SEROLOGICAL EVIDENCE FOR THE OCCURRENCE OF INFECTION WITH HUMAN INFLUENZA VIRUS IN SWINE

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Elkeles (1) and Shope and Francis (2) demonstrated that swine could be infected experimentally with human influenza virus (3). The disease resulting was extremely mild and was similar clinically and at autopsy to that observed in swine infected with swine influenza virus alone (4). When small amounts of a culture of *Hemophilus influenzae suis* (5) were administered with the human virus, a more prostrating febrile illness, similar to true swine influenza although never so severe, usually resulted. Furthermore, the disease induced in swine by the human influenza virus could be transmitted only rarely to normal swine by exposure (2), whereas swine influenza is highly contagious (6). Because of this, the opinion was expressed that it seemed unlikely that the current human influenza virus could become established in swine under field conditions and progress as the cause of any widespread swine disease (2). Within the past year, however, two swine herds that have been under study have furnished evidence to indicate that this opinion may have been at least partially wrong. It is the purpose of this paper to report the findings which indicate that, in these two herds, infection with human influenza virus actually occurred under field conditions as they prevail on eastern farms.

History of Swine Herds Studied

1. Bordentown.[1]—On May 24, 1937, two sick swine from the New Jersey State Prison Farm at Bordentown were brought to the laboratory for diagnosis. The

[1] We are indebted to Mr. J. S. Karlberg, Dr. Howard Wiesler, and Mr. John Grehan for their cooperation in the collection of material and information at the Bordentown farm.

autopsy findings were those of hog cholera. Blood serum from one of the animals was tested for the presence of neutralizing antibodies against the pseudorabies and the human and swine influenza viruses. It failed to neutralize swine influenza or pseudorabies virus but did neutralize human influenza virus. This finding was surprising and entirely unexpected. Sera of swine from a number of sources had in the past been tested against human influenza virus, and neutralizing antibodies had never before been encountered. It was known, however, from earlier work (2) that the sera of swine recovered from experimental infection with human influenza virus contained human virus-neutralizing antibodies. This suggested strongly that the Bordentown pig whose serum neutralized human influenza virus had undergone an earlier human influenza virus infection. A further study of the herd at Bordentown was therefore undertaken.

The farm was visited and the man in charge of the herd interviewed. Only 7 swine remained alive in the pen from which the 2 original sick animals had been taken. However, a nearby pen contained approximately 80 healthy swine of the same general age. The herdsman stated that he had observed no sick pigs in either group prior to the onset of the hog cholera outbreak in the one pen. Blood was obtained by tail bleeding from 6 of the 80 normal swine and from the 7 sick animals. Sera from these 13 pigs were tested for neutralizing antibodies against the swine and human influenza viruses. All 13 sera neutralized human influenza virus; none neutralized the swine influenza virus. These results constituted good evidence that the herd under study had undergone an earlier infection with human influenza virus and that few if any of the animals had escaped infection. The fact that sera from the normal as well as the sick swine contained neutralizing antibodies indicated that the hog cholera outbreak in May was in all probability totally unrelated to and in no way the result of the unrecognized human influenza virus infection.

All of the swine on the farm had been born prior to November, 1936, and had thus lived through a winter during which epidemic influenza was known to have been prevalent (7, 8). In order to obtain sera for control purposes from swine on the same farm, but from animals born long after influenza may have been present in the prison farm human population, further studies were postponed until October and November, 1937. By this time the fall pigs could be bled for control sera. Blood was obtained by tail bleeding from 8 of these young pigs born after July, 1937, and by throat bleeding at slaughter from 15 more of the old hogs born prior to November, 1936. These sera were all tested for neutralizing antibodies against the human and swine influenza viruses. The results obtained, together with those obtained with the sera drawn in May and June, will be outlined subsequently in Table I.

2. *Jamesburg.*[2]—The swine herd on the farm of the New Jersey State Home for Boys at Jamesburg contained, when first seen in November, something over

[2] We are indebted to Mr. William Mills for his cooperation in the collection of material and information at the Jamesburg farm.

