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

IV. The Demonstration of Masked Swine Influenza Virus in Lungworm Larvae and Swine under Natural Conditions

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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 build-up in numbers of cases is extremely rapid, and one gains the impression that the disease has arisen at many different spots almost simultaneously. Because of the large numbers of cases at or near the onset of the epizootic, swine influenza has gained the reputation of being a highly communicable disease, the causative agent of which is capable of almost unbelievably rapid spread over large areas. After the first widespread outbreak of the disease fresh swine droves are infected in smaller and smaller numbers until by late December or early January the epizootic appears to have run its course and swine influenza largely disappears as a farm infection until the following October or November. The whereabouts of the swine influenza virus during 9 or 10 months of the year has been a baffling feature of the epidemiology of the disease.

The recent discovery that the swine lungworm can serve as intermediate host for the swine influenza virus (1, 2) has suggested the possibility that the interepizootic sojourn of the virus might be spent in this worm host. The demonstration that the virus is capable of surviving in the lungworm for almost 3 years (2), over twice the period elapsing from one epizootic of swine influenza to the next, falls in with this conception. The field experiments to be described in the present paper were conducted in order to learn the bearing of these facts upon the epidemiology of the disease.

Plan of Field Experiments

There appeared to be two practical approaches to the problem of determining whether or not the swine lungworm serves as reservoir host for swine influenza virus under field conditions. Both have been employed in the present experiments. Utilizing the first, an attempt was made to learn whether swine influenza virus is present in third-stage lungworm larvae in earthworms dug in or near the pig pens on farms with a history of swine influenza annually. Using the second approach, an attempt was made to find whether during interepizootic periods the pigs on such farms are carriers of masked influenza virus capable of inducing the disease on appropriate provocation. The two groups of experiments will be separately discussed.

EXPERIMENTAL

Swine that had been reared on earthworm-proof concrete platforms and that were demonstrably free of lungworms were used in the portion of this work dealing with the demonstration of masked influenza virus in lungworm larvae in earthworms. The method employed in infesting these swine with the lungworms has been described in the preceding paper (2). The procedures used to provoke influenza virus infections in animals infested with the field lungworms were also the same as already outlined (2).

Attempts to Demonstrate Masked Swine Influenza Virus in Pooled Lungworm Larvae in the Earthworms of Several Farms

As already stated, the farms from which earthworms were collected were chosen because of a history that influenza was of annual occurrence in their swine droves.[1] About 50 earthworms were dug in or about the hog lots on each farm and were taken to the laboratory in 1 lb. coffee tins, together with some of the dirt in which they had lived. Three separate collections of earthworms were made at different times of the year. In May of 1939 collections were made from 28 Iowa farms and again in October of the same year earthworms were obtained from 24 other farms. In April of 1940 cans of earthworms were dug on yet another 28 Iowa farms.

The calciferous glands and hearts of two earthworms from each farm were examined microscopically for the presence of lungworm larvae.[2] It was found that, of the earthworms dug in April of 1939, those from 21 of the 28 farms were infested with lungworm larvae. The infestation rate among the earthworms obtained in October of 1939 was considerably less, only 6 of the 24 farms yielding infested specimens. The infestation rate among the earthworms dug in April of 1940 was higher, 19 of the 28 farms having earthworms infested with third-stage lungworm larvae. A seasonal fluctuation in the degree to which earthworms are parasitized by larval lungworms is suggested by these findings. It is possible that among those samples in which examination of two earthworms failed to reveal the presence of lungworm larvae, careful study of larger numbers might have demonstrated their presence. However, since the experimental

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[2] I am indebted to Drs. H. P. Smith and William M. Hale for making space available to me in their laboratories in the State University of Iowa Medical School.

plan called for the use of pooled samples of worms, a determination of the approximate degree of lungworm infestation seemed sufficient.

In order to conserve the isolation facilities required in working with swine influenza, the earthworms from each of the three separate collections of farms were divided into two groups of those deriving from 12 or 14 farms depending upon their original number and designated for convenience as groups 1 and 2 of the particular collection.

