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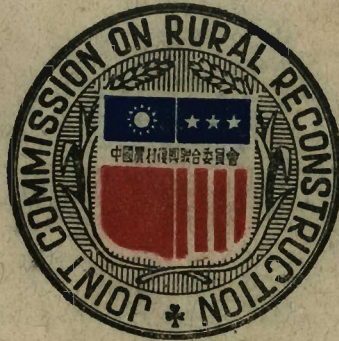
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Joint Commission on Rural Reconstruction

A PRELIMINARY REPORT ON THE
LAPINIZED HOG CHOLERA VACCINE IN TAIWAN

By

Robert C. T. Lee



TAIPEI, TAIWAN, CHINA

October 1954

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*Report prepared by Robert C. T. Lee
Data compilation assisted by P. C. Lin*



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A PRELIMINARY REPORT ON THE LAPINIZED HOG CHOLERA VACCINE IN TAIWAN

1. Introduction

Hog cholera is the most devastating hog disease in Taiwan. Its existence is still an exceedingly serious threat to the industry of hog raising, the most important rural side-line industry of this island. Early in 1949, before the Joint Commission on Rural Reconstruction moved its headquarters to Taiwan, the first project approved by the Joint Commission for this island was the "Production of Crystal Violet Hog Cholera Vaccine" sponsored by the Taiwan Provincial Veterinary Serum Institute. Following the success in the manufacture of the crystal violet vaccine, this vaccine has been generally adopted and widely used in the island-wide mass hog vaccination program during the period of 1950 to the present (1954). However, the veterinaries of Taiwan have never stopped in seeking for a better vaccine for combating hog cholera, a more effective, cheap, yet safe vaccine.

In fact, after some success in propagating the hog cholera virus in non-specific hosts by Zichis (1939), Tenbroeck (1941), Koprowski (1946), Baker (1946) and others, there was a prevailing interest by some workers (Ching 1948, Kwong and Lee 1948, Coronel 1950 and others) in looking for a new vaccine especially one attenuated through rabbit passages. In almost all instances, those host specific viruses, such as rinderpest virus and hog cholera virus, when serial passages were made in a different species resulted in the attenuation of their virulence for the corresponding original hosts. Consequently, such a highly attenuated and modified virus could be used as an immunizing vaccine. Pasteur's fixed rabies virus and Nakamura's lapinized rinderpest virus strain No. III, which were both adapted to rabbits are two good illustrations. Following this clue, workers of various countries conducted

experiments on propagation of the hog cholera virus through rabbits. After many years' research, some laboratories in the United States have finally succeeded in perfecting a new type of vaccine*. It is, in fact, a modified hog cholera live virus vaccine, so modified that it can no longer cause cholera in swine but does establish a high degree of solid immunity. However, each of these laboratories has its own way of production as well as the administration of their new products.

On December 12, 1952, with the kind permission of the Bureau of Animal Industry of the Philippine Government, a rabbit inoculated with the lapinized hog cholera virus was brought back to Taiwan by the late Dr. Isaac E. Newsom and the writer, after their trip to Manila. From this single rabbit, a series of experiments were energetically conducted. In this respect, the Joint Commission has also rendered its full technical and financial assistances. After more than one year's laboratory experiments and field tests, this new virus has now been finally used in the whole of Pingtung Hsien under the project of the "Pingtung Hog Cholera Control Demonstration Program" which started in February, 1954. The result thus far is so promising that it might eventually bring the hog cholera control work of this island into a new era.

It is very true that ever since the introduction of this new virus, it has been put under intensive study by a group of veterinary workers of Taiwan. Most of the work and experiments, however, were designed from the practical view point of how to apply it in the field rather than as a real academic study. Moreover, the experience of these veterinaries with the Nakamura's lapinized rinderpest virus enabled them to tackle this problem much easier. After a continuous effort of twenty-one months, the general practical problems of this virus, as a matter of fact, have mostly, if not entirely, been solved. The final composition of the new vaccine adopted is an aqueous preparation of the blood, spleen, lymph glands and liver of the inoculated rabbits, or the so-called "fresh fluid vaccine", probably new to the American and European countries, yet not to the Asian technicians. The findings of all the experiments and their experience in using it are hereby summarized in this report.

However, this report is in no way intended to be a systematic or a complete paper, but is intended to make available to the other countries all the latest information on the lapinized virus in Taiwan during

* Rovac of Lederle Lab., Swivax of Allied Lab., MLV of Fort Lab., Novac of Norden Lab., Corvac of the Corn States Serum Company, Viracine of Cutter Lab., True Vac of Anchor Serum Co. (Some of them require simultaneous administration of anti-serum).

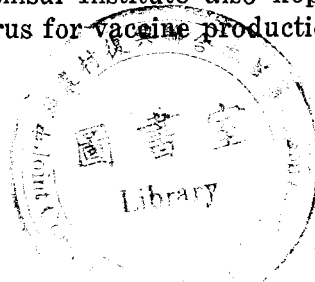
the period from December 1952 to September 1954. Most of the work stated in this report was sponsored by the Taiwan Provincial Veterinary Serum Institute and the Pingtung Hsien Veterinary Diagnostic Center under the projects of the Joint Commission. The writer hopes that the material compiled herein will be of some valuable assistance to the veterinary workers of other countries, especially those in the Far East, because this report proves that by the use of a very simple technique and limited equipment, the wet preparation of this lapinized vaccine can be produced locally and used in the field successfully. It is understood that such a wet preparation of the lapinized hog cholera vaccine has never been tried on such a big scale by any of the other countries.

2. Serial Passages of Lapinized Virus in Taiwan Rabbits

The technique of transmitting the lapinized hog cholera virus through rabbits is quite simple. The rabbit is usually sacrificed on the third day after inoculation. The blood is withdrawn by heart puncture. The spleen, and the mesenteric lymph glands are collected aseptically. One c.c. of defibrinated fresh blood or of 10% emulsion of either spleen or lymph glands in saline is then injected intravenously into at least two healthy rabbits, about two kg. each in weight. Temperature of each inoculated rabbit is taken twice or three times daily. On the third day one of the two rabbits is again killed. And the blood, spleen and lymph glands are then collected, processed, and passed as before.

From December 12, 1952 to September 6, 1954, the Tamsui Veterinary Serum Institute has been able to pass this lapinized virus introduced from the Phillipines through the native Taiwan rabbits for 144 successive passages, using a total of 765 rabbits. The inoculated rabbits might or might not have the thermal response, but in either case these were found good for virus passages or vaccine production. The fever reactions of the 765 inoculated rabbits of Taiwan are summarized in Table 1. (page 27). From Table 1, it can be seen that the thermal response of the Taiwan rabbits to the lapinized virus has undergone quite a change. The percentage of moderate and severe fever increased as the number of serial passages increased. The increase was most marked after the 71st passage.

As the lapinized vaccine has been put under field application in Pingtung since February 1954, so the Pingtung Veterinary Diagnostic Center under the supervision of the Tamsui Institute also kept quite a number of rabbits inoculated with this virus for vaccine production. From



February 10, 1954 to September 20, 1954 the Pingtung Veterinary Diagnostic Center has inoculated 621 rabbits, the thermal reactions of which are presented in Table 2. (page 28). Though the percentage of severe fever revealed by Table 2 (35.90%) is much higher than that of the corresponding serial passages (111th to 144th) in Tamsui Institute (7.52%), yet the total percentage of moderate and severe fever remained quite close (85.02% and 87.97% respectively).

Moreover, another quite interesting phenomenon is that autopsy findings of the inoculated rabbits also seems to have some changes, which however still could not be identified conclusively so far as characteristics are concerned. At the beginning, almost no naked-eye discerned lesion in the hog-cholera infected rabbit could be detected. Later some swellings were observed in spleen, mesenteric lymph glands, appendix, Peyer's patches, etc. The autopsy findings of 31 rabbits killed on September 16 to 20, at the Pingtung Veterinary Diagnostic Center are summarized in Table 3 (page 29). Of all these changes, the most interesting one is the slight swelling of the lymphoid tissue. Such swelling was not so frequently observed in the earlier passages in Taiwan rabbits. It is understood, however, that some normal rabbits also show, after killing, a similar swelling in the lymphoid tissue such as in **Sacculus rotundus**, **Tonsilla cecalis major** etc., but always of less marked degree. It is very difficult to distinguish such autopsy findings from those of hog cholera infected rabbits with fewer swelling changes. So, whether these changes are due to the individual differences or to some specific causes needs more study and longer observation. Besides, cultures of these inoculated rabbits were always found to be negative, which at least indicated that such changes had no connection with **Salmonella typhimurium** infection.

Pictures showing the different autopsy findings of normal rabbits and rabbits inoculated with Nakamura's lapinized rinderpest virus strain No. III and the lapinized hog cholera virus are shown in this report (pp. 52-53). The changes in the lymphoid tissue of hog cholera infected rabbits are far less marked than those of rinderpest infected rabbits. They show only slight swelling and appear to be somewhat opaque or pale white color, which could easily be differentiated from the typical yellow, necrotic lesion of the Peyer's patches and the other lymphoid tissue of the rinderpest infected rabbits. But, the difference between the hog cholera infected rabbit and the normal is not so distinct. Sometimes, in some cases it might be misleading.

As to the other symptoms, most of the inoculated rabbits showed no observable symptom other than fever which was also not typical. As a rule, all the rabbits, if not killed, recovered.

3. Keeping Quality of Lapinized Virus

Experiments on the keeping quality of this lapinized virus in various tissues under different conditions were carried out at the Tamsui Institute (Lin and Cheng 1953). In these experiments, blood was taken from the heart of the rabbit on the third day after inoculation, and was defibrinated. The spleen and lymph glands were removed aseptically at the same time. It was found that the virus in spleen and lymph glands so collected could survive at room temperature (10°-17° C) for three days and at normal refrigeration temperature (5° C) for 33 days. It was also revealed by the experiments that the virus in that blood could be kept viable at room temperature for only five hours, while in the 50 times dilution of spleen, lymph glands or liver emulsion could survive for 24 hours under the same condition. From these experiments, it can be seen that the lapinized hog cholera virus is not very resistant to temperature above the normal refrigeration. Its viability also remains longer in spleen and lymph glands than in blood.

Another test at Tamsui on the persistence of the virus in the inoculated rabbits showed that the lapinized hog cholera virus could be detected on the eighth day but not on the twelfth day after inoculation in the rabbit.

4. Immunity Development by Lapinized Virus

The lapinized hog cholera virus is a modified live virus. It is generally agreed by all the workers and amply demonstrated by experimental procedures that a live virus vaccine always produces dependable, long-lasting, solid immunity. A preliminary experiment at Tamsui (Lin and Cheng, 1953) showed that a solid immunity developed about four days after vaccination. Two cc. of 10,000 times dilution of spleen tissue, 5,000 times dilution of lymph gland tissue, or 500 times dilution of blood or liver tissue still could induce a solid immunity in the hogs vaccinated and protect them against the challenge of one c.c. fresh virulent hog cholera blood. Later, the Tamsui Institute under the project of the Joint Commission continued working on this problem and the findings and the procedures of these tests are summarized in the following.

As of the end of August, 1954, the Tamsui Institute has injected 314 head of hogs with this new lapinized vaccine, of which 298 head have been tested for their potency. Under these experiments of the Tamsui Institute, small pigs representing pigs with a weight of 8-25 kg. (mostly under 16 kg.) purchased from the nearby farms of Tamsui Chen, and mature hogs representing hogs with a body weight of around 60 kg. procured mostly from the southern Taiwan through the hog dealers were used. All the pigs and hogs were put under inspection for about one week and only those found to be healthy were used in the tests. All the hogs (including pigs) used were F1 hybrids from the Berkshire and the native cross. All the hogs, small or mature, received under these experiments a dose of two c.c. per head, subcutaneously or intramuscularly, from a preparation of blood and organ emulsion of an inoculated rabbit bled on the third day. The emulsion originally consisted of spleen and lymph glands one part, defibrinated blood nine parts, and normal saline 40 parts. Later, after March 1953, the normal saline was increased to 90 parts. Since 1954, the liver was added to the vaccine and a finalized composition was reached which will be mentioned in the next section. After vaccination, the hogs were kept under close observation. Temperatures of the animal were recorded twice daily during the experimental period. Challenge was usually done by injecting one c.c. of fresh virulent blood subcutaneously generally on the 7th day after vaccination.

