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

V. Provocation of Swine Influenza by Exposure of Prepared Swine to Adverse Weather

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In a series of earlier papers (1–4), data were presented which demonstrated the fact that the swine lungworm serves under natural conditions as a reservoir and intermediate host for the swine influenza virus. The virus is present in a masked form within its worm host and must be provoked to infectivity before it can cause manifest illness in the parasitized swine. Multiple intramuscular injections of parasitized swine with the bacterium, *Hemophilus influenzae suis*, have proven the most regularly effective provocative stimulus in the laboratory. There is presumptive evidence that in nature the provocative stimulus responsible for precipitating attacks of swine influenza is meteorological in character and is in some way associated with sudden changes in weather and especially with the onset of cold, wet, inclement weather. The present paper describes experiments in which influenza was apparently precipitated by the exposure of swine, known to be carriers of masked swine influenza virus, to adverse weather conditions.

EXPERIMENTAL

The earthworms used in the present experiments were housed, as described in an earlier paper (3), in sunken, earth-filled, 50 gallon, aluminum-painted, steel alcohol drums. They had been, 2 to 12 months earlier, fed embryonated lungworm ova from pigs infected with swine influenza virus, and examination of preparations of individual earthworms revealed the presence of numerous third stage lungworm larvae in the hearts and calciferous glands. The earthworms were ordinarily removed from the storage drums just prior to use and were washed thoroughly free of adherent soil before being fed to swine.

The initial portions of the experiments to be reported were conducted essentially as outlined in an earlier paper (2). The earthworms, containing infected third stage lungworm larvae, were minced coarsely with scissors, mixed with dry grain mash, and fed to swine kept in isolation pens. Ordinarily 2 swine were fed at a time in each pen, competition for food thus assuring that the mixture was promptly devoured. Sometimes the earthworms were administered in one feeding, but the more usual practice was to give half of them on 1 day and repeat the feeding on the following day. From 17 to as many as 24 lungworm-infested earthworms were fed to each swine in these experiments and no effort was made to determine accurately the actual numbers of lungworm larvae such doses represented. In no case,

however, was the feeding followed by signs of illness that could be referred to the lungworm infestation itself. The animals were kept under observation in isolation for a variable period of time, but ordinarily long enough for the infesting lungworms to become adults in the swine respiratory tract—about 30 days. After this the animals were considered ready for exposure to the effect of "weather."

For purposes of exposure, two adjoining pens on the roof of our laboratory were utilized. These were protected from the west but were open on the other three sides and had no roof. The floor was of tile and had a drain so that sterilization after use could be effected with scalding water. Animals to be exposed were maintained in these open air pens for from 4 to 24 hours, depending upon the outdoor temperature, and the character of the weather. In no case were animals maintained in the open for more than the minimum time mentioned when the temperature was 28°F. or lower. Most of the exposures were on days or nights that the weather could best be described as "raw" and when there was precipitation either in the form of snow or rain. Ordinarily one pen was used to house swine prepared with lungworms containing masked influenza virus, while in the other pen the control swine, free of lungworms, were kept. A solid metal panel about 3 feet high separated the pens from one another.

A total of 25 swine, infested with lungworms presumed to be carriers of masked swine influenza virus, were exposed on one or more occasions to adverse weather conditions. A total of 8 lungworm-free control swine were similarly exposed in the open to foul weather. The results obtained will be described.

RESULTS

Ten of the 25 "prepared" swine developed either serological or manifest evidence of infection with swine influenza virus as a result of exposure to adverse weather. The remaining 15 prepared swine and the 8 lungworm-free control swine failed to come down with apparent swine influenza and furthermore their sera remained free of swine influenza virus-neutralizing antibodies. Our ability to characterize "weather" was not expert enough to detect differences between that which provoked infections and that which failed to do so. Both positive and negative results were obtained on snowy nights, on windy nights, and on nights when the temperatures were roughly comparable.

Of the 10 prepared swine in which masked swine influenza virus was provoked to infectivity, 6 developed only serological evidence of infection while the remaining 4 became obviously ill. The 6 which failed to show apparent illness, but in whose sera neutralizing antibodies for swine influenza virus appeared, had each been exposed to foul weather on either two or three occasions. Since none of these animals had become sick it was not until some days after their final exposure, when their blood sera were tested and found to contain swine influenza virus-neutralizing antibodies, that it was realized that they had, at sometime during the course of the experiments, nndergone unrecognized infections with swine influenza virus. The exact weather experience responsible for provoking masked virus to infectivity in these animals thus could not be identified. The exposures of the serologically positive swine had been conducted during February and March, a period during which 4 of the 8 lungworm-free control swine had also been repeatedly exposed with negative results.

The findings with the swine which developed obvious signs of illness as a result of exposure to "weather" will be outlined in more detail by describing the two experiments in which the positive results occurred.

