THE SWINE LUNGWORM AS A RESERVOIR AND INTERMEDIATE HOST FOR SWINE INFLUENZA VIRUS

I. THE PRESENCE OF SWINE INFLUENZA VIRUS IN HEALTHY AND SUSCEPTIBLE PIGS

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In this series of papers data will be presented which demonstrate the fact that the swine lungworm serves under natural conditions as a reservoir and intermediate host for the swine influenza virus. The virus can persist in a masked form within its worm host for long periods of time, and months or even years may elapse between its transmission from one swine to the next. The period during which the virus survives in the lungworm is more than adequate to account for its persistence between epizootics of swine influenza. In this first paper facts will be presented which show that healthy susceptible pigs sometimes contain the swine influenza virus and may undergo attacks of influenza if the virus is provoked to activity by multiple intramuscular injections of the bacterium Hemophilus influenzae suis (1).

Of the two agents which act in concert to cause swine influenza (1), it has been shown that the bacterial component, H. influenzae suis, is capable of eliciting an immune response that affords only partial protection against the disease (2). Swine influenza virus vaccines, on the other hand, confer a complete immunity to swine influenza (3). The observations now to be recorded were made as a result of further study of the use of H. influenzae suis vaccines in the prophylaxis of swine influenza.

Swine Influenza Precipitated by Inoculation with Hemophilus influenzae suis Vaccines

Preparation of H. influenzae suis Vaccines.—Cultures 18, 23, and 28 H. influenzae suis, originally obtained from naturally occurring field cases of swine influenza, were pooled for use in the experiments. The 48 hour growths from potato extract-chocolate agar slants were scraped off and suspended in a small amount of physiological saline. These suspensions were then centrifuged in graduated tubes for \(\frac{3}{4}\) hour at 1600 to 1800 R.P.M. The volume of bacterial sediment was noted, after which the sediment was resuspended in sufficient physiological saline to make a final 1 per cent by volume suspension. Part of the suspension was removed to use as living vaccine, while the
remainder was heated at 57°C. for 30 minutes in sealed tubes submerged in a water bath. All heated suspensions proved sterile when planted on media capable of supporting the growth of *H. influenzae suis*.

Two of the strains used, 18 and 23, had been under cultivation sufficiently long that, while still capable of producing influenza when given intranasally to swine in mixture with swine influenza virus, they no longer transferred with the virus from sick to normal animals by contact (4). Strain 28 on the other hand had been but recently isolated and, with the virus, transferred readily from swine to swine by pen contact.

**Source of Experimental Swine Employed.**—Ordinarily, swine reared on the Institute farm are employed in experimental work. However, at the time that the present experiments were being conducted the supply of swine of our own rearing was limited making it necessary to purchase outside animals for use. 27 of these were obtained from a breeder in whose swine drove swine influenza had never appeared, to his knowledge. In a preliminary experiment 2 of these animals were tested for susceptibility to swine influenza by intranasal inoculation with a mixture of swine influenza virus and the bacterium *H. influenzae suis* and found to be fully susceptible. The remainder were bled, and samples of their blood sera tested for the presence of neutralizing antibodies for swine influenza virus. In a serum dilution of 1:2, three serum samples were found capable of partially neutralizing swine influenza virus. These three sera contained sufficient antibody to protect mice against death but not against the production of lesions when the usual neutralization technique was employed (5). This type of finding was different from what might have been expected had the antibodies arisen as the result of previous swine influenza infection; for serum from swine recovered from an attack of swine influenza neutralizes the virus completely in quite high dilution. At the time, the finding of partially neutralizing antibody in the sera of 3 of the animals was tentatively relegated to the vague classification of natural antibody, and the 3 animals supplying the sera were used in experiments other than those under discussion. The remaining swine in the group purchased were considered, on the basis of absence of neutralizing antibody in their sera and the full susceptibility of representative members of the group, not to have had previous experience with swine influenza and to be satisfactory for use in swine influenza experiments. The animals were about 2 months of age when purchased and were kept under observation in semi-isolation for almost 2 months prior to their introduction into the present experiments. They were all found to be infected in varying degrees with ascaris and lungworms, parasites which from past experience were not considered to influence materially the course of a swine influenza infection.