Of the 298 hogs which received only lapinized vaccine, 143 were mature hogs, and 155 head were small pigs. After the potency test, it was found that all the mature hogs survived (100% protection), and nine small pigs out of the 155 head vaccinated died after challenge (94.20% protection) with a total average protection of 96.98% (Please see Table 4. page 30).

The experiments of the Tamsui Institute also showed that the simultaneous administration of anti-hog cholera serum or the tryptaflavin attenuated erysipelas vaccine would not interfere with the immunity development of the lapinized virus against hog cholera (See Table 12b and 13b on pp. 37-38).

As to the duration of immunity produced, a series of experiments were conducted at different intervals by the Tamsui Institute. So far, it has been demonstrated (See Table 5 page 31), that vaccinated pigs were immune against one c.c. of fresh virulent hog cholera blood as long as 12 months after vaccination. The simultaneous injection of anti-

serum (two c.c. per kg.) also does not seem to interfere with the duration of the immunity developed. For this purpose, five small pigs were used (See Table 6, page 32), all of which received the injection of lapinized vaccine and the anti-serum, and were still found immune after six months.

5. Wet Preparation of Lapinized Vaccine Used in the Field and Its Potency Check

The use of wet lapinized vaccine became quite familiar to the Orient during the campaign of rinderpest control. The wet preparation of the lapinized hog cholera vaccine is, in fact, prepared and used in much the same manner as that of the lapinized rinderpest wet vaccine except for the differences in their composition and their nature.

Healthy rabbits of about one and a half to two kg. weight are generally used for the lapinized hog cholera vaccine production. Usually on the third or fourth day after inoculation of the lapinized virus, the rabbit is bled to death from the heart and the blood is defibrinated. The spleen, the mesenteric lymph glands, and the liver are collected aseptically and weighed. The tissues are cut into pieces and ground in a sterile homogenizer. To ten parts of tissue, five parts of defibrinated blood and 200 parts of physiological saline are added. The final suspension is then strained through a layer of sterile gauze, bottled in 100 c.c. containers, and kept under refrigeration for further distribution and use. No preservative is added to the final mixture. The vaccine so produced should be used within ten hours after preparation though there are evidences to show that such preparation is still good when kept in the laboratory for 48 hours at the room temperature (29°-30.5°C) and 96 hours at refrigeration (2° C) (Please see Table 7, page 32). The dosage of the wet vaccine is two c.c. per head subcutaneously or intramuscularly. A two kg. rabbit could yield 500-600 doses. The amount of each batch of the wet vaccine left over after use is discarded on the same day of production.

After some pilot field tests, this wet lapinized vaccine has been finally used in the whole of Pingtung Hsien since Feb. 1954. The local Pingtung Veterinary Diagnostic Center is responsible for the preparation of the wet lapinized vaccine under the supervision of the Tamsui Institute. Early in the morning the vaccine is prepared and distributed in a big container under refrigeration by jeep or bus from the center to the various places where the field veterinaries are waiting. Each field

veterinary has a small cold storage bottle which can contain two bottles of vaccine of 100 cc. each, equivalent to 100 doses, which is enough for one day's work in the field. From February 1954 to September 1954 the Pingtung Veterinary Diagnostic Center has sacrificed 621 rabbits and prepared 80 batches of wet lapinized vaccine with a total yield of 629,110 cc. or 314,555 doses. (See Table 8, page 33).

As neither the thermal reaction nor the lesion of the hog cholera infected rabbits is constant or typical as in the case in rabbits inoculated with Nakamura's lapinized rinderpest virus, so it is very difficult to detect promptly the viability of this virus at the time of vaccine preparation in the field. In order to determine the potency of the vaccine so prepared, a system of spot checking of the batches was devised. During the first month of February, 1954, nearly every batch was tested. Later, only the last batch of the vaccine of the respective manufacturing period was checked. A common manufacturing period consists in general of three successive days, on each of which a certain number of rabbits are inoculated, probably with the same serial passage of virus. Hence, if the vaccine prepared from the last batch of rabbits of that group is proved to be effective, the previous batches of the same period are probably all right also. The sample of vaccine to be tested was picked out at random on the same morning of preparation and was shipped to the Kaohsiung Hsien Farmers' Association Veterinary Serum Plant for potency check. Each batch of such potency test required three pigs, each of which received two c.c. of vaccine subcutaneously and was challenged after one week with one c.c. of virulent blood. During the period of seven months, from February to September, 1954, 48 pigs were used for this purpose. Only one pig of the 48 died on the 28th day after challenge, the cause of this death was proved to be erysipelas infection. The remaining 47 pigs all survived, which evidently indicated that this wet lapinized vaccine prepared in the manner as mentioned in this report is perfectly effective and safe. The data of the potency tests conducted at the Kaohsiung Hsien Farmers' Association Veterinary Serum Plant are compiled in Table 9 (page 34).

If the thermal reaction and the lesion of the rabbits inoculated with the lapinized hog cholera virus could gradually become constant and typical, then the difficulty of detecting the viability of the virus at the time of vaccine manufacturing would probably be nil. In this respect, there is no doubt that a desiccated vaccine is the most desirable as it could easily overcome all the drawbacks and disadvantages of the wet vaccine, especially in the difficult matter of the potency test of each

batch of vaccine issued. Nevertheless, the wet preparation also has some of its own merits. Especially for some of the rural countries where the modern equipment is somehow impossible to be installed, the field workers could then still use this wet lapinized vaccine successfully for combating hog cholera. The technique for its preparation is, as a matter of fact, extremely simple.

6. Reaction of Hogs After Vaccination Under the Laboratory Tests

It is the general weakness of nearly all the live virus vaccines that the inoculated animal always shows a more severe reaction, or a greater flare-up of unrecognized infection than those vaccinated with a killed or inactivated vaccine. So, the lapinized hog cholera vaccine is also not an exception to this general rule. However, a close pre-vaccinal inspection of all the animals to be injected and a small preventive dose of anti-serum to the doubtful cases might correct this disadvantage of the lapinized vaccine to a certain extent.

From December 1952 to the end of August 1954, the Tamsui Institute has inoculated 314 head of hogs with the lapinized vaccine of which 156 head were mature hogs, and 158 head were small pigs. The result is summarized in Table 10 (page 35). From Table 10 it can be seen that 21 head or 13.46% of the vaccinated hogs showed some reaction but none died. Of the 158 small pigs vaccinated, 45 head or 28.48% showed some reaction and two died after vaccination with a mortality of 1.27%. On the average, the percentage of reaction was around 21% and the mortality was approximately 0.64% under the experiment at the Tamsui Institute. Of these two small pigs died after vaccination, one died of hemorrhagic enteritis and croupous pneumonia and the other one died of complication of salmonellosis. However, the great majority of hogs and pigs vaccinated, 78.34%, showed no reaction at all.

After analyzing the 68 head of hogs (including pigs) which showed reaction after vaccination (See Table 11a, 11b, and 11c, page 35), it can be seen that the majority of the hogs showed fever reaction on the third, fourth and fifth days after vaccination (17.65%, 32.35%, and 36.77% respectively; See Table 11a, page 35). None showed any fever after the eighth day. From Table 11b (page 35), it is noticed that of these hogs which had fever reaction, 51.47% was of slight reaction, 33.83% of moderate reaction, 11.76% of severe reaction and 2.94% died

of complication. However from Table 11c (page 35), according to the duration of the fever reaction, most of the mature hogs (33.33%) had a fever lasting for only one day, while most of the small pigs (23.4%) had a fever of two days. On the average, the fever reaction of most of the hogs vaccinated (58.84%) persisted not longer than three days.

In addition, from January 1953 to September 1953, the Tamsui Institute has also used 50 pigs in the experiment of simultaneous administration of anti-serum with lapinized vaccine. Twenty-six pigs were injected with lapinized vaccine and the anti-hog cholera serum. The dosage of anti-serum varied from five c.c. per head to two c.c. per kg. of body weight. The other 24 pigs which were used as a check, received only the lapinized vaccine. All the pigs were small pigs. The 26 tested pigs regardless of the dosage of anti-serum, all remained perfectly normal after vaccination, while in the check lot seven pigs out of the 24 heads showed some reaction. It seemed that five c.c. of anti-serum per head of pig is quite adequate to prevent the post-vaccinal reaction of the lapinized vaccine. All the pigs of the two lots survived after challenge with virulent blood except one in the group of those vaccination with lapinized vaccine alone which died of pneumonia. The result of this test is presented in Table 12a and 12b (pp. 36-37).

In June 1953, another small test of the Tamsui Institute revealed that it was perfectly safe to vaccinate the small pigs simultaneously with the lapinized vaccine and the erysipelas vaccine, and there was also no interference with the immunity developed (See Table 13a, and 13b page 38).

Another interesting test carried by the Tamsui Institute was the reaction of hogs vaccinated with a large dose of the lapinized vaccine. The dosage per head varied from 10 c.c. of a 10% spleen or lymph gland emulsion to as much as about 100 c.c. of a spleen emulsion and the whole blood of a rabbit. Nineteen pigs were involved in this test, of which nine head were injected with a large dose while the other 10 served as a check, receiving only an ordinary two c.c. dose of the wet lapinized vaccine. The result is shown in Table 14 (page 39). It can be seen that though five pigs showed a slight to severe reaction, yet none of the nine tested pigs died after receiving such a large dose, which clearly proved the perfect safety of this lapinized vaccine.

7. Pilot Field Tests in Changhua and Pingtung

Early in March, 1953, a field test was conducted in Shehtou Hsiang of Changhua Hsien, in which 7,100 head of hogs were injected.

The purpose of this test was simply to see the reaction of the new lapinized vaccine under field conditions. Of the 7,100 head vaccinated, 513 head showed quite a reaction of which 432 head were all promptly cured, and 81 head died after vaccination with an average mortality of 1.1%. 69.14% of all the dead ones were small pigs under 30 kg. (See Table 15, page 40).

Later, from August to December 1953, other field tests were carried out in Chu-ju, Likon and Wantan of Pingtung Hsien. These were actually pioneer preparatory field tests before the mass vaccination of lapinized vaccine in Pingtung Hsien was launched. The number of hogs injected under these experiments, 1,567 head in total, was less than the previous test in Changhua, and these were comparatively more carefully planned in order to let the field veterinaries of Pingtung thoroughly understand the nature of the new vaccine to be used and the different reactions under various conditions, i.e., mature hog vaccination vs. small pig vaccination; simultaneous administration of anti-serum vs. lapinized vaccine alone; simultaneous injection of erysipelas vaccine vs. lapinized vaccine alone; weanling pigs vaccination vs. suckling pigs vaccination, etc. Before testing, the health condition of all the hogs was carefully inspected. Hogs of poor condition were discarded from this experiment. All the hogs were closely observed after vaccination, and the temperature of those which showed clinical symptoms was also taken. The data are compiled in Table 16a, 16b, 16c, 17a, 17b, and 17c. (pp. 40, 41, 42 and 43)

It can be noticed that of 933 head of hogs vaccinated in Chu-ju and Likon of Pingtung Hsien, 144 head were of mature hogs (over 60 kg.), 192 head were medium size feeders (30-60 kg.) and 597 head were small pigs (under 30 kg.). The mortality after vaccination of the mature hogs was 0.69%, that of the medium feeders, 1.04%; and that of small pigs reached 2.85%. The average mortality was 2.15% which was higher than the laboratory tests. However, the percentage of the non-reaction of the lot vaccinated with lapinized vaccine alone was 88.06% (Table 16c, page 41) while that of the laboratory test was only 78.34%. By comparison between the 787 head vaccinated with lapinized vaccine alone and the 146 head simultaneously injected with anti-serum (five c.c. per head), the percentage of slight reaction, severe reaction and mortality decreased from 6.61% to 2.74%, 3.04% to 0.68%, and 2.29% to 1.36% respectively. Under this test, 17 head of pigs were also used for contact infection experiment by putting them in the same pen with vaccinated pigs. After an observation of 18 days, none of the 17 non-vaccinated pigs contracted hog cholera.