For each experiment 3 earthworms per farm were used in preparing the pooled specimens of worms from the farms of the group. 2 swine were used in each experiment in testing the earthworms from each group for the presence of masked swine influenza virus. It was realized at the outset that this method of testing for the presence of virus involved a rather long shot unless the incidence of infected intermediate hosts was quite high. For in the first place, although the earthworms had been obtained in pig pastures on farms where influenza had occurred the preceding autumn and although the large majority of them were demonstrably infested with lungworm larvae, we had no assurance that these larvae had developed from ova passed during the time that the swine had been acutely ill, or freshly convalescent from influenza. The period of time during which the swine in a drove on any single farm remained infective, from the standpoint of possessing demonstrably active swine influenza virus in their respiratory tracts probably did not exceed 10 days at the outside, and hence the chances that the larvae demonstrated in the earthworms might have developed from ova laid on some of the remaining 355 days of the year seemed to be about 35 to 1. Secondly, in the cases of a number of the farms from which earthworms were obtained the enclosures in which the swine had been kept during the actual period of their illness were of such a character as not to supply the earthworms sought for. Some of these enclosures were paved with cement; others had been heavily layered with cinders; while a few had been packed so hard by the tramping of livestock as not to be favorable for earthworms. In such cases the direction of drainage from the affected yard was determined and the earthworms were dug in the nearest favorable area that had received this drainage. Again it seemed likely that the conditions had diminished the chances of finding infected lungworm larvae. Yet, although the fact was realized that testing small numbers of earthworms from each of a relatively large group of farms might entail the risk of entirely missing the detection of low incidence infections in the larvae on any single farm, the procedure adopted still seemed the most practical means of dealing with the material at hand. Little importance could be attached to negative experiments, but positive experiments,—even though few in number,—would be of prime significance.

A series of 14 experiments of the sort described were carried out with the various pooled samples of Iowa earthworms. The details and results obtained are shown in Table I.

The record of successful transmission experiments given in Table I is not notably impressive: in only 2 of the 14 experiments was the presence of masked swine influenza virus shown. However, these 2 positive experiments did appear to demonstrate that under conditions prevailing in the field swine lungworms are capable of carrying masked swine influenza virus. Because it seemed possible that the poor showing might have resulted from the use of too small numbers of earthworms from favorable individual farms or from the

TABLE I

Detection of Masked Swine Influenza Virus in Third-Stage Lungworm Larvae from Pooled Samples of Naturally Infested Earthworms

Experiment No.	Earthworms			Swine No.	No. of attempts to provoke infection	Results	Remarks
	Obtained	Group No.	Fed to swine				
1	May, 1939	1	May	2297	3	No infection	
				2360	3	" "	
2	" 1939	2	"	2310	3	" "	
				2358	3	" "	
3	" 1939	1	Sept.	2399	3	" "	
				2406	3	" "	
4	" 1939	2	"	2402	3	" "	
				2418	3	" "	
5	" 1939	1	Nov.	2445	7	" "	
				2425	10	Influenza	Swine influenza virus demonstrated in lung and lungworms by mouse inoculation
6	" 1939	2	"	2451	8	"	" "
				2422	8	"	Recovered; developed antibodies neutralizing swine influenza virus
7	" 1939	1, 2	Jan.	2427			Died on 17th day of noninfluenzal condition
				2483	8	No infection	
8	" 1939	1, 2	Feb.	2437	5	" "	
				2474	5	" "	
9	Oct., 1939	1	Oct.	2421	4	" "	
				2423	7	" "	
10	" 1939	2	"	2429	7	" "	
				2431	7	" "	
11	Apr., 1940	1	Apr.	2471	8	" "	
				2514	8	" "	
12	" 1940	2	"	2512	8	" "	
				2515	8	" "	
13	" 1940	1	Sept.	2607	7	" "	
				2619	7	" "	
14	" 1940	2	"	2550	7	" "	
				2614	7	" "	

inclusion of too many of those from farms upon which conditions for the collection of infected specimens had not been particularly favorable, it was felt that a more clear cut demonstration might be afforded by using worms from a single, well chosen farm.