Experiment 1.—On February 23, 1953, 2 swine, 30-42 and 30-50, were fed a total of 48 earthworms infested with third stage lungworm larvae containing masked swine influenza virus. On the night of the 7th and 8th of March, which was windy and rainy, and during which the temperature ranged around 35°F., they were placed outdoors. They were returned to their isolation room the morning of March 8th. Both animals appeared normal until the afternoon of March 10th, when the temperature of swine 30-42 rose to 40.5°C. The animal appeared depressed and was coughing. The following day its temperature dropped to 39.8°C. for both the morning and afternoon readings, but the animal by now showed typical signs of influenza—prostration, anorexia, and an increase in its respiratory rate. On the following day it was still ill and its temperature in the morning was 40.9°C. It was sacrificed at this time and on autopsy pneumonia involving about two-thirds of both the right apical and the right cardiac lobes and the anterior portion of the right diaphragmatic lobe, in a lobular fashion, was found. This was characteristic of the type of pneumonia ordinarily resulting from infection with the swine influenza virus alone ("filtrate disease") (5), though somewhat more extensive than usual. Immature lungworms were found throughout the respiratory tract. The respiratory tract, below the upper trachea, was bacteriologically sterile. Swine influenza virus, typical in all pathogenic and serological respects, was demonstrated in the pneumonic lung by the inoculation of white mice.

Swine 30-50 remained normal until the afternoon of March 11, the day after 30-42 had shown first evidence of illness, and at this time it appeared depressed and its temperature had risen to 39.8°C. The following day its temperature was 41°C. in the morning and 41.6°C. in the afternoon, and the animal exhibited signs characteristic of swine influenza. It was coughing, prostrate, breathing rapidly, and showed no interest in food. It remained ill for 3 more days and then underwent a rapid and complete recovery. Serum obtained from this animal on its first day of illness failed to neutralize swine influenza virus, while that gotten 12 days later did neutralize the virus completely and in good titre, as tested in white mice.

It seems clear from the findings described that in both of these swine masked swine influenza virus was provoked to infectivity as a result of their exposure overnight to wet inclement weather. Swine 30-42 showed beginning illness on the 3rd day after exposure, while swine 30-50 came down a day later.

Experiment 2.—On September 24 and 25, 1953, swine 30-81 and 30-82 were fed a total of 34 earthworms infested with third stage lungworm larvae containing masked swine influenza virus. On November 6 there was an early fall of wet snow, some of which persisted until the following day. Unfortunately, swine 30-81 and 30-82 were not exposed during the storm. However, on November 7, after the snowfall had stopped, but while wet snow remained on the floors of the exposure pens, they were placed in the pens and were kept there for the afternoon and overnight—a period of 18 hours. During this time the temperature ranged between 35 and 40°F. and the weather was clear but windy. The pigs were in snow during the early part of their exposure, but this thawed before they were returned to their isolation room indoors on the morning of November 8. On November 11, the 4th day after exposure, swine 30-81 appeared depressed and ill, and had a temperature of 41.1°C. (Fig. 1). The following day the animal was still febrile, showed little enthusiasm for food, was depressed, exhibited an increased respiratory rate, and was coughing—all signs characteristic of swine influenza. The animal was killed on the 3rd day of illness, when it seemed

to be showing some signs of improvement. Autopsy revealed the presence of a scant patchy lobular pneumonia, suggestive of "filtrate disease," involving portions of all of the anterior lobes of the lung. In addition, there were rather extensive wedge-shaped areas of pneumonia at the bases of both diaphragmatic lobes in areas of lung surrounding bronchi containing large numbers of adult lungworms. The respiratory tract, below the upper trachea, was bacteriologically sterile. Swine influenza virus, typical in all pathogenic and serological respects, was present in the pneumonic anterior lobes of the lungs, as demonstrated by the inoculation of white mice.

The other animal, swine 30-82, remained apparently normal until November 13, the 6th day after exposure, and 2 days after swine 30-81 had taken ill. At this time it appeared depressed, its temperature had risen to 40.8°C., and its respiratory rate was accelerated. The animal remained febrile and ill and exhibited signs characteristic of swine influenza

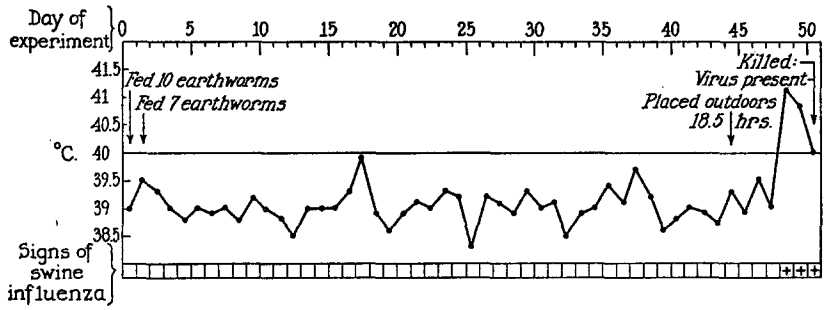

FIG. 1. Swine 30-81 was fed 17 earthworms containing third stage lungworm larvae carrying masked swine influenza virus. 43 days after this feeding it was exposed outdoors to inclement weather for 18 hours. 4 days later the animal came down with an illness with symptoms typical of swine influenza and swine influenza virus was demonstrated at autopsy in its respiratory tract.

for 3 more days and then underwent a rapid and complete recovery. Serum drawn from this animal on the 1st day of its illness failed to neutralize swine influenza virus, while that drawn 10 days later neutralized the virus in good titre, as tested in white mice.