Of the 634 hogs vaccinated in Wantan Hsiang of Pingtung (Table 17a, 17b, and 17c, pp. 42-43), 26 head were sows, 21 head were mature hogs, 222 head were medium feeders, 341 head were small pigs after weaning, and 24 head were suckling pigs (about six weeks old). Though some figures in these tables are too small to be significant, yet they at least can indicate some tendency. It is shown that one sow, none of the large feeders, one medium size feeder, and 11 weanling pigs died after vaccination bearing an average mortality about 2%. The mortality of weanling pigs under this test reached 3.22%, the highest of all the experiments in Taiwan. All of these 610 head of hogs, excluding the 24 suckling pigs, under this test were vaccinated with lapinized vaccine only. Of those 24 suckling pigs, five head were vaccinated with lapinized vaccine alone, six head were injected simultaneously with five c.c. per head of anti-serum, another six head simultaneously received two c.c. of erysipelas vaccine, and seven head simultaneously received five c.c. of anti-hog cholera serum and two c.c. of erysipelas vaccine. As a matter of fact, none of these 24 suckling pigs showed any post-vaccinal reaction. So no comparison could be made between the different lots. However, it might at least show that vaccination of suckling pigs seems to be safer than that of weanling pigs. It was also reported that the potency test of these suckling pigs also showed a solid immunity. The data are presented in Table 17c (page 43). The average percentage of non-reaction under this test came to as high as 91.8% of the total hogs vaccinated. The higher percentage of the non-reaction in the field could be interpreted by the fact that some of the slight reaction could be easily ignored by the field veterinaries because of the restriction of activity. On the other hand, all the hogs showing severe reaction under this field test received medical treatment free of charge. So, it is also understandable that the total percentage of all kinds of reaction was slightly lower than it ought to be.

8. Mass Vaccination with Lapinized Vaccine in Pingtung

After the laboratory and the pilot field tests, it was finally decided that the wet lapinized vaccine would be used in Pingtung Hsien under the Pingtung hog cholera control demonstration program. Pingtung Hsien, located at the southern tip of this island, is well isolated by the central mountain range, the ocean, and the Hsia-tam-sui River. Because of its geographical advantages, it was the intention of the Joint Commission to try an experiment in Pingtung in which not only mass vaccination would be adopted, but also all the modern sanitary measures would be put into practice, such as meat inspection in the slaughter

houses, inter-hsien quarantine, elimination of infected hogs, etc. The operation of this program was started from inter-hsien quarantine and mass vaccination. When the incidence of hog cholera is reduced by the mass vaccination to a point so low that the incineration of any cholera infected hog is economically and practically possible, then the practice of strict slaughtering of infected cases will be enforced so as thoroughly to eradicate hog cholera. The program has been in progress since Feb. 1954 and is still going on.

In respect to the vaccination under this project, it was decided that the first three months, from February to April 1954, would be the mass vaccination period, during which the great majority of the whole hog population of Pingtung (about 180,000 head) would be vaccinated with the new wet lapinized vaccine. From April on, the emphasis was shifted to the vaccination of all the new born pigs in order to maintain the level of protection. The supplemental vaccination of the small pigs would be continued for a certain period until the final strict slaughtering method of all the infected hogs is thoroughly enforced. The whole work of this vaccination has been well planned and scheduled, and the whole Hsien was divided into certain working areas. The health condition of all the hogs in the area to be vaccinated was carefully examined beforehand. The general practices in administration of this new wet lapinized vaccine in the field under this Pingtung project are as follows:

- a) It is imperative that this wet vaccine should be kept under cold condition and should be used within 10 hours after preparation on the same day.
- b) The dosage is two c.c. per head, subcutaneously or intramuscularly, regardless of the size of animal to be vaccinated. A single injection is enough to establish a solid immunity.
- c) The tryptaflavin attenuated erysipelas vaccine is also recommended to be given, at a different site on the animal, simultaneously with the lapinized vaccine in order to control swine erysipelas at the same time. The dosage of the erysipelas vaccine is one c.c. to five c.c. according to the size of the animal.
- d) Pre-vaccinal inspection should be carefully and thoroughly conducted. Animals to be vaccinated must be in a good state of health. All the hogs suffering from poor health condition will be excluded in this vaccination.
- e) All the syringes and needles should be sterilized before use for more

than 10 minutes by boiling in water, no chemical disinfectant is to be used.

- f) It is not recommended that sows in advanced pregnancy and suckling pigs under six weeks should be vaccinated.
- g) It is preferable that small pigs be vaccinated before weaning rather than after weaning because of less reaction as well as for the convenience at the field practice.
- h) To all the small pigs of doubtful cases a dose of five c.c. per head of anti-hog cholera serum will be recommended. However, to all the mature hogs no serum is used.
- i) In the areas which are exposed to virulent virus, the lapinized vaccine could still be used; however, a full preventive dose of anti-serum should be given simultaneously.
- j) All the hogs after vaccination should be checked. Those showing reaction from the third to seventh day after vaccination are generally regarded as undergoing the real post-vaccinal reaction of lapinized vaccine.
- k) In order to pacify the objection of farmers, to all the hogs which show severe reaction treatment is allowed which consists of the anti-hog cholera serum and some anti-biotics. For the rest of the hogs which show slight to moderate reaction, no treatment is needed. As a rule they will recover pretty soon.
- l) To the owner of any hog which died within ten days after vaccination an indemnity fund is paid and the carcass is then incinerated.

As of the end of the August 1954, Pingtung Hsien has been able to vaccinate 223,991 head of hogs using the wet lapinized hog cholera vaccine and the erysipelas vaccine simultaneously. The wet lapinized vaccine was manufactured at the Pingtung Veterinary Diagnostic Center. The result and the data are presented in Table 18 and 19 pp. 44-45). Of the 223,991 head of hogs vaccinated, 15,709 head were breeding hogs, 93,484 were feeder hogs, 86,173 head were small pigs vaccinated with vaccine, and 28,625 head were small pigs vaccinated simultaneously with anti-hog cholera serum. The total number of the vaccinated hogs represented approximately 70.4% of the cumulative hog population of the respective areas at the time of vaccination. If the factor of the suckling pigs under six weeks old, (about 17% of the whole population) which are not likely to contract hog cholera, is taken

into consideration, the constant protected level will then be about 87% of the whole hog population of Pingtung.

It is also worth noting that because of the strict precautions undertaken in this project, the result of this mass vaccination showed a total post-vaccinal reaction of only 3.97%, and an average mortality of 0.65% which is far better than the pilot field tests. The mortality of the breeding hogs, the feeder hogs, and the small pigs was 0.15%, 0.24% and 1.05% respectively, of which that of the small pigs was still the highest. It can also be observed that the percentage of the reaction or the mortality was larger in the small pigs vaccinated with vaccine alone than those vaccinated simultaneously with anti-serum (4.86% vs. 2.53%; or 1.18% vs. 0.65% respectively), which also agrees with the general understanding.

9. Blood Picture of Hog Vaccinated with the Lapinized Vaccine

It is a general practice of the veterinaries of Taiwan to examine the blood picture of the infected hogs and use it as an important clinical aid in the diagnosis of hog cholera. In a typical hog cholera case, the leucopenia and the shift to the left of the neutrophils are very evident and severe. According to Dr. Ogula's classification which is generally adopted in Taiwan, the following terms are used:

One plus (+) denotes stab neutrophils over 20% of the differential W.B.C. count.

Two pluses (++) denote the appearance of meta-myelocyte or stab neutrophils over 40% of the differential W.B.C. count.

Three pluses (+++) denote the appearance of myelocyte.

During the incubation period of the cholera infected hog, or when its fever just begins to rise, the differential leucocyte count always shows a degree of one plus. As the clinical symptom becomes severe, the blood picture also advances to two pluses or three pluses accordingly.

However in the work of vaccination of the lapinized vaccine, it was reported that the blood pictures in the hogs which showed no post-vaccinal reaction remained normal. Those with reaction after vaccination were always marked with a certain degree of shift to the left in the neutrophils. In Table 20 (page 46), the pictures of the differential leucocyte count of 15 pigs are presented, all of which showed some

thermal and clinical symptom from slight to severe. Two pigs (No. 2793 and No. 2791) without any reaction after vaccination are shown as a check. From this table it can be seen that none of the hogs showing slight thermal reaction had any change in the differential leucocyte count. On the other hand, most of the hogs which showed a moderate to severe thermal reaction had a shift to the left in the neutrophils from one plus to two pluses, but none reached to the degree of three pluses. Moreover, as the thermal response subsided, the blood change also recovered to normal. It was also reported that in some severe cases in the blood reaction, leucopenia also set in.

The reaction phase of the pigs vaccinated with the lapinized vaccine during which the thermal response, the leucopenia and the shift to the left in the neutrophils are marked is of special interest to the field veterinary practitioners. It is during this phase that the resistance of the inoculated animal is low and is likely to be overcome much more easily by the infection latent in its body. However, as the great majority (over 90%) of hogs withstood perfectly the reaction period without any observable illness and the percentage of severe reaction is so small (less than 3% in the field test), it is justifiable to conclude that this wet lapinized vaccine is quite safe to be used in the field as far as the weakened defense mechanism during the reaction phase of the vaccinated animal is concerned.

10. Field Findings of Autopsy Records of Pigs which Died After Vaccination.

During the period of mass vaccination in Pingtung (February to April 1954), detailed records of the post-vaccinal check were kept. These are presented in Table 21, 22 and 23 (pp. 47-48). Of the 741 hogs which died after vaccination during the three months, 442 head were diagnosed as cholera, according to the lesion found, some of which might have already been exposed to virulent virus before vaccination. The next two outstanding causes of death were hemorrhagic enteritis (139 cases) and catarrhal pneumonia (51 cases). The experiences of the field veterinaries indicated that such post-vaccinal reaction had no connection with any certain batch of vaccine or any certain area. Whenever there was any trouble of post-vaccinal reaction, it always involved a single litter in the same hog pen. The pigs of the neighboring pen vaccinated on the same day with the same batch of vaccine usually appeared to be perfectly healthy. Moreover, the autopsy findings of some non-vaccinated pigs during the same period in Pingtung also showed

quite a number of hemorrhagic enteritis and catarrhal pneumonia cases. All these evidences pointed to the fact that the post-vaccinal reaction of the lapinized vaccine was not the sole cause of death, though it did act as one of the collective factors. It seems that the condition of the individual or the whole litter had much to do influencing the post-vaccinal results.

From Table 22, and 23, it can also be noticed that most of the vaccinated hogs with post-vaccinal response during the mass vaccination period showed reaction from the third to the seventh day after vaccination which also coincided with the laboratory findings. And most of the deaths occurred on the fifth to the ninth day after vaccination. That the figures in the last column (the tenth day) of both Table 22 and Table 23 showed an increase was simply because some farmers wanted to turn in their hogs which showed some reaction so that they might be killed in order to get the indemnity fund as the tenth day was the deadline for applying for it.

11. Pathogenicity of the Lapinized Virus by Reverse Passages through Swine

The strain of the lapinized virus introduced from the Philippines as reported by the Bureau of Animal Industry of the Philippine Government was obtained directly from the Lederle Laboratory. This strain of virus has been passed in rabbits by Lederle for more than 250 serial passages so that it can no longer cause cholera in swine but establishes a high degree of immunity by a single injection. It was said, that the origin of this strain of virus had been secured from Dr. J. A. Baker who was one of the earliest workers on the problem of propagating hog cholera virus in rabbits.