Tests for the Presence of Masked Swine Influenza Virus in Third-Stage Lungworm Larvae in Earthworms from a Single Farm

The Frank Carson farm in Johnson County, Iowa, was selected.[3] The pig sheds on this place were of a permanent character, and the pasture in which the swine ran consisted of about 20 acres which sloped gently away to the south of the pig sheds, finally leveling off at the south end. A shallow ditch collecting all of the drainage from the area immediately surrounding the sheds ran roughly north to south through the pasture. Swine influenza was of annual occurrence in the swine drove on this farm. The topography seemed ideal for heavy lungworm infestation of earthworms almost anywhere in the pasture but especially along the ditch bisecting the pasture. Consequently an area in and along the ditch was chosen for the collection of the earthworms to be used in the experiments. The first batch was dug in April of 1940. Examination of representative specimens revealed unusually heavy infestations of the hearts and calciferous glands with third-stage lungworm larvae. The counts of larvae ranged from as low as 5 per earthworm to cases in which the larvae were too numerous and active for accurate counting. Other batches of earthworms were obtained from approximately the same site in the pig pasture in December of 1940 and October, 1941, and in these the infestations with lungworm larvae were much lighter: seldom was there more than one larva per earthworm and roughly half the earthworms were uninfested, so far as could be told by calciferous gland examination. All of the earthworms dug on the Carson farm appeared to be of one variety of a single species: *Allolobophora caliginosa* forma *trapezoides* (Dugès.)[4] The finding of much higher infestations of lungworm larvae in earthworms dug in the spring as compared with those dug in the autumn has been regularly observed in these field studies and hence the situation in this respect was not peculiar to the farm under discussion.

Two experiments have been conducted with each of the first 2 groups of earthworms, and one with the third group. The general experimental plan was the same as that for the tests with pooled samples of earthworms, except that each pig—instead of being fed 3 earthworms from each of a group of farms—was fed 20 to 50 earthworms, all from the Carson farm. The swine used had been reared on concrete platforms and were free of lungworms. The results obtained with the earthworms got on the Carson farm are shown in Table II.

The results of experiments with earthworms from the Carson farm, shown in Table II demonstrate far more clearly than those with pooled samples from groups of Iowa farms that third-stage lungworm larvae under field conditions are capable of harboring masked swine influenza virus. Transmission of the virus was shown with all 3 groups of worms, though one of the experiments (Experiment 15), carried out with worms dug in April, gave a negative result. In view of the later successful demonstration of masked virus in these April

[3] I am very grateful to Mr. Frank Carson for his friendly cooperation and for generously supplying me with swine and earthworms from his farm.

[4] I am indebted to Dr. Grace Pickford for the identification of representative specimens of these earthworms.

worms it seems likely that the difficulty may not have been one so much of transmission of masked virus as of its subsequent provocation to infectivity.

The finding of infected lungworm larvae in earthworms on a single farm at such widely separated times of the year as April, October, and December indicates that swine on such farms are subject to a year-round hazard of acquiring masked influenza virus,—a hazard modified however by seasonal conditions making earthworms accessible or inaccessible to swine at various times of the

TABLE II

Detection of Masked Swine Influenza Virus in Third-Stage Lungworm Larvae in Earthworms from a Single Iowa Farm

Experiment No.	Earthworms		Swine No.	No. of attempts to provoke infection	Result	Remarks
	Obtained	Fed to swine				
15	Apr., 1940	Nov.	2663	6	No infection	
			2672	6	" "	
16	" 1940	Mar.	2710	2	Influenza	Recovered; developed antibodies neutralizing swine influenza virus
			2717	2	"	Swine influenza virus demonstrated by mouse inoculation
17	Dec., 1940	"	2708	0*	"	" "
			2714	0*	"	Recovered; developed antibodies neutralizing swine influenza virus
18	" 1940	"	2722	2	"	" "
			2726	2	"	" "
19	Oct., 1941	Dec. and Jan.	2825	6	"	Swine influenza virus demonstrated by mouse inoculation

