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Experimental Infection of Weanling Pigs with A/Swine Influenza Virus*

3. Immunity in Piglets Farrowed by Antibody-bearing Dams Experimentally Infected a Year Earlier

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Pigs experimentally infected as weanlings with swine influenza virus, as described in previous papers, were bred from when mature. Attempts to isolate virus at parturition from the placenta and from different organs of some of the piglets immediately after birth gave negative results. Antibody levels were determined in the sows and remaining piglets at different times after birth, and the clinical course, immunity and antibody formation were studied in some of the piglets challenged with swine influenza virus 10 days after birth. The levels were found to be generally higher in the sows than those in their offspring. Specific antibodies were detected in piglets that had presumably not ingested colostrum, but the possibility of unobserved ingestion of colostrum could not be excluded. Colostrum-fed piglets had specific antibody in their sera ; an increase in antibody titre occurred by the 10th day after birth, remained until the 20th day and decreased steadily to the 30th day. Colostrum-fed piglets receiving antibodies from immune dams were resistant to a challenge of virulent swine influenza virus, and did not respond with an antibody rise during a 30-day observation period after challenge.

After experimental intranasal infection with the influenza strain A/Swine/Měrotin/57, 22 weanling pigs between 42 and 77 days old at the time of infection retained detectable serum antibodies at various levels over a 12-month period of observation and in some cases for a year and a half of observation, (Blaškovič et al., 1970b). The majority of these animals were young sows and these were bred from when sexually mature.

Several questions were investigated concerning the immunological status of these dams and their offspring. A search was made for virus in the placenta at parturition, and in different organs of some of the farrowed piglets exsanguinated immediately after their birth. Antibody titres in the dams and their piglets were compared at different times after the birth of the latter. The clinical course, immunity and antibody formation was followed in some of the piglets challenged with swine influenza virus 10 days after birth.

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MATERIAL AND METHODS

Experimental animals

Attempts at virus isolation were made with the placentas of dams No. 9, 11, 40, 45, 46, 50, 51, 52, 53, 54 (these numbers correspond to the numbers of experimentally infected weanlings described previously; see Blaškovič et al., 1970b). The dams farrowed in September–October 1968. The weight of the piglets ranged from 2.60 kg to 3.10 kg.

Two piglets from each of the above-mentioned dams were exsanguinated after delivery (colostrum-deprived), and attempts to isolate virus were made from suspensions of lung, pericardial fluid, nasal mucosa, and from organ explants as described by Blaškovič et al. (1970a). The remaining piglets (colostrum-fed) were tested by serological assay.

Virus isolation

Organ explants, as described previously (Blaškovič et al., 1970a), were used for placentas and 2 different pieces of lung from 2 farrowed piglets from each dam. The medium from the explants was tested for the presence of virus by adding rooster erythrocytes on the 3rd, 6th, 9th and 15th days after the organ explants had been prepared.

Suspensions (10%) from the lungs and nasal swabs of 2 piglets were prepared in tissue culture medium (Blaškovič et al., 1970a). Pericardial fluid was employed for virus isolation without the addition of antibiotics. This material was inoculated intra-amniotically into 11-day-old embryonating eggs and incubated at 37°C for 3 days. Three successive passages were made. Material from the lungs of 2 piglets was pooled, and the suspension inoculated into 4 eggs.

Infecting virus

The piglets farrowed by dams No. 9 and 52 were infected on the 10th day after birth intranasally with a dose of 0.5 ml in each nostril of the strain A/Swine/Měrotin/57 (HA titre of 1 : 512, and infectious titre of 10^7 EID_{50} ml) after 96 egg, 10 swine and 2 further egg passages. The piglets farrowed by dams No. 40 and 48 were infected at 10 days in each nostril with 0.75 ml of infective allantoic fluid of the same virus passage. The clinical course after infection was followed by daily inspection and by measuring the rectal body temperature.

Serology

The same procedures were used as described in the preceding papers (Blaškovič et al., 1970a, 1970b).

