THE ANTIBODY RESPONSE TO SWINE INFLUENZA*

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(Received for publication, November 4, 1938)

It is well known that antibodies capable of neutralizing swine influenza virus are present in the sera of swine recovered from swine influenza (1–4). However, neither the exact time following infection that these antibodies first appear nor the time at which they reach their highest titer has been determined. The present experiments were conducted in order to obtain this information.

Methods

Five swine were inoculated intranasally with a mixture of strain 15 swine influenza virus (1) and *Hemophilus influenzae suis* (5). Four of these animals developed typical swine influenza, while the 5th had an extremely mild illness like the "filtrate disease" seen in swine infected with virus alone (1). A 6th pig was inoculated intranasally with swine influenza virus alone and developed filtrate disease. These 6 swine were bled just prior to infection and then repeatedly during illness and after recovery, and the sera thus obtained were titrated for antibodies which neutralize swine influenza virus.

Titration of Antibodies for Swine Influenza Virus.—The neutralization tests were conducted in the usual way in white mice (3), employing the supernatant of a 2 per cent suspension of glycerinated infected mouse lung as virus and mixing this in equal parts with the serum dilution to be tested. Strain 15 swine influenza virus was used in all tests. The serum dilutions were prepared in physiologic salt solution, using 0.2 cc. of serum in varying amounts of the diluent. A further twofold dilution occurred, when the serum was added to the virus suspension. Three etherized mice were inoculated, in testing each serum dilution, by dipping their noses in the virus-serum mixture contained in a slightly tilted small Petri dish. Each neutralization experiment was allowed to run for 10 days. Mice which succumbed during the 10 day observation period and showed typical pul-

*This paper is part of a thesis submitted by Dr. Carlos T. Rosenbusch to the Faculty of Iowa State College in partial fulfillment of the degree of Doctor of Philosophy.

monary pathology of influenza at autopsy were considered to have received a non-neutralizing dilution of serum. Those which survived the 10 day period were considered to have received a neutralizing dilution of serum. The final titer of a given serum was taken as the highest dilution which protected all or the majority of the mice against death.

RESULTS

Swine 1993 (Fig. 1) developed typical swine influenza of 7 days' duration, and neutralizing antibodies were first detectible in its serum on the 6th day after infection. They rose to a titer of 1:20 on the 7th day and remained at this level until the animal was killed on the 11th day.

Swine 1974 (Fig. 2) was ill of swine influenza for 7 days following inoculation, and neutralizing antibodies first appeared in the serum on the last day of illness. By the 10th day the antibody titer had reached 1:20, and a titer of 1:60 was attained on the 14th day. Two days later it had decreased to 1:40, and this level was maintained until the termination of the experiment on the 84th day.

Swine 1975 (Fig. 3), inoculated in the same manner with a mixture of swine influenza virus and *H. influenzae suis*, did not show the usual clinical manifestations of swine influenza. Instead it underwent only a brief mild illness characterized clinically by malaise and inappetence of 2 days' duration. The clinical picture was indistinguishable from filtrate disease seen in swine infected with swine influenza virus alone. Infections of this type are of extremely rare occurrence in fully susceptible swine. The exact time at which neutralizing antibodies first appeared in the serum of this pig is unknown, because bleedings were unfortunately omitted on the 8th and 9th days after inoculation. No antibody was detectible on the 7th day, while by the 10th day the titer had reached 1:40. By the 14th day the titer had risen to 1:60, and this level was maintained through the 21st day. On the 27th day the antibody titer was found to have risen to 1:120, and this level was maintained through the 46th day. It had decreased to 1:80 on the 62nd day and was still 1:80 on the 84th day when the experiment was terminated.

Swine 1984 (Fig. 4) was ill of swine influenza for 6 days, and neutralizing antibody first became detectible in its serum on the 7th day. By the 10th day the titer had risen to 1:20 and on the 14th day it reached 1:40. By the 20th day the antibody titer was 1:120. At this time the animal was tested for active immunity to swine influenza by intranasal inoculation with a mixture of swine influenza virus and *H. influenzae suis*. It proved solidly immune. Serum was obtained 6, 12, 24, 50, and 72 hours following the immunity test and titrated for neutralizing antibody, but no significant change in the antibody titer was observed. The titer was still 1:120 when the experiment was terminated on the 31st day.

Swine 1985 (Fig. 5) had a characteristic swine influenza of 7 days' duration, and neutralizing antibody first appeared in the serum on the last day of illness. The titer reached 1:20 on the following day and by the 10th day had risen to 1:80.