* Infection occurred "spontaneously" on 15th day.

year. During the hot dry summer months when earthworms, carrying their infected lungworm larvae, lie deep in the moist subsoil and during the cold winter months when they reside below the frost line they cannot be got by swine, whereas in the moist months of spring and autumn, they are at or near the surface of the soil and can be readily acquired by swine rooting in pasture land.

The Presence of Masked Swine Influenza Virus in Field Swine during an Interepizootic Period

Three approximately 3 months old swine were obtained from the Carson farm in Iowa on September 30, 1940, and sent to Princeton by express. They suffered no ill effects from the trip and remained normal for the first 7 weeks of a period during which they were in rigid isolation at the laboratory. They had been reared in the pig lots and pasture on the Carson farm mentioned earlier as the locale from which the earthworms used in Experiments 15 to 19 were obtained. Swine influenza had not occurred in the swine drove from which they originated since the late autumn of 1939, and blood serum obtained from each of the 3 animals on arrival was free of antibodies neutralizing swine influenza virus. A drove of approximately 50 whole and half brothers and sisters of these animals, which were still on the farm in Iowa, were clinically normal and remained so until almost the middle of November, when they came down with influenza. It is evident from this that the 3 animals were obtained from their home drove during an interepizootic period and that they exhibited no detectable evidence of being infected with influenza virus. They had been born about 7 months after the last clinically recognized case of influenza had occurred on the Carson farm, in the preceding autumn.

The swine were placed together in an isolation unit and kept under observation for a week after arrival. Then each was injected intramuscularly with a suspension of living *H. influenzae suis*. These injections were repeated 3 times at 8 day intervals but no influenza infection was elicited. At this time swine A was moved to another isolation unit alone and swine B and C remained together in the original one. From this time on, the procedures employed to provoke influenzal infection were varied.

After almost 3 weeks' further observation swine A received another intramuscular injection of living *H. influenzae suis* and then 3 days later was fed approximately 40,000 normal embryonated ascaris ova. 5 days later the animal was again injected intramuscularly with living *H. influenzae suis*. On the 2nd day after this injection the animal's temperature rose to 40.2°C. and it appeared mildly ill. The following day it was about the same, and since this reaction had occurred 7 days after the ingestion of embryonated ascaris ova it was felt that it could be accounted for on the basis of the ascaris infestation. However, the next day the animal appeared markedly ill; it was prostrate in its pen, refused food, its respiratory rate was greatly accelerated, and breathing was of the diaphragmatic type seen in influenza. Its temperature rose to 41°C. The animal's condition was unchanged the following day but the next day it appeared improved and thereafter had an uneventful recovery. Blood serum obtained during the height of illness contained no neutralizing antibodies, while that got 9 days later neutralized swine influenza virus. It was evident from this that swine A had undergone an attack of swine influenza virus infection obscured, however, by the coincident ascaris reaction. When swine A eventually came to autopsy a moderate number of lungworms (about 50) were found in the bronchi at the bases of the diaphragmatic lobes.