In view of the results mentioned in the previous sections, it is evident that this lapinized virus has certainly been so attenuated that it has lost its virulence to hogs, yet has fully retained its antigenic structure. However, from the increasing percentage of the thermal reaction and some other evidences in the inoculated rabbits, it is quite doubtful whether this virus has reached a state of so called "fixation", in which the virulence of a virus would remain unchanged even by further passages in the given host. But in the future practical use in the field, the most important question is whether or not this lapinized virus at the present passage level could, in one way or other, revert to its original virulence. Hence, an attempt was made to determine its

pathogenicity by passing it reversely through a course of serial passages in swine.

To determine this matter, the Tamsui Institute started an experiment in November 1953 by injecting three pigs (No. 2793, No. 2791 and No. 2790) with two c.c. each of the wet vaccine prepared from a rabbit of the 97th passage. Direct successive passages were carried out from pigs to pigs by drawing blood from the hearts of the preceding pigs on the fourth to sixth day after inoculation and then injecting it to the next ones. The result is summarized in Table 24 of this report (page 49). All the pigs under this experiment were small pigs purchased from the nearby farmers and had been put under close observation for at least seven days before testing. Each lot or passage generally consisted of two (or three) pigs. From Table 24, it can be seen that this lapinized virus was carried on as far as five passages in pigs without any death but with some moderate reaction in the fifth passage and in one of the two of the fourth passage. However, as of the sixth passage, one of the two inoculated pigs died (No. 2796). The mixed blood drawn from the two pigs (No. 2868 and 2796) of the sixth passage also caused the three pigs of the next passage to have a very severe reaction. Two of the three (No. 2797 and 2870) died, and the other one (No. 2792) recovered after being treated with anti-serum. Another pig (No. 2937) inoculated with eight c.c. of the urine from pig No. 2796 also died. The autopsy findings of these dead pigs showed lesions of hog cholera. Nevertheless, the experiment was still carried on, starting from pig No. 2868, the only one of the two of the sixth passage which survived. Blood of that pig was again drawn, injected and passed further on. Another three passages in pigs were able to continue on without any severe ill-effect until the tenth passage. From the tenth to the 12th passage, all the pigs inoculated died of cholera.

At the same time, all the pigs which survived at the different levels of the reverse passage (from the first to the ninth) were tested for their immunity and were all found to be solidly immune, which at least showed that the swine-passed lapinized virus within a level of the fifth or the ninth passages could still build up a sound immunity in the pigs inoculated.

From this test, it may be concluded that this lapinized virus at the 97th passage (Taiwan passage through rabbits) which is non-pathogenic to hogs may increase its pathogenicity to its original host by the technique of artificial transmission through a series of pigs. However,

whether the chance of the lapinized virus of a much higher level serial passage, if it were reverted to its original pathogenicity to hogs, will be much lessened or almost impossible is a question to be investigated. It is hoped that the continuous passages of the lapinized virus in rabbits will further decrease its virulence to hogs.

12. Contact Infection Experiment

What is stated in the preceding section is material largely of laboratory interest. Such results would never occur under natural field conditions. So, in order further to test right in the field, the possibility of transmitting hog cholera from the hogs vaccinated with the lapinized vaccine to the non-vaccinated ones, an experiment on the contact infection was thus initiated and conducted in a village of Chi-san of Kaohsiung Hsien. This is a quite isolated, small village consisting of about 100 hog pens with a hog population of not more than 400 hogs. This experiment started from June second, 1954 and was put under strict supervision. All the hogs of this village were thoroughly inspected and the pens disinfected before the test began. Almost one half of the pigs of each pen of the village were vaccinated with the lapinized vaccine leaving the other half for contact experiment. All the hogs moved in or out of the village during the experimental period were subjected to quarantine inspection for at least seven days. Some non-vaccinated hogs were able to be contacted twice because of the movement of the hogs of the corresponding pen and the re-vaccination of the newly bought pigs.

As of October 1954, a total of three pigs died, one of the vaccinated lot and the other two of the non-treated lot. The vaccinated one was vaccinated on June 3rd, 1954, and died on August 4th, the cause of which was proved to be tetanus. Each of the other two non-vaccinated pigs died after a contact interval of 30 days and eight days respectively. The causes of deaths were found to be pleurisy and peritonitis, and erysipelas. However, none of the rest of the other non-vaccinated hogs contracted cholera or ever showed any sign of illness during the four months of the contact experiment (See Table 25 A and B, page 50). It was also reported that of the total number of hogs vaccinated under this experiment, 19 hogs showed a post-vaccinal reaction (about 10.6%), most of which occurred on the fifth and sixth day after vaccination of a duration about only one to two days.

As of September 22, 1954, six non-vaccinated pigs were selected and challenged with one c.c. of virulent blood, of which four head were

from the once contact lot at different contact intervals and the other two were from the twice contact lot. The result (see Table 26 page 51), showed that all the pigs died after challenge except two of the four of the once contact lot, one of 110 days contact interval and the other of 21 days contact interval. The latter was bought by the farmer from a hog dealer outside the village, and it was impossible to trace its history to see whether it had been vaccinated or not. However, the former one was raised by one of the farmers in this village and its history was clearly understood. The only reasonable interpretation for this one which survived after challenge seems to be that it, in some way or other, such as by the urine, got the virus from the vaccinated hogs, which caused no illness yet enabled it to produce an immunity, during the period of contact, strong enough to withstand the challenge with virulent blood. If so, then whether or not the virus in the urine under the field conditions, could gain its way through pigs to pigs to such a point that its pathogenicity to swine may also be recovered is an interesting and important problem to be investigated. Yet, under the ordinary condition of Taiwan farming where each pen usually contains only a few hogs, such possibility is likely to be extremely small or entirely nil.

This experiment is still going on now.

13. Discussion

From the above statements, it is clearly seen that this lapinized vaccine is really very hopeful. Since a single rabbit of two kg. could yield 500-600 doses, which is twice the yield of a 50 kg. hog in the production of crystal violet vaccine, it cuts down tremendously the cost of vaccine produced. Moreover, the lapinized vaccine is proved to be double or triple in effectiveness as an immunizing agent as compared to the crystal violet vaccine. Also its development of immunity is astonishingly rapid. All these merits of the lapinized vaccine open the work of hog cholera control of this island to the possibility of a new era. It is understandable that the lapinized vaccine will no doubt cause more post-vaccinal reaction than the crystal violet vaccine. However, the mortality of the hogs (0.65%) as proved in the mass vaccination in Pingtung, does verify the safety of its being used in the field. Because of all these promising results, it is very possible that it will entirely replace the crystal violet vaccine in the work of cholera control some day in the future. In this respect, the writer wishes to stress another point which is also an outstanding advantage of this lapinized vaccine. As is well known, one of the serious drawbacks of the crystal violet vaccine

manufacturing here in Taiwan is the cumbersome handling for disposal of all the carcasses of the virus donor hogs. Especially is this true while under a large production program as is now the case. The loose sanitary control in the veterinary serum institutes, especially in the two non-governmental plants, will gradually develop into some troublesome problem which without strict supervision and prompt correction, might become a menace to the whole hog cholera work. If the lapinized vaccine is to be used, this annoying inconvenience will be entirely eliminated, as the lapinized virus will not transmit cholera. This single merit, it seems to the writer, is sufficient to compensate for all its disadvantages.

However, the lapinized vaccine used at the present in Taiwan is only a fresh wet preparation which is not so stable and can only be used within ten hours after preparation. Moreover, the potency check of every batch issued is also most troublesome. To correct these handicaps, the only way is to make a desiccated vaccine. Nevertheless, under some situations, in which it is impossible to set up a desiccated vaccine laboratory, this report at least could prove that by the experience and the method adopted in Taiwan this wet lapinized vaccine, from a disease control standpoint, could still be used successfully in the field.

In view of the increasing percentage of the thermal reaction in the rabbits inoculated with the lapinized virus, it is justifiable to suspect that the pathogenicity of this virus to rabbits at the present level of serial passage is still undergoing some changes. However, it is now hoped to continue the passage of this lapinized virus in rabbits in order to secure a more attenuated strain or even a "fixed" one. On the other hand, the possibility that this lapinized virus instead of being "fixed", might become over attenuated by continuous long serial passages is also an interesting problem to be considered. In this case, if at a certain level of passage which is proved to be the most desirable, the desiccation of this lapinized virus would then be urgently needed. It will eliminate the necessity for frequent serial passages as two or three passages each year will probably be sufficient to ensure its viability.

During the 21 months since the lapinized virus was introduced here, too much emphasis of all the studies was placed on the practical aspects as all the veterinary workers concerned were so eager to see it being used in the field. Consequently, because of facility, labor and financial limitation, some fundamental investigations seemed to have been ignored which will greatly affect its future application. Among

these, some which need to be studied as soon as possible are suggested hereunder:

- a) The titration test to determine the M.I.D. (minimal infective dose) of the virus in different tissue of the inoculated rabbit at various passage level, to see whether there will be any change after long serial passage.
- b) The persistence of the lapinized virus in the body as well as in the excretion of the vaccinated hogs at different intervals after inoculation.
- c) Detailed study on pathogenicity of the lapinized virus by reverse passages through swine by using blood and urine with different routes of administration.
- d) Test on development and duration of immunity in suckling pig vaccination.
- e) Problem of desiccation of lapinized virus.

Besides, it is also of some interest to test the adaptability of the lapinized hog cholera virus in the goat. This is not only of academic interest but also of some practical value. If the goat adaptation is possible and its antigenicity for hogs is retained, the goat will surely provide a great amount of tissue and facilitate the vaccine production in large quantities. Moreover, goats are available nearly everywhere in Taiwan, their population is steadily increasing during recent years, and their price is very cheap.

14. Summary

1. The lapinized virus introduced from the Philippines in December 1952 has been successfully passed through Taiwan rabbits for 144 serial passages as of September 1954. The inoculated rabbits have so far shown no characteristic lesion. The percentage of thermal reaction of the inoculated rabbits indicated a tendency of gradual increase as the level of passage went higher. As of September 1954, 87.97% of rabbits of 111-144th passages showed thermal response, mostly of not a longer duration than 24-36 hours. Potency tests of the virus in the rabbits killed on the third or the fourth day after inoculation regardless of the fever reaction were all proved to be effective.
2. A two c.c. emulsion of 10,000 times of spleen, 5,000 times of lymph glands or 500 times of liver or blood from an inoculated rabbit

could still induce a sound immunity against cholera in the hog vaccinated. The lapinized virus in the cholera-infected rabbit could be detected as long as eight days after inoculation. Virus in spleen and lymph glands of the rabbit collected on the third day after inoculation is still viable after three days at room temperature and 33 days under refrigeration. The virus in blood of the rabbit is more liable to die under the same condition.

3. The lapinized vaccine adopted in Taiwan is a wet preparation, the composition of which is:

Spleen, lymph glands and liver	10 parts
Blood	5 "
Saline	200 "

A two kg. rabbit could yield approximately 1,000-1,200 c.c. of vaccine. One dose consists of a single injection of two c.c. of this preparation, subcutaneously or intramuscularly, regardless of the size of hog to be vaccinated.

4. The wet lapinized vaccine used in the field was prepared at the local hsien veterinary diagnostic center. From February 1954 to September 1954, the Pingtung Hsien Veterinary Diagnostic Center has produced 80 batches of the wet vaccine with a total yield of 629,110 c.c. or 314,555 doses. The potency tests showed that the lapinized vaccine so produced was effective.
5. As of the end of August, 1954, a grand total of about 243,000 head of hogs (including data other than that of Pingtung Hsien) were successfully vaccinated with the wet lapinized vaccine (mostly simultaneously with erysipelas vaccine). The average mortality was approximately 0.65%. The great majority of hogs (about 90%) did not show any noticeable post-vaccinal reaction. Those which showed reaction after vaccination had the thermal response which usually occurred on the third to the fifth day with a duration of not longer than three days, followed by a rapid recovery. Simultaneous injection of anti-serum with the lapinized vaccine is not necessary except for sub-condition pigs.
6. The wet preparation of the lapinized vaccine has been proved to be still effective after keeping it for 48 hours under room temperature. However, for the purpose of safety it is recommended to be used within ten hours after preparation in the field under refrigeration.
7. During the period of fever reaction in the hog vaccinated, the differential count of the white blood cells in some cases showed a

degree of shift to the left. The intensity of the shift to the left always corresponded to the degree of the fever and the clinical symptoms.