**Attempted Vaccination of Swine with Heated and Living *H. influenzae suis***.—During December of 1936 each of 4 swine was given three intramuscular injections at 8 day intervals of heat-killed *H. influenzae suis*; a second group of 4 swine received injections similarly of living *H. influenzae suis*. The amount of the first dose administered was 1 cc., while the two succeeding doses were 2 cc. each. No noteworthy reaction was observed in any of the 8 swine following either their first or second injections of vaccine. However, after the third injection, in the cases of all 8 animals, a surprising and puzzling reaction
occurred. Since its character varied depending upon whether the animals had received living or heat-killed vaccine, the two groups will be discussed separately.

Reaction in Swine Vaccinated with Living \textit{H. influenzae suis}.—On the 2nd day after the third injection, the temperature of swine 1843 rose to 40.9°C, and the animal appeared ill. The following day the animal was prostrated and had labored breathing. By the next day it appeared extremely ill, and it was moribund on the following day. It died on the 4th day after its initial temperature rise and the findings at autopsy were strongly suggestive of fatal swine influenza. 2 other animals, swine 1840 and 1847, exhibited temperature elevations to 40.9° and 40.4°C., respectively, on the 3rd day after their third injections of living \textit{H. influenzae suis}. Swine 1840 was ill for 6 days with what clinically could not be distinguished from swine influenza. The illness of swine 1847 clinically resembled mild swine influenza and lasted for 4 days. The 4th animal in the group, swine 1844, developed a temperature of 41.2°C. on the 4th day after its third injection of \textit{H. influenzae suis} and exhibited for 5 days an illness that was clinically indistinguishable from swine influenza.

Reaction in Swine Vaccinated with Heat-Killed \textit{H. influenzae suis}.—All 4 of the swine injected with heat-killed \textit{H. influenzae suis} exhibited an extremely mild and indefinite illness for 2 or 3 days, beginning on the 2nd or 3rd day after their third injection. The clinical picture shown by these 4 animals was characteristic of that seen in “filtrate disease” (1) and would probably have entirely escaped notice had not the 4 swine receiving the living \textit{H. influenzae suis} vaccine been ill at the same time.

Experiments to Determine the Cause of the Disease Resulting from Multiple Injections of \textit{H. influenzae suis}.—Pieces of lung of swine 1843, the animal which had died on the 4th day, were tested for the presence of swine influenza virus by mouse inoculation (6). An agent typical in all respects of swine influenza virus was demonstrated. Blood serum was obtained from the remaining 7 swine following their recovery, and all seven samples neutralized swine influenza virus completely, although failing to exert any effect on the PR8 strain of human influenza virus. Furthermore, the 7 recovered swine were subsequently tested for immunity to swine influenza and found to be fully immune. It thus seemed clear that the reaction observed in all 8 of the experimental animals following their third injection of \textit{H. influenzae suis} had as its basis infection with the swine influenza virus. The disease observed in the animals injected with living \textit{H. influenzae suis} was true swine influenza in that both the virus and the bacterial component were active; while the disease developing in the animals inoculated with heat-killed \textit{H. influenzae suis} was “filtrate disease,” such as is caused by experimental infection with the swine influenza virus alone (1), and apparently precipitated in the present instance by the inoculation with heated \textit{H. influenzae suis}. No explanation of the source of the swine influenza virus responsible for these infections was apparent from consideration of the experiments just discussed.
Confirmation of the Findings