Efforts to provoke influenza infections in swine B and C were continued about 2 weeks after the removal of swine A from their isolation unit. Each was given another intramuscular injection of living *H. influenzae suis*, and then, beginning 3 days later, received 100 cc. of 95 per cent ethyl alcohol mixed in its ground grain mash daily for 4 days. Both ate this alcoholized mash with a relish that increased with each day and they appeared quite drunk for as long as an hour after completing their meal. Swine B developed severe paroxysms of coughing each time soon after she partook of the alcohol. Another intramuscular injection of living *H. influenzae suis* was given 8 days after the preceding one and 2 days after the daily administration of alcohol had been discontinued. On the 2nd day after this injection swine B became very ill. She lay prostrate in her pen, refused food, and exhibited the accelerated, thumping, diaphragmatic type of respiration seen in influenza. Her temperature reached 40.9°C. The next day her clinical condition was unchanged except that her temperature had risen to 41.1°C. She was killed. At autopsy an atelectatic type of pneumonia involving large portions of all the anterior lobes of the lung was found. In addition the bases of both diaphragmatic lobes were extensively consolidated, and in the bronchi of these consolidated basal areas there were myriads of lungworms. Swine influenza virus of average pathogenicity was demonstrated in a suspension of pneumonic lung and lungworms by mouse inoculation, indicating that the illness induced in swine B had swine influenza virus as its basis. *H. influenzae suis* was not isolated from the respiratory tract of the animal though bacteria of other types were present at all sites cultured.

The day after swine B was killed, swine C that had been in the same isolation unit became ill. This was on the 4th day after the *H. influenzae suis* injection that appeared to have provoked swine B's infection. Clinically swine C was less ill than had been its unit mate. On the 1st day it was moderately depressed, its appetite was diminished, its respiratory rate accelerated and thumpy, but its temperature reached only 39.9°C. The following day the clinical condition of swine C was unchanged except that its temperature had risen to 40.5°C. It was killed and autopsied then. The lungs presented a moderately extensive lobular atelectatic pneumonia involving predominantly the anterior lobes on the left side, and there was only scant consolidation at the bases of the diaphragmatic lobes. Lungworms were present in small numbers in the bronchi at the bases. Swine influenza virus of average pathogenicity was demonstrated in a suspension of the anterior pneumonic lobes of lung by mouse inoculation. The lungworms were not tested for the presence of virus. It was evident that the illness of swine C had influenza virus as its basis. However, because this animal had been in the same isolation unit with swine B, which became ill first, one cannot tell whether C acquired its infection by exposure to B or whether C's disease was merely provoked later than B's. The first explanation seems the more likely.

It is evident from the experimental data just presented that swine influenza virus infections were elicited in 2 out of 3 swine picked at random during an interepizootic period from a drove of about 50 animals on an Iowa farm. An assumption which seems warranted, in view of the findings with lungworm larvae in earthworms from the same farm, is that these swine were carriers of masked swine influenza virus when they were received at the laboratory, and that this masked virus existed in association with lungworms acquired before they left home. There is good reason to suppose that the swine influenza virus responsible for the disease which developed in these animals was the same of which their 50 whole and half brothers and sisters sickened about a week earlier back on the home farm in Iowa.

DISCUSSION

The necessity for predicating an intermediate host to account for the dissemination of so highly contagious a disease as swine influenza is not at once apparent. Ordinarily an intermediate host is sought for or considered requisite only in those diseases that do not transmit naturally by contact. However, one period of the epidemiologic cycle of swine influenza, the interepizootic phase, would become more understandable if the services of an intermediate host capable of maintaining the virus from one epizootic to the next could be invoked. In the present experiments, evidence has been presented indicating that the lungworm serves as reservoir and intermediate host for swine influenza virus under natural conditions.

In the group of experiments carried out with pooled samples of earthworms from groups of Iowa farms, in which only a few earthworms in a sample derived from any single farm, evidence that swine influenza virus was transmitted was meager. Two of the 14 experiments yielded positive results. Of the 12 negative experiments 4 were conducted at a time of the year during which, to judge from experiments with known infective worms, prepared swine were refractory to provocation (2). These were Experiments 1 and 2 in which the swine received their lungworm infestations in May and Experiments 11 and 12 in which the swine were fed their worms in April but were not submitted to provocative stimuli until the next month. In the remaining 8 negative experiments the swine were fed and attempts to provoke infections were made at a time of the year when other experimental swine were not refractory and it would seem reasonable to conclude that masked swine influenza virus was not transmitted. It was present in the lungworms used in Experiment 5 and was provoked to infectivity in one of the 2 swine employed for test. The negative animal (swine 2445), sacrificed after seven unsuccessful attempts to provoke an influenzal infection, might have eventually come down had efforts to activate a masked virus in it been continued for the tenth attempt, which succeeded in the case of its companion, swine 2425. In Experiment 6 masked virus was present and caused influenza in both swine following the eighth provocative stimulus. Whether any significance can be attached to the fact that a long series of attempts to provoke infections were carried out before success was finally attained in these experiments is not known. It is conceivable that such observations may indicate that only a very few of the lungworms infesting the experimental swine were carriers of the masked virus.