8. A solid immunity developed about four days after vaccination in the hog showing no post-vaccinal reaction. The duration of the immunity established by this lapinized vaccine so far as the experiment showed could last as long as 12 months.
9. Simultaneous injection with erysipelas vaccine or anti-hog cholera serum does not seem to interfere with the development of immunity of the lapinized vaccine.
10. It is justifiable to say that the lapinized vaccine will not transmit hog cholera to the non-vaccinated hogs in the field.

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Table 1

**Figures Showing Thermal Reaction of Rabbits
Inoculated with Lapinized Hog Cholera Virus**

(at T.V.S.I.)

Serial Passage No.	Date	No. of Rabbits Used (head)	Thermal Response (head)				Total
			(1) —	(2) ±	(3) +	(4) ‡	
1 — 10	Dec. 12 '52 — Jan. 6 '53	77	27	22	26	2	77
11 — 20	Jan. 9 '53 — Feb. 5 "	45	12	19	14	—	45
21 — 30	Feb. 9 " — Mar. 9 "	70	19	23	28	—	70
31 — 40	Mar. 12 " — Apr. 8 "	58	16	23	19	—	58
41 — 50	Apr. 11 " — May 8 "	60	32	20	8	—	60
51 — 60	May 11 " — June 7 "	48	9	18	21	—	48
61 — 70	June 10 " — July 7 "	57	25	18	14	—	57
Sub-total		415	140	143	130	2	415
Percentage:			33.73	34.46	31.33	0.48	100.0
71 — 80	July 10 '53 — Aug. 9 '53	91	2	13	45	31	91
81 — 90	Aug. 12 " — Sep. 15 "	46	1	1	26	18	46
91 — 100	Sep. 20 " — Nov. 16 "	43	1	4	25	13	43
101 — 110	Nov. 23 " — Jan. 18 '54	37	2	3	26	6	37
Sub-total		217	6	21	122	68	217
Percentage:			2.76	9.68	56.22	31.34	100.0
111 — 120	Jan. 22 '54 — Mar. 15 '54	45	1	5	36	3	45
121 — 130	Mar. 24 " — June 1 "	42	3	3	30	6	42
131 — 140	June 7 " — Aug. 9 "	33	—	4	29	—	33
141 — 144	Aug. 16 " — Sep. 6 "	13	—	—	12	1	13
Sub-total		133	4	12	107	10	133
Percentage:			3.01	9.02	80.45	7.52	100.0
Grand Total		765	150	176	359	80	765
Percentage:			19.61	23.00	46.93	10.46	100.0

Remarks: (1) — — No reaction.
 (2) ± — Doubtful or slight thermal reaction, a) having fever curve, but maximum temperature not over 40°C, b) no fever curve, but maximum temperature over 40°C for one day.
 (3) + — Moderate thermal reaction, having definite fever curve, with a duration about one day, maximum temperature over 40°C.
 (4) ‡ — Severe thermal reaction, having definite fever curve, with a duration of more than one day, maximum temperature over 40°C or more.

Table 2

**Figures Showing Thermal Reaction of Rabbits Used
For Lapinized Hog Cholera Vaccine Production
in Pingtung Hsien**

(at P.H.V.D.C.)

Period	Serial Passage No. of Virus	Batch No. of Vaccine	Date	No. of Rabbits Used (head)	Thermal Response (head)				
					-	±	+	††	Total
February 1954	114	1—3	Feb. 10—12	50	-	9	30	11	50
	116	4—6	20—22	39	-	3	18	18	39
March	117	7—10	Mar. 2—5	50	2	15	20	13	50
	119	11—13	12—14	48	-	1	13	34	48
	121	14—17	22—27	44	-	-	15	29	44
April	122	18—20	Apr. 1—3	47	-	3	19	25	47
	123	21—23	6—8	27	-	1	8	18	27
	123	24—26	11—13	29	-	-	3	26	29
	124	27—29	21—23	26	-	4	18	4	26
May	126	30—32	May 6—8	12	-	-	3	9	12
	129	33—38	25—31	38	-	2	27	9	38
June	130	39—43	June 1—5	18	4	10	4	-	18
	132	44—48	16—20	24	-	5	14	5	24
July	136	49—58	July 11—20	68	5	14	46	3	68
August	139	59—63	Aug. 1—5	19	-	5	7	7	19
	141	64—68	16—20	33	-	2	30	1	33
	142	69—70	27—28	2	-	-	2	-	2
September	143	71—75	Sep. 1—5	16	-	4	9	3	16
	145	76—80	16—20	31	-	4	19	8	31
Total				621	11	82	305	223	621
Percentage:					1.78	13.02	49.12	35.90	100.0

Remarks: Temperature charts of six rabbits inoculated in March, May, and June for vaccine production were missed; another six temperature charts of rabbits inoculated in April for purpose other than vaccine production were substituted.

Table 3

Thermal Reactions & Autopsy Findings of 31 Cholera Infected Rabbits

(at T.V.S.I.)

Rabbit No.	Sex	Inoculum*	Date of Inoculation	Date of Bleeding	Interval in days	Thermal Reactions				Autopsy Findings			
						Incubation Period (hrs.)	Period of recovery (hrs. after inoculation)	Duration of Fever (hrs.)	Classification	Conges- tion of lungs	Swell- ing of Spleen	Swelling of Mesen- teric lymph glands	Swell- ing of Appen- dix
654	♂	dose 1	Sep. 13, '54	Sep. 16, '54	3	28	31	3	+	—	+	+	+
657	♂	dose 1	Sep. 13, '54	Sep. 16, '54	3	28	46	18	+	—	+	+	+
658	♂	dose 1	Sep. 13, '54	Sep. 16, '54	3				±	+	+	+	±
661	♀	dose 1	Sep. 13, '54	Sep. 16, '54	3	22	42	20	+	—	+	+	+
665	♀	dose 1	Sep. 13, '54	Sep. 16, '54	3	24	62	38	±	—	+	+	+
667	♂	dose 1	Sep. 13, '54	Sep. 16, '54	3	28	42	14	+	—	+	+	+
669	♀	dose 1	Sep. 13, '54	Sep. 16, '54	3	28	52	24	+	—	±	+	+
655	♂	dose 1	Sep. 13, '54	Sep. 17, '54	4	28	66	38	±	—	±	+	+
659	♂	dose 1	Sep. 13, '54	Sep. 17, '54	4	22	66	44	±	—	+	+	+
662	♂	dose 1	Sep. 13, '54	Sep. 17, '54	4				±	—	+	+	+
663	♂	dose 1	Sep. 13, '54	Sep. 17, '54	4	22	66	44	+	—	+	+	+
668	♀	dose 1	Sep. 13, '54	Sep. 17, '54	4	31	66	35	+	—	+	+	+
670	♀	dose 1	Sep. 13, '54	Sep. 17, '54	4	46	70	24	+	—	±	+	+
674	♂	dose 1	Sep. 13, '54	Sep. 17, '54	4	28	70	42	+	—	+	+	+
656	♂	dose 1	Sep. 13, '54	Sep. 18, '54	5	28	66	38	+	—	+	+	+
660	♂	dose 1	Sep. 13, '54	Sep. 18, '54	5	28	76	48	±	—	±	+	+
664	♂	dose 1	Sep. 13, '54	Sep. 18, '54	5	46	76	30	+	—	±	±	+
666	♂	dose 1	Sep. 13, '54	Sep. 18, '54	5	22	66	44	+	—	+	+	+
671	♀	dose 1	Sep. 13, '54	Sep. 18, '54	5	22	90	68	+	—	±	+	+
672	♂	dose 1	Sep. 13, '54	Sep. 18, '54	5	28	70	42	±	—	+	+	+
673	♂	dose 1	Sep. 13, '54	Sep. 18, '54	5	28	90	62	±	—	±	+	+
676	♂	dose 2	Sep. 16, '54	Sep. 19, '54	3	24	62	38	+	—	+	±	+
677	♂	dose 2	Sep. 16, '54	Sep. 19, '54	3	24	57	33	+	—	±	+	+
680	♂	dose 2	Sep. 16, '54	Sep. 19, '54	3	33	57	24	+	—	+	+	+
682	♂	dose 2	Sep. 16, '54	Sep. 19, '54	3				±	—	—	±	+
675	♂	dose 2	Sep. 16, '54	Sep. 20, '54	4	24	68	44	+	—	+	+	+
678	♂	dose 2	Sep. 16, '54	Sep. 20, '54	4	33	72	39	±	—	+	+	+
679	♂	dose 2	Sep. 16, '54	Sep. 20, '54	4	48	54	6	+	—	+	+	+
681	♂	dose 2	Sep. 16, '54	Sep. 20, '54	4	33	68	35	±	—	±	+	+
683	♂	dose 2	Sep. 16, '54	Sep. 20, '54	4	44	68	24	+	—	+	—	+
684	♂	dose 2	Sep. 16, '54	Sep. 20, '54	4				±	—	+	+	±
Average:						30	64	34					

* Remarks: *Dose 1—1 c.c. of 10% spleen emulsion from rabbit of 144 passage level, I.V.

Dose 2—1 c.c. of 10% spleen emulsion from rabbit No. 665 and 669, I.V.

— : Negative

± : doubtful

+ : slight

±± : moderate

Table 4

Immunity Test of Hogs Vaccinated with Lapinized Virus
(at T.V.S.I.)

Kinds of Animal	No. of tested animal used (head)	Termination After Challenge with 1 c. c. Hog Cholera Virulent Blood (head)					Death ‡	Total pigs Used (head)	Control	
		No. re- action	Slight reaction	Moderate reaction	Severe reaction	No. of			Terminna- tion	
		—(1)	±(2)	+(3)	‡(4)	pigs Used (head)				
Mature Hogs (about 60 kg.)	143	101	36	4	2	0	143	76	All died	
	Percentage:	70.63	25.17	2.80	1.40	0	100.0			
Small pigs (8-25 kg.)	155	101	36	8	1	9	155	22	„ „	
	Percentage:	65.16	23.23	5.16	0.65	5.80	100.0			
Total	298	202	72	12	3	9	298	98	„ „	
	Percentage:	67.79	24.16	4.03	1.00	3.02	100.0			

Remarks:

- (1) —: remaining normal after challenge.
- (2) ±: Maximum body temperature slightly over 40°C, with a fever of less than 1-2 days.
- (3) +: Maximum body temperature 40.5°-41°C, with a fever of about 3-4 days.
- (4) ‡: Maximum body temperature over 41°C, with a fever of more than 4 days.

Table 5

Duration of Immunity Produced by Lapinized Vaccine
(at T.V.S.I.)

Interval between vaccination & challenge	Kinds of animal	No. Used (head)	Vaccination			Date	Dose	Strain of virulent blood	Challenge						Control	
			Date	Batch No. of vaccine	No. of				Dose	Dose	Termination	No. of Pigs	Used	minia-		tion.
									(1)	(2)	(3)	(4)	(5)	(6)	(head)	(head)
3 months	Mature hogs	25	Jan. 27 1953	No. 15	2 c.c.	Apr. 27 1953		A.L.D. No. 905	18	3	2	1	1	(6)	2	All died
3 months	"		Jan. 30 1953	No. 16	i.m.	Apr. 30 1953	"	A.L.D. No. 1192	"							
3 months	Small pigs	2	Feb. 2 1953	No. 17	"	May 4 1953	"	A.L.D. No. 1121	1	-	1	-	-	-	1	"
7 months	"	2	Mar. 27 1953	No. 31	"	Oct. 29 1953	"	A.L.D. No. 2666	1	1	-	-	-	-	2	"
9 months	"	7	Jan. 30 1953	No. 16	"	"	"	"	5	2	-	-	-	-	2	"
12 months	"	7	Jan. 30 1953	No. 16	"	Jan. 26 1954	"	A.L.D. No. 25	7	-	-	-	-	-	2	"

Remarks: (1) - No reaction
 (2) ± Slight reaction
 (3) + Moderate reaction
 (4) †† Severe reaction
 (5) ††† Death
 (6) Died of complication of cholera and salmonellosis.