RESULTS

Virus isolation

All attempts to isolate swine influenza virus, by the methods described above, from 9 dams infected as weanlings approximately a year previously, and from their offspring, gave negative results.

Haemagglutination-inhibiting (HI) and virus-neutralizing (VN) antibodies in dams and their offspring

The levels of serum antibodies in dams on the date of farrowing were compared with those of their piglets at 0, 10, 20 and 30 days after delivery. Because the piglets from dams No. 9, 40, 48 and 52 were reinfected with virulent Měrotin strain and their antibodies checked later, they are not included in this group.

The results obtained are summarized in Table 1.

In 7 out of 8 dams and their piglets a comparison can be made between HI and VN antibodies. With the exception of those of dams No. 13, 50 and 51, all offspring had lower serum antibody titres immediately after delivery than later and showed a significant rise in titre at the 10th day. This is also shown in Fig. 1, where the geometrical mean titres are presented.

Clinical and immunological response of colostrum-fed piglets to infection with swine influenza virus

The offspring of dams No. 9, 40, 48 and 52, colostrum-fed, were infected intranasally with 10^7 EID_{50} of the Měrotin strain 10 days after delivery. The piglets were observed and their rectal temperature measured daily. There was no significant difference between 2 litters in which one-half of the piglets were infected and the other remained as controls for contact infection. None of the piglets showed any signs of disease.

The immunological response of these piglets is shown in Table 2 and in Fig. 2. It will be seen that the challenge virus given to piglets 10 days old (or 18 days old in the case of those from dam No. 9) did not cause any rise in HI or VN titre up to a month after the challenge dose, at which time the observations were terminated.

DISCUSSION

There were discrepancies in the levels of both HI and VN antibodies among the dams and their offspring. On the whole, the antibody titres in the blood of the offspring taken on the day of delivery were lower than those of their dams. In one case the antibody titres were approximately equal in the sera of the dam (No. 54) and her litter. Two exceptions (dams No. 50 and 51 and their offspring) are difficult to explain: higher titres were found in the piglets' than in the dams' sera in both HI and VN tests. The differences were perhaps due to the presence of non-specific neutralizing substances, or the offspring ingested a large volume of colostrum after delivery.

One would expect that, because of the type of the placenta in pigs, no antibody would be present in the sera of offspring at the time of delivery and before ingesting colostrum. Antibody was detected, however, at the time of delivery, and this may be explained by the fact that, despite a careful watch at the time of delivery, some of those piglets that

VN AND HI ANTIBODIES IN SOWS AND THEIR COLOSTRUM-FED OR COLOSTRUM-DEPRIVED OFFSPRING UP TO 30 DAYS AFTER DELIVERY [a]

No. of animal	Days after delivery			
	0	10	20	30
Dam 11	64 64	ND		
Piglet 1	<8* 4*	E		
2	16* 4*			
3	<8* 16*	128 64	128 64	32 16
4	⩽8* 16*	32 64	32 32	16 8
5	⩽8* 8*	128 128	64 32	32 16
6	⩽8* 8*	128 64	128 32	64 16

No. of animal	Days after delivery			
	0	10	20	30
Dam 13	ND	32 16	ND	ND
Piglet 1		64 16	32 16	8 4
2		128 16	64 16	16 8
3		32 16	32 16	16 8
4		64 32	64 8	16 8
5		64 16	32 16	16 16
6		64 16	32 16	16 16
7		64 8	32 16	16 16

No. of animal	Days after delivery			
	0	10	20	30
Dam 45	128 32	ND		
Piglet 1	8* 2*	E		
2	8* 4*			
3	8 2	64 64	64 32	32 8
4	8 2	128 64	256 32	32 16
5	8 4	32 64	128 32	64 16
6	8 2	128 128	64 32	32 8
7	8 4	128 128	128 32	64 8
8	8 4	256 64	128 32	64 8