Late in January of 1937 4 more swine were placed in isolation and injected intramuscularly, as in the preceding experiments, with 1 per cent suspensions of heat-killed *H. influenzae suis*. On the 3rd day following the second injection this time, 2 of the 4 animals developed temperatures in the neighborhood of 41°C. and appeared mildly ill. The other 2 animals appeared mildly ill also, but their temperatures remained within normal limits. One of the febrile swine was killed on the 2nd day of fever and the other one on the 3rd day of fever, and at autopsy the findings in the respiratory tract were characteristic of a filtrate disease more extensive than usual. However, the lesions, instead of being limited to the anterior lobes as is usual in swine infected intranasally with virus, were diffusely scattered throughout the lung and were especially numerous at the bases of the diaphragmatic lobes. Swine influenza virus typical in all respects was demonstrated in both respiratory tracts by mouse inoculation. The 2 afebrile swine were kept under observation. They remained mildly ill for 2 days. They were bled 11 days later, and the serum of each neutralized swine influenza virus completely but was without effect on the PR8 strain of human influenza virus. It seemed clear that the reactions following the second injection in this group of experiments had been due to infection with the swine influenza virus. They thus confirmed the previous observations. The clinical picture exhibited by 2 of the animals was characteristic of filtrate disease; while in the remaining 2 which developed febrile reactions of 41°C. the clinical pictures were more severe than is ordinarily seen in swine infected with virus alone. The characteristics of the findings presented at autopsy were, however, typical of an extensive filtrate disease.

With this confirmation of the original observations it seemed that a regularly reproducible phenomenon was being dealt with. The situation, as it appeared from the data available at the time, could be summarized as follows: Apparently normal swine, given multiple intramuscular injections of suspensions of living *H. influenzae suis*, developed typical swine influenza in which both *H. influenzae suis* and swine influenza virus participated as infective agents. Similar swine given multiple intramuscular injections of heat-killed *H. influenzae suis* developed filtrate disease, in which the swine influenza virus was the sole infective agent. In neither set of experiments had swine influenza virus knowingly been introduced, and the origin of the virus infecting the swine was obscure.

Possible Sources of Virus

At the time, four possible sources of the virus were considered, either to be studied further or discarded as impossibilities. These may be briefly summarized as follows.

1. The virus might have been present as a contaminant of one of the cultures of *H. influenzae suis* used. This possibility could be eliminated on three grounds. First, direct test of the cultures by the intranasal inoculation of swine or mice failed to reveal virus; second, the heat-killed bacterial sus-
pensions had been heated well above the thermal death point of the virus; and lastly, had virus been present in the bacterial suspensions it should have immunized swine when given intramuscularly rather than induced infection (3).

2. The isolation technique might have been inadequate to prevent accidental infection. This possibility did not seem to furnish a reasonable explanation because at the time the experiments under discussion were conducted there were no cases of swine influenza in the laboratory. Furthermore, the isolation technique employed was the same as that used here for 8 years of more or less continuous investigation of swine influenza without an accidental cross-infection.

3. The swine used may have been carriers of swine influenza virus. This possibility was not considered very likely, because at the time no way of introducing swine influenza virus into swine was known that did not cause either infection or the acquisition of immunity. It had been established that virus given intranasally induced infection regularly, while administered by any other route it regularly immunized without causing recognizable infection. Since the swine used in the present experiments proved fully susceptible to infection and their sera were free of neutralizing antibodies, it had been concluded that they had not had a previous experience with swine influenza virus and thus could not be carriers of the virus. The possibility that virus might have gained access to the swine without either infecting or immunizing seemed remote.

4. The virus may have arisen de novo as a result of the experimental procedures to which the swine had been submitted. This possibility was included to be considered seriously only in case one of the three preceding was not found applicable.