100 hogs over 1 year of age, weighing from 200 to 300 pounds apiece, and about the same number of small pigs born during the autumn of 1937. All animals appeared in fine physical condition nor was there any history of past illness. Blood was obtained by tail bleeding from 7 of the small pigs and by throat bleeding at slaughter from 20 of the old hogs. The sera thus obtained were tested for neutralizing antibodies against the human and swine influenza viruses and the results will be outlined subsequently in Table II.

Recent Respiratory Tract Disease History of Human Populations from Which the Swine Herds Could Have Acquired Human Influenza Virus

1. Bordentown.—The garbage fed to the swine at Bordentown came from the dining rooms of the prison at Trenton and the prison farm at Bordentown, and the swine were tended by prison inmates. Dr. Howard Wiesler, resident physician, found on examining his records, that he had seen some 45 respiratory tract conditions among the Bordentown inmates during December, 1936, and January, 1937. Most of these cases were simple coryzas. Only 4 had been febrile and ill enough to go to bed. One of these 4 febrile cases was, at the time of his illness, assigned to work at the pig lots. He reported sick on January 19, 1937, and remained in bed for 3 days. At the Trenton prison during the same period, there were 3 cases that were clinically suggestive of influenza, and 2 pneumonias. The incidence of upper respiratory tract ailments and of influenza in the Trenton district as a whole was above average during the corresponding period.

2. Jamesburg.—The garbage fed the swine at Jamesburg came from the dining rooms of the New Jersey State Home for Boys and the swine were tended by inmates of the institution. Fortunately, so far as the present experiments are concerned, a definite history of influenza among the inmates of the institution is furnished by the studies of Stokes, McGuinness, Langner, and Shaw (8) conducted during the fall and winter of 1936–37. Among a population of 550 inmates they record the occurrence of 219 cases of upper respiratory tract disease. 55 of these cases were febrile, and 164, afebrile. Influenza virus, typical in all respects, was isolated, by ferret inoculation, from one of the febrile cases.[3]

EXPERIMENTAL

The neutralization tests were conducted in white mice, using a technique previously described (9). The human influenza viruses used were Francis' PR 8 strain (10) and the P-37-9 strain of Stokes and his coworkers (8). The swine influenza virus employed was strain

[3] We are indebted to Stokes and his coworkers for furnishing a mouse-adapted strain of this Jamesburg virus (strain P-37-9) for use in studies of the neutralizing antibodies in sera from the Jamesburg swine herd.

15 (Iowa, 1930). All three viruses had been well adapted to white mice and regularly killed these animals in the dosages used in the present experiments.

The supernatant of a 2 per cent suspension of infected mouse lung was employed as virus in the cases of the PR 8 strain human virus and the strain 15 swine virus, and of a 5 per cent suspension in the case of the P-37-9 strain. Virus was mixed in equal parts with the undiluted sera to be tested, and the mixtures stored for 2 hours in the refrigerator prior to their administration to the test mice. 3 etherized mice were inoculated in each test by dipping their noses in the virus-serum mixture contained in a slightly tilted small Petri dish. Surviving mice were killed on the 7th day and their lungs, together with those of mice dying earlier, were examined for the presence of influenza lesions (10–12). Mice which succumbed during the 7 day observation period and showed typical influenza pulmonary pathology at autopsy were considered to have received a non-neutralizing serum. Those which survived the 7 day period were considered to have received a neutralizing serum. The 7 day period of observation was chosen because experience has shown that, with the dosage of virus employed, no further deaths occur after that period. Within individual groups, where results were split and only one or two of the mice died, judgment of the neutralizing capacity of the serum under test was determined by the survival or death of the majority of the mice in the group. Usually, however, the results obtained were clear cut and all mice in the group either died or survived.

RESULTS

1. Bordentown Swine.—The results obtained with the sera from the Bordentown farm swine are outlined in Table I.

As shown in Table I, the sera of none of the swine born after July, 1937, neutralized the human influenza virus, whereas the sera of all 29 of those born prior to November, 1936, did neutralize. Roughly half the sera of these older animals not only prevented death but neutralized the virus so completely that lung lesions were not encountered in the surviving test mice when these were autopsied on the 7th day.