No. of animal	Days after delivery			
	0	10	20	30
Dam 46	128 32	ND		
Piglet 1	8* 2*	E		
2	8* 2*			
3	16* 16*	64 64	64 64	64 32
4	16* 8*	128 64	64 32	128 16
5	16* 8*	256 64	128 32	64 16
6	32* 2*	128 8	Died	
7	16* 4*	256 64	256 64	128 16
8	32* 2*	128 64	128 32	64 8
9	16* 2*	128 64	128 64	128 16
10	16* 2*	256 64	128 64	128 16
11	16* 4*	256 64	128 32	64 16

No. of animal	Days after delivery			
	0	10	20	30
Dam 50	64 16	ND		
Piglet 1	128 64	E		
2	128 64			
3	128 32	64 16	16 8	16 4
4	256 64	Died		
5	128 32	16 ND	Died	
6	32 16	16 8	16 4	8 4
7	128 64	64 16	32 8	16 4

No. of animal	Days after delivery			
	0	10	20	30
Dam 51	8 4	ND		
Piglet 1	64* 32*	E		
2	64* 32*			
3	32 16	16 64	8 4	4 <4
4	128 16	Died		
5	64 16	16 32	8 4	4 <4

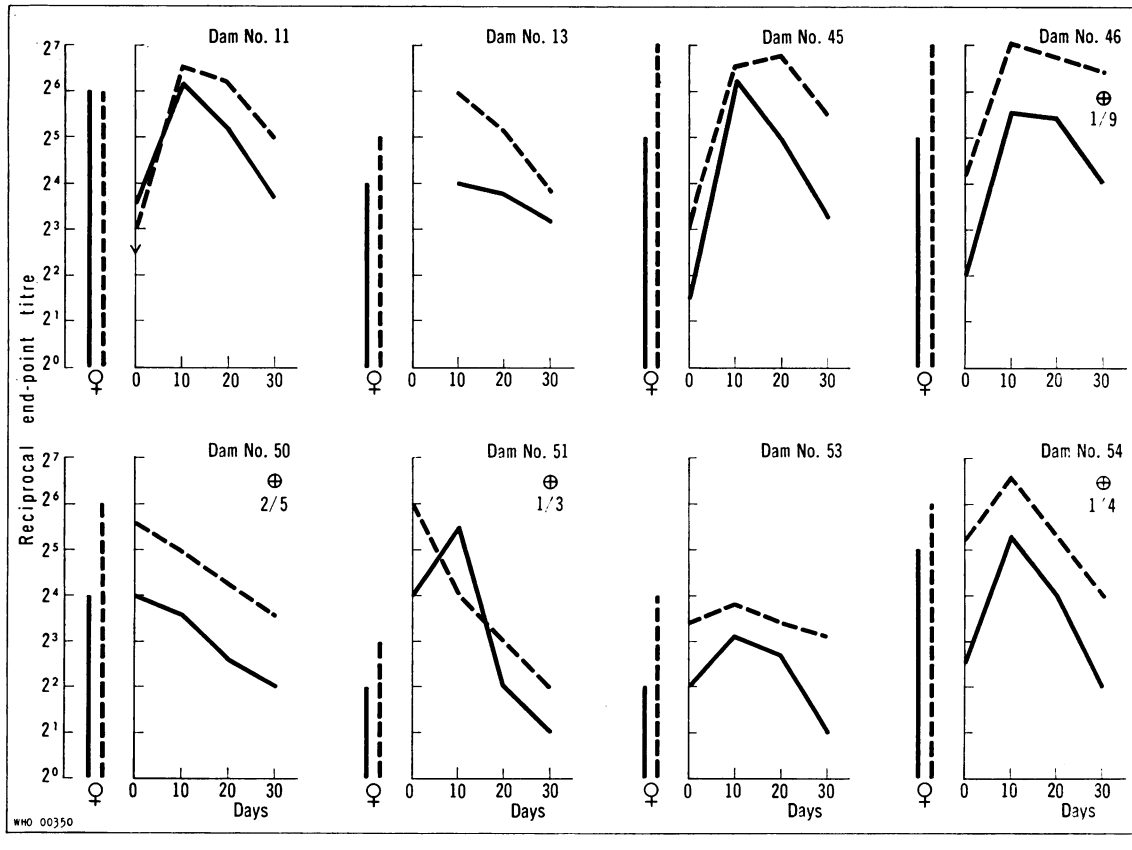
No. of animal	Days after delivery			
	0	10	20	30
Dam 53	16 4	ND		
Piglet 1	8* 2*	E		
2	4* 4*			
3	16* 4*	16 8	16 8	8 <4
4	16* 4*	16 8	16 8	16 <4
5	16* 4*	32 32	8 8	8 <4
6	16* 4*	16 16	8 8	4 <4
7	16* 4*	8 8	16 8	16 <4
8	4* 4*	16 4	8 4	8 <4
9	4* 4*	8 4	8 4	8 <4