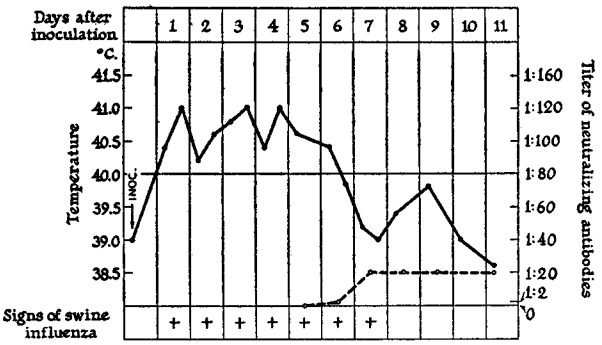

FIG. 1. Swine 1993. Inoculated intranasally with swine influenza virus + *H. influenzae suis.*

Figs. 1 to 6. The full line represents temperature; the broken line, neutralizing antibody titer.

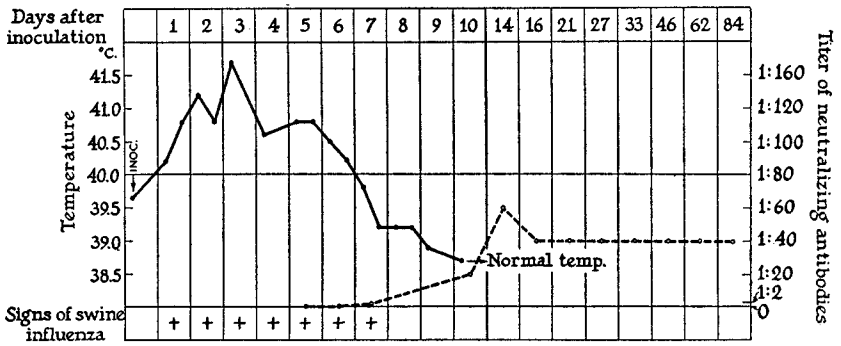

FIG. 2. Swine 1974. Inoculated intranasally with swine influenza virus + *H. influenzae suis.*

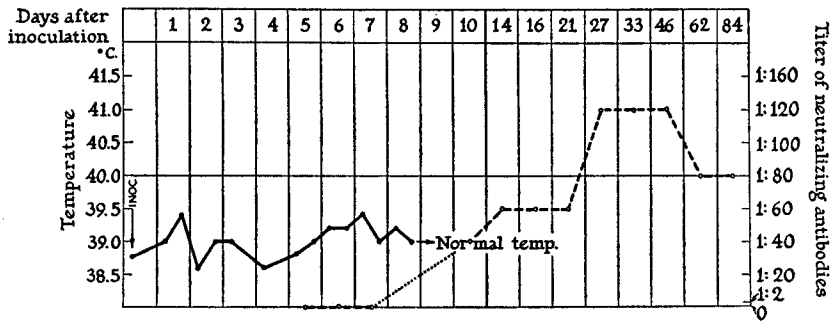

FIG. 3. Swine 1975. Inoculated intranasally with swine influenza virus + *H. influenzae suis.*

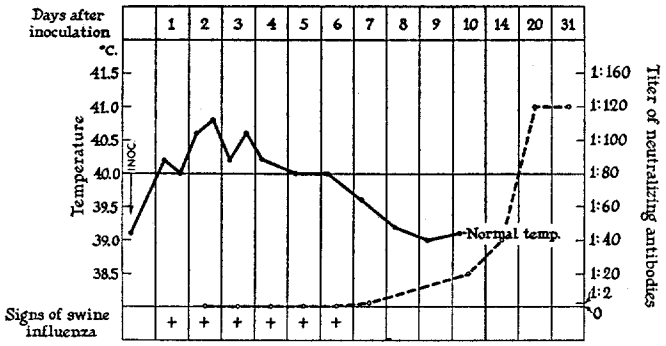

FIG. 4. Swine 1984. Inoculated intranasally with swine influenza virus + *H. influenzae suis.*

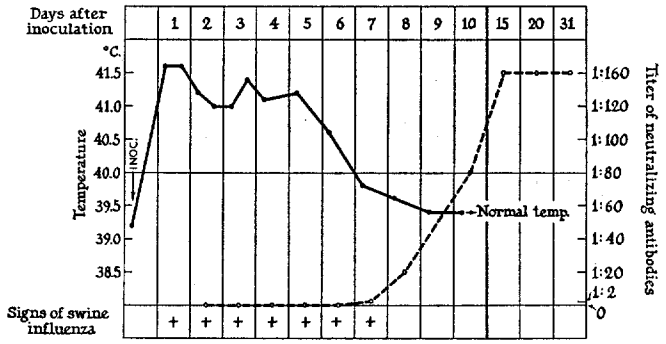

FIG. 5. Swine 1985. Inoculated intranasally with swine influenza virus + *H. influenzae suis.*

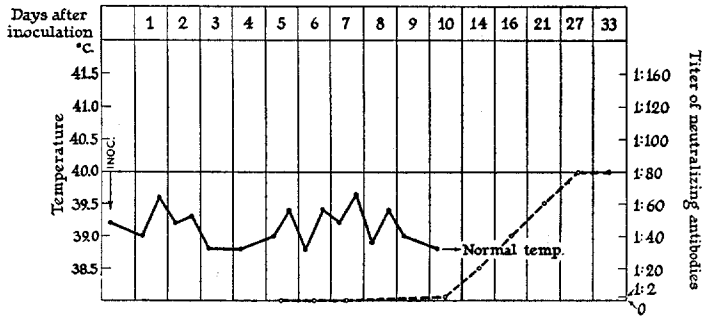

FIG. 6. Swine 2002. Inoculated intranasally with swine influenza virus alone.