It seems clear from the findings described that the 2 pigs in this experiment underwent swine influenza virus infections following exposure to adverse weather conditions. It is believed that these infections resulted from the provocation of masked influenza virus in lungworms in the respiratory tracts of the animals and that this provocation had been in some manner "triggered" by the adverse "weather" to which the parasitized swine had been exposed.

The severity of the illnesses shown by the 4 swine involved in the two experiments just outlined require some comment in view of the fact that only swine influenza virus was involved and concomitant infection with *H. influenza suis* was not demonstrated. One might have expected that the illnesses should have been the relatively mild "filtrate disease" (5) that one sees in animals infected intranasally with swine influenza virus alone. An explanation for the difference lies in the greater pulmonary involvement observed in swine in

which virus is provoked than in those infected by virus administered intranasally. As has been indicated in previous papers (2, 6), swine in which virus has been provoked exhibit a more extensive pneumonia and one with a considerably different distribution than do those infected with virus by the nasal route. In animals infected intranasally with virus alone, scant scattered lobular areas of pneumonia are ordinarily seen only in one or two of the anterior lobes of the lung and the obvious signs of illness are extremely mild (5). In animals in which virus is provoked on the other hand, pneumonia in the basal lobes of the lung in areas surrounding lungworm-filled bronchi, is the rule (2, 6), and, in addition, there are scattered lobular areas of pneumonia in the anterior lobes. This increased amount of pneumonia probably contributes to enhancing the manifest severity of the illness even though, as in "filtrate disease," infection with only the swine influenza virus alone is involved.

DISCUSSION

The experiments outlined indicate that certain adverse weather conditions result in the provocation of masked swine influenza virus to infectivity. It is not apparent from the findings obtained why, out of 25 "prepared" swine exposed to adverse weather, only 10 of them responded to provocation while the remainder failed to react. Neither is it clear why 4 of these 10 swine developed apparent illness while the remaining 6 underwent only unrecognized infections. This incidence of unrecognized infections following provocation by weather is considerably greater than was obtained in earlier work in which masked virus was provoked to infectivity by multiple intramuscular injections of suspensions of *H. influenzae suis* (2, 3). However, the numbers of animals involved are probably too small to lend significance to the difference.

Ordinarily swine influenza epizootics are of annual occurrence on the farms in our Midwestern swine-raising states. It is characteristic of them to begin explosively, either late in October or early in November. The onset of an epizootic is ordinarily preceded by meteorological changes which farmers in the Midwest have come to refer to as "hog flu weather"—the first cold blustery weather of the late autumn usually accompanied by rain or snow. At that time of the year there are many susceptible swine on the farms, born since the previous year's influenza epizootic. These animals have become heavily infested with lungworms containing masked influenza virus through devouring earthworms rooted out of pastures during the late summer and early autumn. They are identical to our "prepared" swine in the laboratory as has been shown in an earlier paper (4) and their masked virus is ready to be "triggered" to infectivity by the first provocative stimulus that comes along. Circumstantial epizootiological evidence has long incriminated cold wet inclement weather, the so called "hot flu weather," as the responsible provocative stimulus.

The findings reported in the present paper furnish experimental support for

this field evidence that adverse weather conditions can be responsible for provoking masked swine influenza virus to infectivity. Such a provocative stimulus, prevailing as it does over wide geographical areas, adequately accounts for the sudden appearance of swine influenza throughout large parts of the Middlewest almost simultaneously each year.

The sudden onset of the disease and its prompt involvement of many swine herds in large geographical areas during the course of 2 or 3 days have given swine influenza a reputation for almost miraculously rapid spread. It would appear, in the light of the experimental evidence presented, that this seemingly rapid spread of the disease does not represent spread at all in the usual epidemiological sense of that expression. Rather, it would seem that masked swine influenza virus, which had been widely preseeded prior to the beginning of an epizootic, is merely provoked almost simultaneously by a stimulus common to a wide geographical area. This mass provocation of virus with resultant outbreaks of disease appearing at almost the same time in many swine droves in an area would give an illusion of rapid spread of the infection from place to place. It is felt that the findings recorded in the present paper furnish verification, under experimental conditions, of a situation that has long been presumed on epizootiological grounds: namely, that adverse weather is the provocative stimulus responsible for starting swine influenza outbreaks each autumn.

SUMMARY

Twenty-five swine, infested with lungworms infected with masked swine influenza virus, were exposed to adverse weather conditions on one or more occasions. Of these, 4 came down with apparent swine influenza, while 6 others developed serological evidence of infection with swine influenza virus. The remaining 15 prepared swine, as well as 8 lungworm-free control swine, failed to show evidence of swine influenza virus infection, despite repeated exposures to adverse weather.

The data presented indicate that, in the 10 swine in which swine influenza virus infections were elicited, some feature of the weather to which the animals were exposed was responsible for provoking masked influenza virus to infectivity. The exact constituent of the meteorological complex comprising "weather," responsible for the provocation, cannot be determined from the data obtained.

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