In the experiments with lungworms from the Carson farm, masked swine influenza virus proved more readily demonstrable and in only one of these (Experiment 15) was it not detected. In view of the ease with which virus was later demonstrated in Experiment 16 in worms from the same source it seems likely that some explanation other than actual absence of virus must have been accountable for this failure. In contrast to the experiments with pooled samples of Iowa worms in which a long continued series of attempts to provoke were required, those with worms from the Carson farm yielded infections more promptly. Only in Experiment 19 in which the animal received a second feeding of earthworms after its initial infestation were more than 2 provocative injections required and in one experiment (Experiment 17) a swine influenza virus infection resulted on the 15th day after infestation with lungworms in swine to which no known provocative stimulus had been applied. If, as was reasoned in the case of the pooled Iowa worms, long delay in the response to provocation indicates a low incidence of masked virus carriers among the infesting lungworms, then the prompt provocation in the cases of the worms from the Carson farm might indicate a very high incidence of lungworms that were carriers of masked influenza virus. Regardless of what may be the explanation for the variations encountered, the experiments leave no doubt that virus is present in lungworms from the field and can be unmasked in test swine by appropriate procedures. The lungworm is thus removed from the category of an interesting intermediate host capable of transmitting swine influenza virus under the highly artificial conditions of the laboratory and placed in that of hosts playing a true rôle in the natural epidemiology of the disease. It is believed that the lungworm is the agent responsible for the perpetuation of swine influenza virus from one epizootic to the next.

The experiments with the 3 Carson swine brought from Iowa and in which influenza virus infections were provoked under controlled conditions at the laboratory are instructive in at least two directions. First, they indicate that apparently normal swine on Midwestern farms may be carriers of masked swine influenza virus and that this masked virus can be provoked to infectivity by procedures found effective in our own swine experimentally infested with lungworms carrying masked swine influenza virus. This finding, considered in conjunction with the demonstration of masked virus in lungworms obtained from the pens in which these 3 animals had been reared, supports the contention already advanced that the lungworm is the responsible intermediate host for swine influenza virus under field conditions.

The other direction in which these experiments are instructive relates to the natural incidence of masked virus infection as indicated by the findings with the 3 field swine just mentioned. They had been picked at random from a drove of about 50 animals and there is no reason to suspect that they represented an unusual sample of the drove. It was therefore surprising to find that 2 at least of the 3 animals were carriers of masked virus. The results suggest that the popular conception of the epidemiology of swine influenza may be far from the true state of affairs. It has been quite generally assumed, since the disease can be shown to be highly contagious under experimental conditions, that in a naturally occurring epizootic of swine influenza most of the cases result from direct exposure to previous cases. Though this conception has not fitted the known epidemiological facts, it has gained acceptance because it coincides with the accepted epidemiologic patterns of other contagious diseases of animals and man. However, it does not take into account, in the case of swine influenza, the explosive appearance of the disease which appears practically simultaneously among large groups of animals over wide areas. The rapidity of spread of the disease throughout a given area and even in a single drove cannot readily be accounted for by the successive infection of swine since the known incubation period is 2 to 7 days (usually 4) (3). The present experiments with the pigs from the Carson farm would suggest that most cases of swine influenza represent instances of provoked masked virus infections and that under natural conditions the spread of influenza from animal to animal, though readily accomplished, is the exception rather than the rule. This conception fits the known epidemiological facts and makes easier to understand the so called "influenza farms" and the appearance of influenza in dozens of localities over wide areas of the Midwest almost simultaneously during an epizootic. Instead of the virus going like wildfire from drove to drove with immediate transfer throughout a drove, it is probably already widely seeded and merely provoked almost simultaneously. On this conception the great rapidity of spread is more apparent than real and represents a delusion resulting from the provocation of widely disseminated masked virus by a stimulus common to wide areas. So far as can be learned by the direct observation of epizootics the effective provocative stimulus in the field consists in wet cold changeable weather coming after the swine have passed their refractory state of summer. Thus far I have not succeeded in imitating in the laboratory the type of "weather" associated with the provocation of swine influenza infections in the field.