Table 6

**Immunity Test of Pigs at Six Months Interval After Simultaneous
Vaccination with Lapinized Vaccine and Anti-serum**
(at T.V.S.I.)

Kind of animal	No. used (head)	Interval	Simultaneous Vaccination		Challenge				
			Date	Batch No. of wet lap. vaccine	Batch No. of anti-serum	Date	Strain of virulent blood	dose	Termination
small pig	5	6 months	Sept. 25 '53	No. 53 2cc i.m.	No. 3 2 cc per kg subc.	Mar. 27 '54	ALD No. 164	1 cc subc.	No reaction

Table 7

Preservation Tests on Wet Preparation of Lapinized Vaccine
(at T.V.S.I.)

Kind of animal	No. used (head)	Date of vaccine prepared	Vaccination		Date of challenge	Potency Test		Reaction after challenge — ± + ††
			Batch No. of wet lap. vaccine	Keeping condition of vaccine		Strain of virulent blood		
small pig	2	Mar. 20 '54	No. 71	Room temp. (29°C) for 8 hrs.	Apr. 7 '54	ALD No. 207 1 cc Subc.	2 - - -	
"	2	May 6 '54	No. 73	Room temp. (29°C) for 12 hrs.	May 15 '54	ALD No. 493 1 cc Subc.	- 2 - -	
"	2	"	"	Room temp. (29°C) for 24 hrs.	"	"	2 - - -	
"	2	July 7 '54	No. 80	Room temp. (30.5°C) 36 hrs.	July 21 '54	ALD No. 944 1 cc Subc.	2 - - -	
"	2	"	"	Room temp. (30.5°C) 48 hrs.	"	"	1 - - 1	
"	2	Mar. 20 '54	No. 71	Refrigeration (2°C) 24 hrs.	Apr. 7 '54	ALD No. 493 1 cc Subc.	2 - - -	
"	2	"	"	Refrigeration (2°C) 48 hrs.	"	"	2 - - -	
"	2	May 6 '54	No. 73	"	May 15 '54	ALD No. 207 1 cc Subc.	1 1 - -	
"	1	"	"	Refrigeration (2°C) 72 hrs.	"	"	1 - - -	
"	2	July 7 '54	No. 80	"	July 21 '54	ALD No. 944 1 cc Subc.	2 - - -	
"	2	"	"	Refrigeration (2°C) 96 hrs.	"	"	1 1 - -	

Table 8**Figures Showing Amount of Wet Lapinized
Vaccine Prepared in Pingtung****(at P.H.V.D.C)**

Period	No. of Bathches	No. of rabbits used (head)	Total yield of vaccine (c.c.)
February '54	6	89	83,400
March	11	144	135,400
April	12	123	119,150
May	9	51	51,610
June	10	45	48,400
July	10	68	78,050
August	12	54	57,600
September	10	47	55,500
Total	80	621	629,110
Average		1	1,013

Table 9

Potency Tests of Lapinized Vaccine Prepared in Pingtung

(at K.H. FAs. V.S.P.)

Period	No. of batches checked	No. of hogs used		Amount of vaccine injected	Post-vaccinal reaction (head)					Virulent blood challenged	Result after challenge													
		Kind	Head		Clinical symptom				Termination		Reaction (head)					Termination (head)								
					-	±	+	Total	Survival		Death	Total	-	±	+	++	+++	Total	Survival	Death	Total			
February	4	Medium hog	2	2 c.c. Subc.	2	-	-	2	2	-	2	1 c.c. Subc.	1	1	-	-	-	2	2	-	2			
					Control	2	Large hog	2	-	-	-	-	-	-	-	-	6	1	-	-	-	7	6	1†
March	3	Med. hog	1	2 c.c. Subc.	1	-	-	1	1	-	1	„	1	-	-	-	-	1	1	-	1			
					Control	3	Pig	8	-	-	8	8	-	8	„	8	-	-	-	-	8	8	-	8
					Control	3	Large hog	8	-	-	-	-	-	-	-	-	-	-	1	7	8	-	8*	8
April	4	Pig	12	2 c.c. Subc.	10	-	2	12	12	-	12	„	12	-	-	-	-	12	12	-	12			
					Control	4	Large hog	12	-	-	-	-	-	-	-	-	-	-	-	12	12	-	12*	12
May	2	„	6	2 c.c. Subc.	5	1	-	6	6	-	6	„	6	-	-	-	-	6	6	-	6			
					Control	2	„	3	-	-	-	-	-	-	-	-	-	-	3	3	-	3*	3	
June	1	„	3	2 c.c. Subc.	2	-	1	3	3	-	3	„	3	-	-	-	-	3	3	-	3			
					Control	1	„	1	-	-	-	-	-	-	-	-	-	-	1	1	-	1*	1	
July	1	„	3	2 c.c. Subc.	3	-	-	3	3	-	3	„	3	-	-	-	-	3	3	-	3			
					Control	1	„	1	-	-	-	-	-	-	-	-	-	-	1	1	-	1*	1	
August	1	„	3	2 c.c. Subc.	3	-	-	3	3	-	3	„	3	-	-	-	-	3	3	-	3			
					Control	1	„	1	-	-	-	-	-	-	-	-	-	-	1	1	-	1*	1	
September	1	„	3	2 c.c. Subc.	3	-	-	3	3	-	3	„	3	-	-	-	-	3	3	-	3			
					Control	1	„	1	-	-	-	-	-	-	-	-	-	-	1	1	-	1*	1	
Total	17	L. hog	18	2 c.c. Subc.	44	1	3	48	48	-	48	„	46	2	-	-	-	48	47	1	48			
					Pig	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control test total	15	L. hog	29	-	-	-	-	-	-	-	-	-	-	-	-	2	27	29	-	29*	29			

Remarks: † Died of erysipelas

* Bled to death

Table 10

Showing Reactions of Hogs Vaccinated with Lapinized Virus

(at T.V.S.I.)

Kind	Period	No. of animal used (head)	No reaction		Reaction		Death	
			Head	%	Head	%	Head	%
Mature hogs	Dec. '52	156	135	86.54	21	13.46	0	0
Small pigs	Aug. '54	158	111	70.25	45	28.48	2	1.27
Total		314	246	78.34	66	21.02	2	0.64

Table 11a

Distribution of Reaction Hogs According to Day showing Fever Reaction after Vaccination

(at T.V.S.I.)

Kind	Day after Vac.		3rd	4th	5th	6th	7th	8th	Total
	1st	2nd							
	Head	%	Head	%	Head	%	Head	%	
Mature hog	0	1 4.76	4 19.05	6 28.57	9 42.86	0	1 4.76	0	21
Small pig	0	0	8 17.02	16 34.04	16 34.04	4 8.51	2 4.26	1 2.13	47
Total	0	1 1.47	12 17.65	22 32.35	25 36.77	4 5.88	3 4.41	1 1.47	68

Table 11b

Classification of Hogs with Post-Vaccinal Reaction

(at T.V.S.I.)

Kind	Slight reaction ±		Moderate reaction +		Severe reaction ††		Death †††		Total
	Head	%	Head	%	Head	%	Head	%	
Mature hog	10	47.62	9	42.86	2	9.52	0	0	21
Small pig	25	53.18	14	29.79	6	12.77	2	4.26	47
Total	35	51.47	23	33.83	8	11.76	2	2.94	68

Table 11c

Showing Duration of Fever of Hogs with Post-Vaccinal Reaction

(at T.V.S.I.)

Kind	Days										Total								
	1	2	3	4	5	6	7	8	9 & over										
	Head	%	Head	%	Head	%	Head	%	Head	%	Head	%							
Mature hog	7	33.33	3	14.29	3	14.29	0	5	23.81	0	1	4.76	1	4.76	47				
Small pig	8	17.02	11	23.40	8	17.02	8	17.02	3	6.38	4	8.51	2	4.26	1	2.13	68		
Total	15	22.05	14	20.60	11	16.18	8	11.76	8	11.76	4	5.88	3	4.41	3	4.41	2	2.94	21

Table 12a

**Experiment on Simultaneous Injection of Lapinized
Vaccine and Anti-Serum**
(at T.V.S.I.)

Date of Inoculation	Lot	Simultaneous Injection		Lap. Vaccine alone (check)					
		Doses	No. of pigs used (head)	Reaction (head)	Doses	No. of pigs used (head)	Reaction (head)		
Jan. 21, 1953	A	Vaccine (No. 13) 2 cc i.m. Serum (No. 124) 2 cc per kg Subc.	5	5	0	Vaccine (No. 13) 2 cc i.m.	11	10	1
Apr. 25, 1953	B	Vaccine (No. 35) 2 cc i.m. Serum (No. 4) 10 cc per head Subc.	6	6	0	} Vaccine (No. 35) 2 cc i.m.	13	7	6
"	C	Vaccine (No. 35) 2 cc i.m. Serum (No. 4) 5 cc per head Subc.	5	5	0				
June 16, 1953	D	Vaccine (No. 39) 2 cc i.m. Serum (No. 4) 5 cc per head Subc.	5	5	0	-	-	-	-
Sept. 25, 1953	E	Vaccine (No. 53) 2 cc i.m. Serum (No. 3) 2 cc per kg Subc.	2	2	0	-	-	-	-
"	F	Vaccine (No. 39) 2 cc i.m. Serum (No. 3) 10 cc per head Subc.	3	3	0	-	-	-	-
Total			26	26	0		24	17	7

Table 12b

**Potency Tests on Pigs Simultaneously Vaccinated with Lap.
Vaccine & Anti-Serum**
(at T.V.S.I.)

Date of Challenge	Strain of virulent blood	Lot J/	Result after Challenge										Control	
			Reaction of Simultaneous group					Reaction of Lap. vaccine alone group					Non-treated pigs (head)	
			-	±	+	++	Total	-	±	+	++	Total	Total	±
Feb. 31, 1953	ALD 1 cc Subc.	A	2	1	1	1	5	6	-	-	-	6	3	3
May 11, 1953	"	B	5	-	1	-	6	5	1	1	2	12	3	3
"	"	C	3	1	1	-	5	5	-	-	-	-	-	-
July 7, 1953	"	D	4	-	1	-	5	-	-	-	-	-	-	-
Total			14	2	4	1	21	11	5	1	1	18	6	6

Remarks: 1/ Lot E & F are not included
2/ Died of Pneumonia
3/ Died of hog cholera

Table 13a

**Experiment on Simultaneous Vaccination with Lap. Vaccine
and Erysipelas Vaccine**
(at T.V.S.I.)

Date of Inoculation	Kind	Simultaneous injection of Lap. vaccine & erysipelas				Lap. vaccine alone						
		Dose	Post-vaccinal reaction (head)		Dose	Post-vaccinal reaction (head)						
			-	±		+	‡	Total	-	±	+	‡
June, 13, 1953	pig	Lap. vaccine (No. 38) 2cc i.m.	2	-	-	2	Vaccine (No. 38) 2cc i.m.	1	1	-	-	2
		S.E. vaccine (No. 33) 5cc Subc.										
June, 16, 1953	pig	Lap. vaccine (No. 39) 2cc i.m.	1	2	-	3	Vaccine (No. 39) 2cc i.m.	3	-	2	-	5
		S.E. vaccine (No. 33) 5cc Subc.						4	1	2	-	7
Total												

Table 13b

**Potency Test on Pigs Vaccinated Simultaneously with Lap.
Vaccine and Erysipelas Vaccine**
(at T.V.S.I.)

Date of Challenge	Strain of virulent blood	Result after Challenge				Control											
		Reaction of Simultaneous group (head)		Reaction of Lap. Vaccine group (head)		Non-treated pigs (head)											
		-	±	+	‡	Total	-	±	+	‡	Total	-	±	+	‡	Total	
July 7, 1953	ALD 1 cc Subc.	2	-	2	1	5	1	1	1	2	2						

Table 14

Showing Reaction of Hogs Received Large Amount of Lap. Vaccine
(at T.V.S.I.)

