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TRANSMISSION OF SWINE INFLUENZA VIRUS BY LUNGWORM MIGRATION*·‡

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Shope (1, 2) reported that *Metastrongylus* spp. under specific conditions have the capacity to transmit swine influenza virus to pigs. Since the authors are not aware of published confirmation of Shope's observations by other laboratories, experiments in which swine influenza virus was transmitted by infected lungworms in pathogen-free, antibody-devoid pigs are here reported.

Materials and Methods

Virus.—Shope's S-15 strain of swine influenza virus maintained in the laboratory by 232 successive mouse and 23 chick embryo passages was used throughout these experiments. Allantoic fluid from infected eggs was the virus source.

Parasites.—The *Ascaris* eggs used were prepared and embryonated as described by Underdahl and Kelley (3).

For the extract, 105 gm. of adult tissue of *Ascaris suum* body wall was ground in 400 ml. of 0.1 M sodium phosphate in physiological saline and centrifuged at 2000 R.P.M. for 15 minutes at 4°C. The supernatant was collected, the pH adjusted to 7.0, and passed through a sephadox G-25 column to remove salt. The first 80 ml. of eluate, which contained 3.2 mg. of protein per ml., was retained and stored at -20°C. until used.

Lungworm eggs were obtained from laboratory infected pigs 1 week after they had been inoculated with influenza virus. Eggs were separated from feces or taken directly from the worm and placed in a mixture of sphagnum moss and soil kept in perforated coffee cans. 100 mature earthworms, *Eisenia foetida*, collected from an area on the Nebraska Station which had not been contaminated with swine feces, were placed in each of the culture cans. The lungworm larvae reached the infective stage 3 to 4 weeks after the eggs were ingested by the earthworms. Distilled water was used to wash the external debris from the infective earthworms. Following the washing, the worms were laid on a board and minced by slicing with a razor blade. The resulting *brei* was suspended in 10 ml. of distilled water and given to the pigs. The dose of infective larvae was estimated by counting the number of larvae in aliquots from the total material. Lungworm larvae and *Ascaris* eggs were given by gavage.

Experimental Pigs.—Pathogen-free, colostrum-deprived pigs were used throughout the experiments. These were obtained by hysterectomy as described by Young *et al.* (4). The pigs were raised in individual isolation units and fed a diet of pasteurized cow's milk, egg, and

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TABLE I

Results of Experiments Conducted to Demonstrate Transmission of Influenza Virus by Lungworms

Group	Pig No.	Treatment		Recorded temperature	Results*		
		Lungworms	Provocation		Pig	Mouse	Egg
Experiment 1, fall and winter							
I	1	1200 uterine origin	<i>Ascaris</i> extract†	106.2 on 24th day	+	+	+
	2			Normal	+	+	-
II	3	1000 fecal origin	Calcium chloride§	Normal	-	-	-
	4			Normal	-	-	-
III	5	1200 uterine origin	<i>Ascaris</i> eggs	106.7 on 25th day	+	-	+
	6			Normal	+	-	-
	7	1000 fecal origin	<i>Ascaris</i> extract¶	Normal	-	-	-
	8	1200 uterine origin	<i>Ascaris</i> extract¶	Normal	-	-	-
Experiment 2, spring							
I	1	800 uterine and fecal origin	<i>Ascaris</i> eggs**	108.4 on 16th day	+	+	+
	2			Normal	-	-	-
II	3	800 uterine and fecal origin	None	Normal	-	-	-
	4			Normal	-	-	-
III	5	None	<i>Ascaris</i> eggs**	Normal	-	-	-
	6			Normal	-	-	-
Experiment 3, summer‡‡							
	2	1500 fecal and uterine origin	<i>Ascaris</i> eggs§§	NT	+	NT	+
	3	1500 fecal and uterine origin	<i>Ascaris</i> eggs§§	NT	+	NT	+
	4	None	<i>Ascaris</i> eggs§§	NT	-	NT	-

* Typical influenza lesions in pigs and mice with virus isolation in eggs. All the egg virus isolated was neutralized by anti-S-15 mouse antiserum.

† 1, 2 and 3 ml. subcutaneous injections at 2 day intervals prior to lungworm larvae and three 3 ml. weekly injections following the lungworms.

§ 5 ml. 1 per cent CaCl₂ directly into chest cavity 19th day post lungworms.

|| 24,000 *Ascaris* eggs on 8th and 20th days following lungworms.

¶ 3, 3 ml. injections (subcutaneously) following lungworm infection.

** 225,000 *Ascaris* eggs 10 days post lungworm infection.

‡‡ This experiment was performed by Douglas Krous, National Science Foundation Fellow.

§§ 24,000 *Ascaris* eggs 15 days following lungworms.

||| NT, not taken.

mineral for the duration of the experiment. The pigs in Experiments 1 and 2 were killed and autopsied after a temperature increase had taken place in 1 pig in the group. In Experiment 3 the pigs were killed 7 days following *Ascaris* treatment. The lungs were examined for lesions of influenza. A lung extract was prepared from the consolidated lesions or, in normal appearing lungs, from the right apical lobe. The extract was treated with penicillin and streptomycin before inoculation of mice and eggs. Allantoic fluid was harvested from eggs 48 hours after inoculation to assay for virus. Mice were necropsied 10 days following inoculation.

RESULTS

Results of the experiments (Table I) are in agreement with those previously reported by Shope (1, 2). In the three experiments conducted, 7 of 14 pigs fed lungworm larvae from pigs infected with influenza virus themselves came down with influenza. Virus was isolated by egg inoculation from 5 of these. The masked virus present in the lungworms was elicited for infection purposes by multiple injections of *Ascaris* extract or by migrating *Ascaris* larvae following infection with lungworms of influenza origin. Positive identification of the influenza virus was accomplished by isolation of it and neutralization by known anti-S-15 serum.

As shown by Table I, the pigs given calcium chloride injections into the chest cavity or *Ascaris* eggs alone, remained normal. The same held true of swine that received lungworm larvae of non-influenza origin. When these pigs were examined at necropsy there were no lung lesions and the virus could not be demonstrated by mouse and egg inoculation.

DISCUSSION

Shope (1, 2) reported that he was able to elicit an influenza infection only during the fall and winter and not during the spring and summer. In the experiments reported here, conducted during the fall, winter, spring, and summer, some of the pigs in each experiment became infected with influenza. The repeatability of these experiments can be attributed to the experimental animals used, namely pathogen-free, colostrum-deprived pigs obtained by hysterectomy and raised in isolation. Such hosts will be used to further explore the nature of latent virus in lungworm larvae.

SUMMARY

Experimental evidence is presented which confirms the reports by Shope (1, 2) that swine lungworms can serve as an intermediate host in transmitting swine influenza virus to pigs. The virus is present in a masked non-infective form as he showed and a provocative stimulus is necessary to initiate infection. Multiple injections of *Ascaris* extract or the migration of *Ascaris* larvae furnished the needed provocation. The virus could be elicited in the spring and summer as well as fall and winter, from the pathogen-free, colostrum-deprived pigs.

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