The conception just outlined, according to which the causative virus is disseminated in a masked form by an intermediate host, separates chronologically the acquisition of the etiological agent and the onset of the epidemic. Ordinarily widespread dissemination of a causative agent is thought to coincide approximately with the onset of the epidemic. In the case of swine influenza, however, it seems likely that masked virus is acquired by pigs from lungworm larvae that they get in earthworms brought to or near the dirt surface during late spring and early autumn rains. The presence of this masked virus does not become clinically evident, though, until it is provoked to infectivity in late October or November. The onset of a swine influenza epizootic would appear to be determined, not by the time of acquisition of the causative virus, but by meteorological or physical influences which favor activation of the virus.

The months of November and December, during which swine influenza epizootics ordinarily occur, are not the most favorable ones for the provocation of the masked virus. As pointed out in the preceding paper (2), this is more readily effected during the first 4 months of the year,—a period during which swine influenza is either absent or of low prevalence in the field. The widespread prevalence of influenza during the last 2 months and its absence during the first 4 months of the year would therefore at first sight seem incongruous on the basis of the findings with swine experimentally infested with lungworms carrying influenza virus. As indicated in the preceding paper (2), however, swine under field conditions may be in quite another category than are our experimental animals. They have probably already acquired masked influenza virus *via* the lungworm by the time the refractory period of summer and early autumn is past, and the virus is available for provocation at the first favorable opportunity. This is ordinarily presented in November. After they have recovered from their infections they are immune. Thus the majority of swine that remain on the farms during the later period, which experimental evidence indicates to be the most favorable for provoking masked infections, go into that period protected by a solid immunity. It seems likely that if, for any reason, field infections failed to be provoked during the usual months of November and December and the swine thus remained susceptible into the early months of the year, an epizootic of swine influenza might occur at any time until May.

If, as certain evidence indicates and as at least two workers have maintained (4, 5), the swine influenza virus represents a surviving prototype of the 1918 pandemic human influenza virus, then that agent was fortunate indeed to find awaiting it an intermediate host in the swine lungworm, capable of perpetuating it so effectively. Had no such effective reservoir host been available swine influenza could scarcely have appeared after its first epizootic year of 1918—unless indeed the virus is maintained by other means as yet unrecognized.

SUMMARY

1. The presence of masked swine influenza virus has been demonstrated in lungworm larvae from earthworms dug on Midwestern farms.

2. Swine influenza virus infections were provoked in 2 of 3 swine obtained from an Iowa farm during an interepizootic period.

3. The evidence presented has been interpreted as indicating that the swine lungworm is the reservoir and intermediate host for swine influenza virus in the field.

4. A concept of the epidemiology of swine influenza in which the causative virus is represented as being maintained and disseminated in a masked form by its lungworm intermediate host has been presented.

BIBLIOGRAPHY

1. Shope, R. E., *J. Exp. Med.*, 1941, **74,** 49.
2. Shope, R. E., *J. Exp. Med.*, 1943, **77,** 111.
3. Shope, R. E., *J. Exp. Med.*, 1931, **54,** 349.
4. Laidlaw, P. P., *Lancet*, 1935, **1,** 1118.
5. Shope, R. E., *Harvey Lectures*, 1935–36, **31,** 183.