Date of Injection	Kind	Serial passage No. of lap. vac.	Dose	Reactios (head)		Date of Chal-enge	Strain of virulent blood	Reaction of challenge (head)		Control			
				-	+ ††			- ‡	‡ Total	Dose	No. of pig used (head)		
Apr. 26 1953	Small pig	45th	10% spleen emulsion	-	-	1	ALD	1	-	1	2cc of lap.	No	
			10cc i.m.	-	-	1	1cc Subc.	-	1	1	vaccine i.m.	4 reaction	
			10% ly. gl. emulsion	-	-	1	"	-	1	1			
Jan. 7 1954	Mature hog	108th	Def. blood 10cc i.m.	1	-	-	"	-	-	-			
			10% tissue emulsion	1	-	-	"	1	-	1	"	2	"
Jan. 21 1954	Mature hog	110th	Def. blod 10cc Subc.	1	-	-	"	1	-	1			
			Spleen 5gm + blood	1	-	-	"	2	-	2		2	"
			50cc (total amount	1	1	-	"	2	-	2		2	"
Feb. 7 1954	Small pig	113th	105cc emul.) Subc.	-	-	2	"	1	-	1			
			Liver 30gm in 300cc Subc.	-	-	1	"	1	-	1		2	"
Total			Spleen 4gm + liver 6gm + blood 8cc (total 80 cc emul.) Subc.	-	-	1	"	1	-	1		2	"
				4	3	1	1	9	7	2	9	10	

Table 15

**Classification of Deaths after Lapinized Vaccine Injection
according to Body Weight**

(in Shehtou of Changhua Hsien)

Kind of animal	N o. of death (head)	Percentage
Small pig under 30 kg	56	69.14
Medium hog 30—60 kg	23	28.40
Large hog over 60 kg	2	2.46
Total	81	100.0

Remarks: Total hogs vaccinated: 7100 head

Table 16a

**Figures Showing the Vaccination of Lapinized
Vaccine in Chu-ju and Likon**

(P.H.V.D.C.)

Kind	Vaccinated with lap. vaccine (head)	Simultaneous injection with anti-serum* & vaccine (head)	Total (head)	Non-treated hogs for contact experi- ment (head)	Grand total (head)
Mature hog (over 60 kg.)	142	2	144	8	152
Medium feeder (30-60kg.)	171	21	192	4	196
Small pig	474	123	597	5	602
Total	787	146	933	17	950

* 5cc of anti hog cholera serum/head

Table 16b

Classification of Post-vaccinal Reactions of Hogs According to Size of Animal in Chu-ju and Likon

(P.H.V.D.C.)

Kind	No. of animal used (head)	Post-vaccinal Reaction			(head)
		No Reaction	Slight Reaction (1-2 dys)	Sever Reaction (over 3 dys)	Death
Mature hog	144	124	13	6	1
	% (100.0)	(86.11)	(9.03)	(4.17)	(0.69)
Medium feeder	192	175	11	4	2
	% (100.0)	(91.15)	(5.73)	(2.08)	(1.04)
Small pig	597	533	32	15	17
	% (100.0)	(89.28)	(5.36)	(2.51)	(2.85)
Total	933	832	56	25	20
	% (100.0)	(89.17)	(6.00)	(2.68)	(2.15)

Table 16c

Classification of Post-vaccinal Reactions of Hogs according to Methods of Vaccination in Chu-ju, and Likon

(P.H.V.D.C.)

Kind	No. of pigs used (head)	Post-vaccinal Reaction			(head)
		No Reaction	Slight Reaction (1-2 dys)	Sever Reaction (over 3 dys)	Death
Lap. Vaccine alone	787	693	52	24	18
	% (100.0)	(88.06)	(6.61)	(3.04)	(2.29)
Simultaneous with anti-serum & vaccine	146	139	4	1	2
	% (100.0)	(95.21)	(2.74)	(0.68)	(1.36)
Total	933	832	56	25	20

Table 17a

Figures Showing the Vaccination of Lap. Vaccine in Wantan
(P.H.V.D.C.)

Kind	No. of animal vac. with lap. vaccine (head)	Vaccination of suckling pigs (head)				Total
		A	A B	A C	A B C	
Breeding sow	26	-	-	-	-	26
Mature hog (over 60 kg)	21	-	-	-	-	21
Medium feeder (30-60 kg)	222	-	-	-	-	222
Small pig (under 30 kg)	341	-	-	-	-	341
Suckling pig (above 6 weeks)	-	5	6	6	7	24
Total	610	5	6	6	7	634

- A. with lap. vaccine
- B. „ anti-hog cholera serum (5cc/head)
- C. „ erysipelas vaccine

Table 17b

Figures Showing Post-vaccinal Reactions of Hogs in Wantan
(P.H.V.D.C.)

Kind	No. of animal inoculated (head)	Post-vaccinal Reaction (head)			
		No Reaction	Slight Reaction (1-2 dys)	Severe Reaction (over 3 dys)	Death
Breeding sow	26	24	1	-	1
	% (100.0)	(92.30)	(3.85)		(3.85)
Mature hog	21	19	2	-	-
	% (100.0)	(90.48)	(9.52)		
Medium feeder	222	203	15	3	1
	% (100.0)	(91.44)	(6.76)	(1.35)	(0.45)
Small pig	341	312	15	3	11
	% (100.0)	(91.50)	(4.40)	(0.88)	(3.22)
Suckling pig	24	24	-	-	-
	% (100.0)	(100.0)			
Total	634	582	33	6	13
	% (100.0)	(91.80)	(5.21)	(0.94)	(2.05)

Table 17c

**Immunity Tests of Suckling Pigs Vaccinated with
Lapinized Vaccine
(P.H.V.D.C.)**

Pig No.	Sex	Body Wt.	Kind	Inoculation and Result				Potency Test					
				Date of Inoculation	Lap. Vaccine	Anti-cholera serum	Erysi-pelas vaccine reaction	Post-vaccinal reaction	Termination	Date of Challenge	Strain of virulent blood injected	Reaction	Termination
1	♀	7.8 kg	Suckling pigs	Sept. 18 1953	2cc Subc.	5cc Subc.	-	No reaction	Normal	Nov. 25 1953	ALD No. 842-86 1cc subc.	Slight	Survived
2	♀	8.4	"	"	"	"	-	"	"	"	"	No	"
3	♂	10.8	"	"	"	-	2cc Subc.	"	"	"	"	No	"
4	♀	8.1	"	"	"	-	"	"	"	"	"	Slight	"
5	♂	8.1	"	"	"	5cc Subc.	"	"	"	"	"	No	"
6	♂	7.8	"	"	"	"	"	"	"	"	"	No	"
7	♂	20.4	Weanling pigs	"	"	-	"	"	"	"	"	No	"
8	♀	27.6	"	"	"	-	"	"	"	"	"	No	"
C. 861		102.8	Control									Severe	Died *
C. 862		78.6	"									Severe	Died *

* Bled to death

Table 18
Figures Showing Mass Vaccination with Lapinized
Vaccine in Pingtung
 (P.H.V.D.C.)

Period	Prevac- cinal health survey (head)	No. of hogs not vaccinated (head)	No. of hogs vaccinated * (head)				Amount of biologics used (c.c.)				
			Breed- ing hogs	Feeder hogs	By vac- cine	Small pigs With anti- serum simult.	Total	Lapinized Vaccine	Erysipelas 'Vaccine'	Anti-hog cholera serum	
February '54	46,175	16,959	3,075	16,046	4,757	5,338	10,095	29,216	56,904 cc	78,599 cc	27,241 cc
March	81,391	28,450	3,725	29,166	15,145	4,905	20,050	52,941	93,915	140,806	24,007
April	67,077	18,750	2,752	30,153	11,799	3,623	15,422	48,327	94,056	136,009	18,891
May	32,767	11,876	1,941	3,389	11,928	3,633	15,561	20,891	41,762	47,974	16,648
June	22,603	6,047	1,267	2,001	9,713	3,575	13,288	16,556	33,264	37,496	18,446
July	42,779	8,239	1,970	8,522	19,212	4,836	24,048	34,540	68,952	82,995	24,807
August	25,552	4,032	979	4,207	13,619	2,715	16,334	21,520	42,996	50,172	14,420
Total	318,344	94,353	15,709	93,484	86,173	28,625	114,798	223,991	431,849	574,042	144,460
	(100%)	(29.64%)						(70.36%)			

* With erysipelas vaccine also

Table 19.

**Figures Showing Post-vaccinal Reactions of Hogs in
Mass Vaccination of Pingtung**

(at P.H.V.D.C.)

Period	Post-vaccinal reaction (head)				Death after vaccination (head)					
	Breeding hogs	Feeder hogs	By Vac- cine	Small pigs With anti- serum simult. total	Total	Breeding hogs	Feeder hogs	By Vac- cine	Small pigs With anti- serum simult. total	Total
February '54	49	557	291	56	347	10	42	96	26	174
March	51	661	808	65	873	5	57	247	27	336
April	86	2,009	880	181	1,061	1	73	191	36	301
May	29	57	481	105	586	4	10	130	12	156
June	21	74	489	129	618	-	5	87	34	126
July	24	262	817	146	963	3	27	187	42	259
August	5	92	425	44	469	-	10	83	9	102
Total	265	3,712	4,191	726	4,917	23	224	1,021	186	1,454
% as of total hogs vaccinated	1.69	3.97	4.86	2.54	4.28	0.15	0.24	1.18	0.65	0.65

Table 20

**Differential Leucocyte Count of Pigs Vaccinated with
Lapinized Vaccine**
(at T.V.S.I.)

Pig No.	Kind	Degree of post vaccinal reaction	Days of blood examination after vaccination	Shift to the left	Differential Leucocyte Count (%)						
					Bas.	Eos.	Neutrophils			Lym.	Mon.
						MyL	Meta.	Stab.	Seg.		
No. 2281	Small pig	Severe	6 th	††	1.0	1.0	30.0	28.0	38.0	2.0	
"			11	-	1.0		6.0	34.0	52.0	7.0	
No. 2286	"	Severe	6	††	2.0		63.0	13.0	23.0	4.0	
"			10	-	1.0	3.0	4.0	57.0	32.0	3.0	
No. 370	Mature hog	Severe	4	††	0.5	0.5	0.5	64.5	16.5	15.0	2.5
"			5	††			8.0	54.0	5.0	28.0	5.0
"			11	-			3.0	33.0	53.0	10.0	
"			16	-	2.0		9.0	30.0	52.0	7.0	
No. 375	Mature hog	Moderate	4	+	1.0	6.0	25.0	20.0	46.0	2.0	
No. 372	"	"	4	††		0.5	53.5	9.5	29.0	7.5	
No. 2405	"	"	6	+	2.0	2.0	21.0	9.0	59.0	7.0	
"			9	-	2.0	4.0	9.5	32.5	47.0	5.0	
No. 2409	"	"	6	††			1.0	47.0	34.0	16.0	2.0
No. 217	"	"	4	††			1.0	43.0	16.0	39.0	1.0
No. 2932	Small pig	"	3	††			1.0	38.0	38.5	32.0	5.5
No. 210	"	"	3	††	2.0	3.0	1.0	27.0	9.0	58.0	0
No. 1725	"	"	6	-	2.0		2.0	49.0	41.0	6.0	
No. 2283	"	Slight	6	-		9.0	10.0	26.0	47.0	8.0	
No. 2287	"	"	6	-	1.0	6.0	6.0	18.0	62.0	7.0	
No. 2285	"	"	6	-	1.0	6.0	6.0	18.0	62.0	7.0	
No. 1451	"	"	8	-	2.0	1.0	4.0	24.0	56.0	13.0	
C.No. 2793	"	No	4	-	1.0	4.0	5.0	36.0	51.5	2.5	
C.No. 2791	"	No	4	-	3.0	4.0	2.0	38.0	50.0	3.0	

Table 21

**Autopsy Findings of 741 Pigs Died after Lapinized Vaccine
Injection in Pingtung**

(P.H.V.D.C.)