While there had been no history of a swine influenza infection in the Bordentown herd during the winter of 1936–37, the sera obtained were all tested for their capacity to neutralize swine influenza virus in order to eliminate from consideration the possibility that the human influenza virus-neutralizing antibodies might really represent cross-neutralizing antibodies resulting from an earlier swine influenza infection. As shown in Table I, the sera of none of the Bordentown

TABLE I

Neutralization Tests against the Viruses of Swine and Human Influenza with Sera from Swine on New Jersey State Prison Farm, Bordentown, New Jersey

Serum from swine No.	Serum tested for capacity to neutralize					
	PR 8 strain human influenza virus			Strain 15 swine influenza virus		
	Mouse No.			Mouse No.		
	I	II	III	I	II	III
(*a*) Swine born after July, 1937						
T- 1	D 4*	D 4	D 4	D 4	D 4	D 4
T- 2	D 4	D 5	D 5	D 4	D 4	D 4
T- 3	D 2	D 3	D 7	D 5	D 5	D 5
T- 4	D 2	D 2	D 4	D 3	D 4	D 4
T- 5	D 2	D 2	D 6	D 4	D 4	D 4
T- 6	D 2	D 5	D 5	D 3	D 3	D 3
T- 7	D 4	D 6	D 6	D 4	D 4	D 4
T- 8	D 2	D 4	D 6	D 3	D 3	D 3
(*b*) Swine born before November, 1936						
S- 1	S	S	S	D 4	D 4	D 4
S- 2	S	S	S	D 4	D 5	D 5
S- 3	S	S	S	D 6	D 6	D 7
S- 4	S	S	S	D 5	D 5	D 6
S- 5	D 6	S	S	D 3	D 4	D 4
S- 6	S	S	S	D 4	D 6	S
S- 7	S	S	S	D 4	D 5	D 6
S- 8	S	S	S	D 5	D 5	D 6
N- 1	S	S	S	D 5	D 5	D 5
N- 2	S	S	S	D 5	D 5	D 6
N- 3	S	S	S	D 2	D 4	D 7
N- 4	S	S	S	D 2	D 4	D 4
N- 5	S	S	S	D 5	D 5	S
N- 6	S	S	S	D 4	D 4	D 4
N- 7	S	S	S	D 5	D 6	D 6
N- 8	S	S	S	D 3	D 3	D 3
N- 9	S	S	S	D 4	D 5	D 7
N-10	S	S	S	D 4	D 5	D 6
N-11	S	S	S	D 4	D 5	D 6
N-12	S	S	S	D 4	D 4	D 4
N-13	S	S	S	D 6	D 6	D 7
N-14	S	S	S	D 5	D 6	D 6
N-15	S	S	S	D 4	D 5	D 6
N-16	S	S	S	D 5	D 6	D 6
N-17	S	S	S	D 7	D 7	S
N-18	S	S	S	D 6	D 6	D 7
N-19	S	S	S	D 6	D 7	S
N-20	S	S	S	D 4	D 4	D 4
N-21	S	S	S	D 3	D 4	D 5

* D 4 = died on 4th day.

S = survived. (Experiment terminated on 7th day.)

TABLE II

Neutralization Tests against the Viruses of Swine and Human Influenza with Sera from Swine on Farm of New Jersey State Home for Boys, Jamesburg, New Jersey