On the 15th and 20th days the antibody titer was 1:160. The animal was tested for active immunity to swine influenza on the 20th day and found to be solidly immune. Serum drawn 6, 12, 24, 50, and 72 hours after the immunity test was titrated for antibody, but no significant change was found. The titer remained at 1:160 when the experiment was concluded on the 31st day.

Swine 2002 (Fig. 6) was infected with swine influenza virus alone and underwent an attack of the mild filtrate disease. There was no significant temperature elevation, and clinically the illness was characterized by malaise and inappetence of 2 days' duration. Neutralizing antibody first became detectible in the serum of this animal on the 10th day. The antibody titer rose gradually to 1:80 on the 27th day and persisted at this level on the 33rd day when the experiment was terminated.

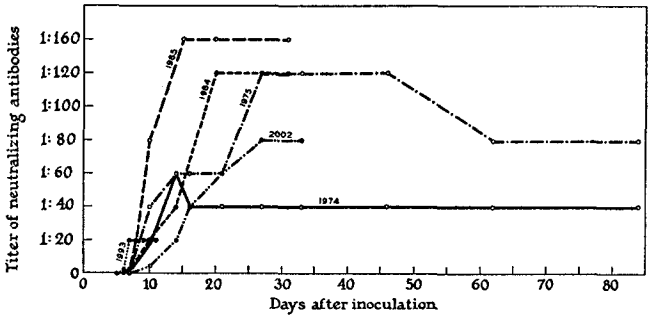

FIG. 7. Comparative antibody response of the 6 swine to swine influenza.

DISCUSSION

The results summarized in Fig. 7 illustrate the variable extent of the antibody response of individual swine to swine influenza. In the 4 animals that underwent typical attacks of the disease, antibodies were present by the 7th day after infection, while in the 2 that suffered only the mild filtrate disease, antibodies did not appear until sometime after the 7th day. Similarly the time required to reach the highest antibody titer appeared to be influenced by the clinical severity of the disease. Excluding swine 1993, observed for only 11 days, the animals with typical influenza reached their maximum titers on the 14th, 15th, and 20th days after infection. The 2 swine that underwent attacks of the mild filtrate disease, on the other hand, did not reach their maximum titers until the 27th day after infection. There was no apparent relationship between clinical severity of disease and the

maximum antibody titers eventually reached. These ranged from 1:60 to 1:160. In 2 animals kept under observation for 84 days there was some decrease in titer from the highest level attained.

The present findings concerning the antibody response in swine influenza are similar to those noted by investigators of human influenza. Smith and Stuart-Harris (6) observed that the antibody titer of a human case had risen considerably by the 8th day after onset of illness, reached a peak between the 16th and 31st days, and had declined slightly by the 44th day. Francis and his coworkers (7) noted, in another human case, that the antibody titer rose abruptly on the 7th day, reached a peak on the 14th day, and then gradually declined. Smorodintseff and his coworkers (8) reported 25 to 100-fold increases in the neutralizing antibody titers of the sera of their volunteers 10 to 15 days after experimental infection with human influenza virus.

In the 4 swine that underwent typical attacks of swine influenza, the appearance of neutralizing antibodies coincided rather closely with defervescence and clinical recovery, suggesting that the antibodies may have contributed materially to the cessation of signs of illness. It is known, furthermore, that swine influenza virus is, as a rule, no longer demonstrable in the swine respiratory tract 7 or more days after infection. The anatomical changes produced in the lung by the infection, however, persist for a variable period of time after recovery is clinically apparently complete. The possibility that the appearance of circulating virus-neutralizing antibody is the sole cause of clinical recovery is rendered unlikely by the findings in the cases of the 2 mildly ill animals, in which, though signs of clinical infection persisted for only 2 days, neutralizing antibody did not become detectible until after 7 days.

SUMMARY

Antibodies that neutralize swine influenza virus became detectible in the serum of swine on the 6th or 7th day after infection with swine influenza. Their appearance corresponded rather closely with clinical recovery. In swine with the milder filtrate disease, neutralizing antibodies did not appear until sometime between the 7th and 10th days. The maximum antibody titers ranged from 1:60 to 1:160 and were attained on from the 14th to the 27th days after infection.

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