subcutaneously died, while the two receiving the virus in feed were only slightly affected. The third lot received

virus fifty-eight days after serum treatment, the two receiving virus hypodermically died of cholera, the two receiving virus in feed were only slightly affected. The fourth lot of four hogs brought in were exposed to cholera seventy-four days after the serum treatment. One was placed in an infected pen with hogs that were very sick, and later died from cholera, while the remaining three were each infected with what is known as treated virus. The hog exposed in the infected pen was off feed two or three days, while the hogs receiving the treated virus showed no symptoms of any infection.

Hogs were lost in this experiment forty-two days after the serum treatment when inoculated subcutaneously with fresh hog cholera virus, while they were able to withstand exposure in infected pens for seventy-four days after serum-alone treatment. Practically no difference was noted in the potency of the source from the two laboratories designated as No. 1 and No. 2.