Serum from swine No.	Serum tested for capacity to neutralize								
	P-37-9 strain human influenza virus			PR 8 strain human influenza virus			Strain 15 swine influenza virus		
	Mouse No.			Mouse No.			Mouse No.		
	I	II	III	I	II	III	I	II	III
(*a*) Swine born after July, 1937									
YS 1	D 4*	D 5	D 6	D 3	D 4	D 4	D 3	D 3	
YS 2	D 4	D 5	D 5	D 4	D 4	D 5	D 3	D 4	D 4
YS 3	D 3	D 4	D 4	D 3	D 4	D 4	D 3	D 3	D 4
YS 4	D 4	D 5	D 6	D 6	D 6	D 7	D 3	D 3	D 4
YS 5	D 4	D 5	D 5	D 4	D 6	D 6	D 3	D 3	D 4
YS 6	D 4	D 4	D 4	D 3	D 4	D 4	D 3	D 3	D 4
YS 7	D 3	D 3	D 4	D 4	D 4	D 6	D 3	D 3	D 3
(*b*) Swine born before November, 1936									
OS 4	D 6	D 7	S	D 6	D 7	D 7	D 3	D 4	D 4
OS 6	D 6	D 6	D 6	D 5	D 6	D 6	D 3	D 3	D 4
OS 7	S	S	S	D 4	D 4	D 5	D 4	D 4	D 4
OS 9	D 5	D 5	D 5	D 5	D 5	D 5	D 3	D 3	D 3
OS 15	D 4	D 5	D 6	D 3	D 4	D 4	D 3	D 3	D 4
OS 1	S	S	S	S	S	S	D 3	D 3	D 3
OS 2	S	S	S	S	S	S	D 3	D 4	D 5
OS 3	S	S	S	S	S	S	D 3	D 4	D 4
OS 5	S	S	S	S	S	S	D 3	D 4	D 4
OS 8	S	S	S	S	S	S	D 4	D 4	D 4
OS 10	S	S	S	S	S	S	D 3	D 3	D 3
OS 11	S	S	S	S	S	S	D 3	D 4	D 5
OS 12	S	S	S	S	S	S	D 4	D 4	S
OS 13	S	S	S	S	S	S	D 4	D 4	D 5
OS 14	S	S	S	S	S	S	D 3	D 3	D 3
OS 16	S	S	S	S	S	S	D 3	D 4	D 5
OS 17	S	S	S	S	S	S	D 5	D 6	D 6
OS 18	S	S	S	S	S	S	D 2	D 5	S
OS 19	S	S	S	S	S	S	D 3	D 3	D 4
OS 20	S	S	S	S	S		D 3	D 4	D 5

* D 4 = died on 4th day.
S = survived. (Experiment terminated on 7th day.)

swine, either old or young, contained neutralizing antibodies for swine influenza virus. The swine had thus clearly not undergone a previous infection with swine influenza, and the neutralizing antibodies for human influenza virus encountered in the sera of the older animals had not resulted from such infection.

2. Jamesburg Swine.—The sera from the swine on the Jamesburg farm were tested for their capacity to neutralize, not only the PR 8 strain and strain 15 viruses, but the P-37-9 human strain as well, since this virus had originally been recovered from a case of influenza occurring in one of the institution inmates. It was conceivable that the P-37-9 virus might be more appropriate to use, under the circumstances, than the PR 8 strain. The results obtained are given in Table II.

As shown in Table II, the sera of none of the 7 young swine neutralized either strain of human influenza virus. However, the sera from 16 of the old hogs neutralized the P-37-9 strain human virus while the sera of 15 neutralized the PR 8 strain. One serum sample from an old hog (OS 7) neutralized the P-37-9 strain very effectively but failed to neutralize the PR 8 strain. Repeated tests of this serum against the two human viruses have confirmed the correctness of this result. While roughly half of the Bordentown swine sera had neutralized the human virus so thoroughly that lung lesions were completely suppressed in the test mice, none of the Jamesburg sera achieved such solid protection against the PR 8 strain. However, against the P-37-9 strain, 5 of the 16 neutralizing sera neutralized so completely that no lung lesions were encountered in the surviving test mice when these were autopsied at the end of the experiment.

None of the Jamesburg sera neutralized swine influenza virus, indicating that, as in the case of the Bordentown herd, the human influenza virus-neutralizing antibodies had not resulted from earlier infection of the herd with swine influenza.