Attempts to Extend the Observation and to Determine the Nature of the Phenomenon

Further experiments of the type described earlier were carried out in the hope of learning more of the phenomenon and determining the source of the swine influenza virus responsible for the infection that followed multiple injections of H. influenzae suis. At this phase of the investigation swine of our own rearing were again available and the supply of those purchased outside and used in the original experiments had been exhausted. Consequently in subsequent experiments our own swine were used. The first of these experiments failed completely to duplicate the original observation. So did the second and the third groups of experiments. Swine were given multiple intramuscular injections at 8 day intervals but remained perfectly normal throughout, neither acquiring swine influenza nor developing antibodies neutralizing swine influenza virus in their sera. As a result of this group of unsuccessful experiments the possibility was considered that the phenomenon
might be more closely related to the source of swine than had been considered likely in the beginning. Because of this, 8 more swine were purchased from the outside breeder who had furnished the original animals. These were of the same stock as purchased before but from later farrowings. After determining that their blood sera were free of swine influenza virus-neutralizing antibodies they were given multiple intramuscular injections of *H. influemae suis*. No illness resulted from a long continued course of injections at 8 day intervals, nor did the animals develop swine influenza virus-neutralizing antibodies. With these failures it seemed apparent that a new attack on the problem was indicated.

Consideration of the experimental factors which might have changed between the time of the earlier positive experiments and the current negative ones suggested *H. influemae suis* itself as probably the most labile. Because of the possibility that the cultures employed might have varied it was decided to obtain some fresh field strains for use. Seven strains were isolated in Iowa from naturally occurring cases of swine influenza. These seven cultures were pooled and administered intramuscularly to swine at 8 day intervals; but they, too, failed to induce a swine influenza virus infection in the experimental animals.

With the apparent exhaustion of the possibility that the source of swine or the cultures of *H. influenzae suis* themselves were responsible for the failure to duplicate the original experiments, other possibilities were considered. The original swine had been kept, prior to experimental use, in rather crowded quarters in a pen indoors, and it seemed that this fact might conceivably furnish a clue to the character of their peculiar reactivity to multiple injections of *H. influenzae suis*. Because of the crowding, cleaning of the pens had not been as scrupulous as it might have been under less crowded conditions, and it was reasoned from this that more than the usual opportunity had been afforded for the building up of heavy parasitic infections. It was furthermore reasoned, on the possibility that virus might have been made to arise *de novo*, that it would probably have been generated at the intramuscular site of injection of *H. influenzae suis*, under which circumstance it would have had to be transported in some way to the susceptible tissues of the respiratory tract. It seemed possible that the failure of the later experiments might have been due to a lack of this hypothetical transporting agent. Because the swine ascaris fitted the picture of a parasite whose larval stage migrated widely throughout the body before eventually becoming established in the gastrointestinal tract, experiments were planned in which wandering ascaris larvae would be present in the animals at the time of their second or third injections of *H. influenzae suis*. To this end, swine were fed embryonated swine ascaris ova (7) 2 or 3 days prior to their second or third injections of *H. influenzae*.
suis. Usually on the 8th day, occasionally somewhat later, after the ascaris feeding, the animals exhibited clinical signs of respiratory tract involvement. They became depressed, their respiratory rates were accelerated, and their temperatures were elevated to fever level. However, at autopsy the findings in the lung were only those characteristic of an ascaris pneumonia, and swine influenza virus could not be demonstrated in the respiratory tracts. Furthermore, swine that had been treated in this way and allowed to recover failed to develop swine influenza virus-neutralizing antibodies in their blood sera upon recovery. It thus seemed evident that the ascaris infestation had not furnished the requisite factor.

A number of other things were tried. Swine were kept in dirty pens, others were underfed, some were kept in cold isolation units, and others were kept in unusually warm isolation units; but under none of these conditions did multiple injections of *H. influenzae suis* exert the slightest effect so far as inducing a swine influenza virus infection was concerned.

In an accompanying paper experiments which explain the phenomenon will be reported.

**SUMMARY**

Multiple intramuscular injections of *H. influenzae suis* were found to precipitate swine influenza virus infections in a group of apparently normal swine. The most likely explanation of the phenomenon seemed to be that the animals, though healthy and susceptible, harbored the virus in some unknown manner. The factors possibly determining the phenomenon were explored experimentally but without success.

**BIBLIOGRAPHY**


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