Diagnosis	Period			Total	Percentage	Culture findings
	February	March	April			
Hog cholera	89	184	169	442	59.65	Sal. suispestifer 20 cases E. coli 3 „
Swine erysipelas	-	2	4	6	0.81	Erysipelothrix rhusiopathiae 6 „
Swine plague	1	4	-	5	0.67	Past. suisepctica 5 „
Catarrhal pneumonia	9	28	14	51	6.88	
Croupous pneumonia	-	4	3	7	0.94	
Suppurative pneumonia	1	-	-	1	0.14	
Fibrinous pneumonia	1	-	-	1	0.14	
Haemorrhagic enteritis	55	61	23	139	18.76	
Necrotic enteritis	3	4	-	7	0.94	
Fibrinous peritonitis	-	2	-	2	0.27	
Suppurative peritonitis	1	1	-	2	0.27	
Suppurative hepatitis	-	1	1	2	0.27	
Malnutrition	5	13	4	22	2.97	
Misc.	9	21	24	54	7.29	
Total	174	325	242	741	100.00	

Table 22

**Distribution of Reaction Hogs According to Day Showing
Post-vaccinal Reaction in Pingtung**

(P.H.V.D.C.)

Period	Day after vaccination										Total
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
February	30	22	47	71	111	174	153	136	99	110	953
March	42	56	127	173	234	319	275	156	211	223	1,816
April	48	42	144	237	280	387	465	372	339	356	2,670
Total	120	120	318	481	625	880	893	664	649	689	5,439
%	2.21	2.21	5.85	8.84	11.49	16.18	16.42	12.21	11.93	12.66	100.0

Table 23

**Distribution of Deaths According to Day After
Vaccination in Pingtung**

(P.H.V.D.C.)

Period	Day after vaccination										Total
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
February	14	5	11	16	14	16	16	25	20	37	174
March	9	14	31	27	38	35	38	33	52	48	325
April	16	9	8	11	26	21	30	41	24	56	242
Total	39	28	50	54	78	72	84	99	96	141	741
%	5.26	3.78	6.75	7.29	10.53	9.72	11.34	13.36	12.95	19.02	100.0

Table 24

**Showing Pathogenicity of Lapinized Virus thro
Reverse Passages in Pigs**

(at T. V. S. I.)

Serial passage No. in pig	Date of inoculation	Time of bleeding in days after inoculation	Pig No. & Result
1.	Nov. 2 '53	5	2793 N.R. → 2791 N.R. 2790 N.R.
2.	Nov. 7 "	4	2788 N.R. → 2789 R.±
3.	Nov. 11 "	4	2798 R.± → 2799 R.±
4.	Nov. 15 "	4	2785 R.± → 2786 R.+
5.	Nov. 19 "	4	2795 R.+ → 2794 R.+
6.	Nov. 23 "	4 & 6	2868 R.± → 2796 R.## Urine 8c.c.
7.	Nov. 27 "		2792 R.## 2797 R.## 2870 R.## 2937 R.##
	Nov. 29 "	3	2933 N.R. → 2926 R.±
8.	Dec. 2 "	5	2927 N.R. → 2936 N.R.
9.	Dec. 7 "	4	2935 * → 2928 R.##
10.	Dec. 11 "	4	2929 R.## → 2925 R.##
11.	Dec. 15 "	4	2924 R.## → 2931 R.##
12.	Dec. 19 "		2949 R.## → 2948 R.##

Remarks: Figure in bracket denotes pig No.
 N.R. No Reaction after inoculation
 R.± Slight reaction after inoculation
 R.+ Moderate reaction after inoculation
 R.## Severe reaction after inoculation
 R.### Death after inoculation
 * Death due to bleeding from heart

Table 25

Showing Contact Infection Experiment of Lapinized Virus

(Chi-san, Kaohsiung)

A. Once Contact

Initial date of contact	No. of hogs involved	No. of hogs used (head)			Period of observation (day)	Result of non-treated hogs
		Vaccinated	Non-treated	Total		
June 3, 1954	65	108*	127*	235	124	Negative
June 17, 1954	4	8	13	21	110	"
July 12, 1954	11	18	25	43	85	"
Aug. 2, 1954	2	3	3	6	64	"
Aug. 16, 1954	8	14	15*	29	50	"
Sept. 1, 1954	7	19	21	40	35	"
Total	97	170	204	374		

* One of which died; causes: tetanus, peritonitis, and erysipelas ...

B. Twice Contact

Initial date of 1st contact	Initial date of 2nd contact	No. of hogs (head)	Interval of 1st observation (days)	Result	Interval of 2nd observation (days)	Result
June 3, 1954	Aug. 2, 1954	3	60	Negative	64	Negative
"	July 12, "	2	40	"	85	"
"	Aug. 16, "	1	75	"	50	"
June 17, 1954	July 12, "	3	26	"	85	"
Total		9				

Table 26

Immunity Test of Non-treated Pigs of Contact Experiment

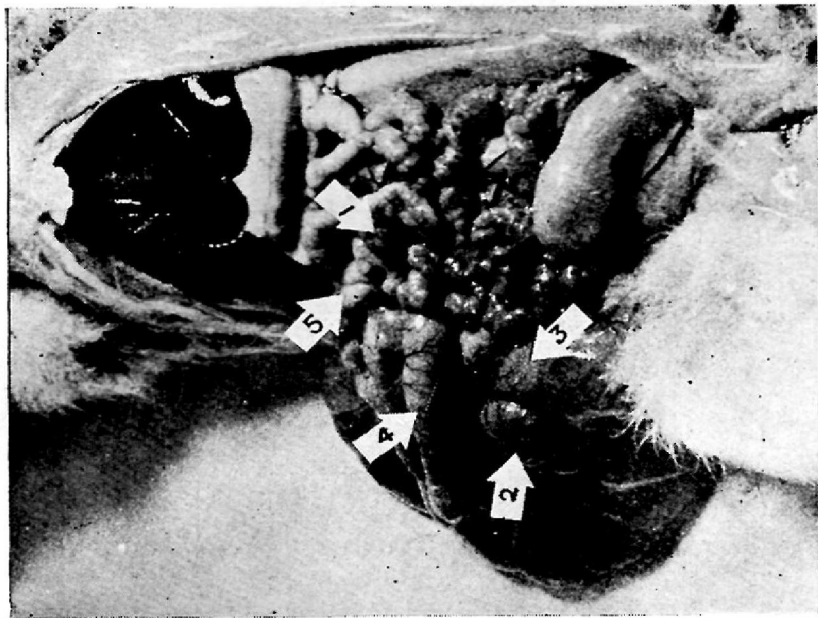
(K.H. FAs V.S.P.)

Pig No.	Body wt.	Condition of contact	Result after challenge				
			Date of challenge	Strain of virulent blood	Inoculum	Reaction	Termination
44	45.0 kg.	Once contact, 110 days interval	Sept. 22, 1954	ALD, 100	1c.c. Subc.	+	Survived
81	60.6 kg.	"	"	"	"	‡	Died (Sept. 30)
120	14.4 kg.	Once contact 96 days interval	"	"	"	‡	Died (Sept. 30)
371	8.4 kg.	Once contact, 21 days interval	"	"	"	+	Survived
32	19.0 kg.	Twice contact, 60 & 50 days intervals	"	"	"	‡	Died (Oct. 3)
268	42.6 kg.	Twice contact, 26 & 71 days intervals	"	"	"	‡	Died (Oct. 2)
C.423	90.6 kg.	Control	"	"	"	‡	Died



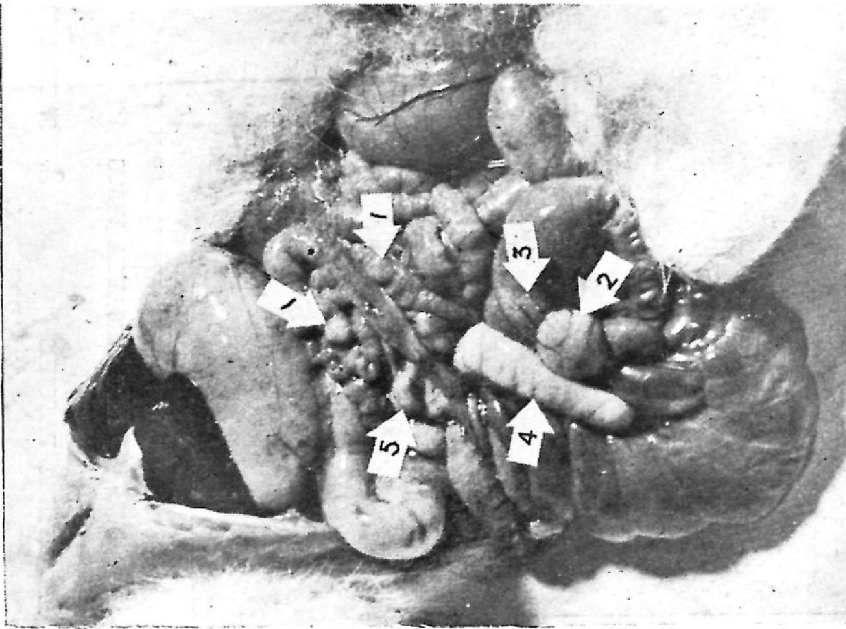
RABBIT INFECTED WITH LAPINIZED
HOG CHOLERA VIRUS

1. PEYER'S PATCHES 2. SACCULUS ROTUNDUS
4. APPENDIX



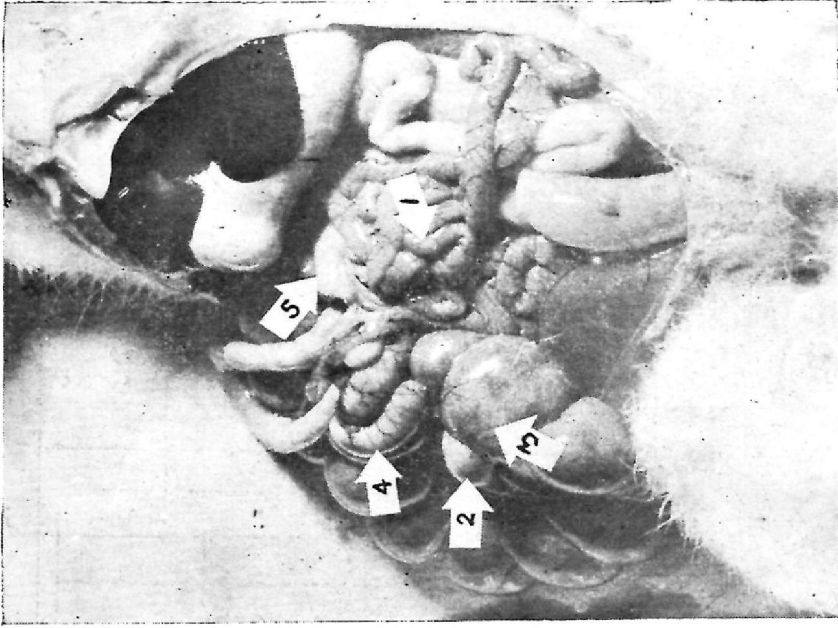
RABBIT INFECTED WITH LAPINIZED
HOG CHOLERA VIRUS

3. TONSILLA CECALIS MAJOR
5. MESENTERIC LYMPH-NODE



RABBIT INFECTED WITH LAPINIZED
RINDERPEST VIRUS

1. PEYER'S PATCHES
2. SACCULUS ROTUNDUS
4. APPENDIX
5. MESENTERIC LYMPH-NODE



NORMAL RABBIT

1. PEYER'S PATCHES
2. SACCULUS ROTUNDUS
3. TONSILLA CECALIS MAJOR
4. APPENDIX
5. MESENTERIC LYMPH-NODE

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