DISCUSSION

The sera from two age groups of swine on two New Jersey institution farms have been studied for their capacity to neutralize the swine and human influenza viruses. The sera from none of the young swine, born since July, 1937, neutralized either virus. However, the sera

of all of the old hogs studied on one farm and of three-fourths of those studied on the other farm neutralized human influenza virus, although failing to neutralize swine influenza virus. These older animals had all been born prior to November, 1936, and had thus lived through a winter when human influenza was known to have been unusually prevalent. The presence of human influenza virus-neutralizing antibodies in the sera of the older animals was not an age phenomenon, because sera from swine of corresponding ages from other sources have failed to neutralize human influenza virus. It is believed, on the basis of the known behavior of swine to experimental infection, that the antibodies in the sera of the older animals resulted from actual infection with human influenza virus and that, in both herds studied, a widespread infection of human origin had occurred. The failure to recognize either outbreak is not surprising because, even under conditions of experimental infection of swine with large doses of human influenza virus alone, the resulting disease is so mild and ill defined as to be difficult of certain recognition (1, 2). How the virus was transferred to swine is unknown, though presumably it was either by direct exposure to human cases or through the medium of garbage contaminated by virus. In either event the initial infection must have been so extensive as to involve all of the swine on the Bordentown farm and three-fourths of those on the Jamesburg farm, unless human influenza virus infection of swine under farmyard conditions is more highly contagious than it could be shown to be in the laboratory (2).

One apparent discrepancy in the results obtained with the Jamesburg sera deserves comment. The serum from swine OS 7 neutralized the P-37-9 strain human influenza virus but failed to neutralize the PR 8 strain. Certain antigenic differences between various strains of human influenza virus have recently been observed (13, 14) and it seems possible that the discrepancy with the OS 7 serum may indicate that more than one strain of virus was prevalent in the human population at Jamesburg and that more than the one strain was transmitted to the swine. The same suggestion is afforded by the results with the Bordentown sera. Here roughly half of the sera neutralized the PR 8 virus completely, as evidenced by protection of the test mice not only

against death but against the development of any lung lesions as well. The other half of the sera protected against death but did permit the development, in the lungs of the test mice, of a varying amount of influenza virus pneumonia. While these differences may have been dependent solely upon quantitative differences in the amounts of antibody contained by the sera, they may equally well reflect differences in the antigenic structure of the viruses responsible for their generation. Thus one virus may have been of an antigenic type very similar to the PR 8 strain and have caused the production of antibodies that completely neutralized the PR 8 virus while the other virus may have been of a slightly different antigenic type and have produced antibodies only partially neutralizing the PR 8 strain. Whatever the antigenic compositions of the influenza viruses infecting the swine at Bordentown and Jamesburg may have been, they were definitely and quite completely different from that of ordinary swine influenza virus.

Previous to the experiments just described there has been no concrete evidence that influenza virus could be transmitted from man to swine under natural conditions. As long ago as 1918, however, there was, in the Middle West, the popular belief, first voiced by Dr. J. S. Koen, that swine could acquire influenza from man and that swine influenza had had its origin from man during the 1918 pandemic (15, 16). Numerous similarities between the viruses of swine and human influenza, together with the history that swine influenza appeared for the first time during the 1918 human pandemic, led Laidlaw to propound the theory that swine influenza virus represented a surviving form or prototype of the 1918 pandemic human virus (17); a theory to which we subscribed (18). The present experiments, by demonstrating that human influenza virus of the type prevalent during the winter of 1936–37 was transmitted to swine under natural conditions, furnish evidence that a similar transmission from man to swine might readily have occurred in 1918. The failure of recent strains of human influenza virus to cause widely disseminated porcine epizootics like those caused annually in the Middle West by swine influenza virus may be explained by the low contagiousness, when in swine, of the current human influenza viruses (2).

SUMMARY

Antibodies capable of neutralizing human influenza virus were present in the sera of old swine on two New Jersey institution farms, but absent from the sera of young swine on the same farms. The old animals had lived through the winter of 1936–37 in which outbreaks of upper respiratory tract disease were prevalent among the human inmates of the two institutions, while the young swine studied were born long after these outbreaks. It is believed that the swine whose sera neutralized human influenza virus had undergone an unrecognized human influenza virus infection acquired from man. The possible bearing of these observations upon the theory that swine influenza was originally of human origin is discussed.

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