No. of animal	Days after delivery			
	0	10	20	30
Dam 54	64 32	ND		
Piglet 1	32* 8*	E		
2	32* 32*			
3	32 4	Died		
4	32 4	64 64	32 16	16 4
5	64 4	128 32	64 16	16 4
6	32 16	128 32	32 16	16 4

[a] In each cell the *upper* value represents the VN antibody titre (reciprocal of end-point serum dilution) and the *lower* value the HI antibody titre. E = exsanguinated immediately after delivery or within 12 hours. ND = not done. * = probably colostrum-deprived.

FIG. 1

GEOMETRICAL MEAN VN AND HI TITRES AMONG PIGLETS OF SOWS ORIGINALLY INFECTED AS WEANLINGS WITH SWINE INFLUENZA VIRUS



HI test results in piglets.

VN test results in piglets.

⊕ No. died after blood sampling/No. sampled.

♀ Vertical solid and broken lines above this symbol indicate the HI and VN titres respectively of each litter's dam at time of delivery.

were farrowed during the night could have sucked colostrum. Alternatively, specific antibody may have leaked through the placental barrier.

The results also show clearly that the antibody titre steadily declined during the 30-day period of observation after delivery.

In a previous communication of this series (Blaškovič et al., 1970a), it was shown that a shedder state existed in weanlings infected 3 months previously. How long this shedder state can persist is not known. We were unable to recover swine influenza virus from these animals when they became sows and were bred from 9–12 months later and delivered their litters 4 months after that. At that time influenza virus could not be isolated either from the placentas or from nasal swabs, lungs or pericardial fluid of the offspring.

It is possible that swine influenza virus may persist in the presence of humoral antibodies, and may even multiply in tissues, as has been observed with herpes viruses. There would then exist the situation that in herds where swine influenza infection is endemic transmission of the virus could continue uninterrupted. The infection might be latent clinically, but a shedder state of virus could occur at times.

TABLE 2

VN AND HI TESTS IN SOWS AND THEIR COLOSTRUM-FED OFFSPRING, THE LATTER CHALLENGED WITH SWINE INFLUENZA VIRUS 10 DAYS AFTER BIRTH [a]

No. of animal	Days after delivery				
	0	10	20	30	40
Dam 40	128 16	ND			
Piglet 1	<8 8	E			
2	<8 16				
3	<8 8	128 64	Died		
4	<8 4	→ 128 64	64 32	32 32	16 8
5	<8 8	→ 256 128	32 32	64 32	16 16
6	<8 8	C 128 128	64 64	128 32	32 16
7	<8 8	C 64 64	64 32	32 16	32 16

No. of animal	Days after delivery				
	0	10	20	30	40
Dam 48	10 8	ND			
Piglet 1	<8 4	→ 8 8	4 4	4 2	4 2
2	<8 8	→ 8 8	8 4	8 4	8 2
3	<8 4	→ 8 16	8 4	4 2	4 2
4	<8 4	C 8 [illegible]	4 4	4 2	4 2
5	<8 4	C 8 8	8 4	4 2	4 2
6	8 4	C 8 8	8 4	8 2	4 2

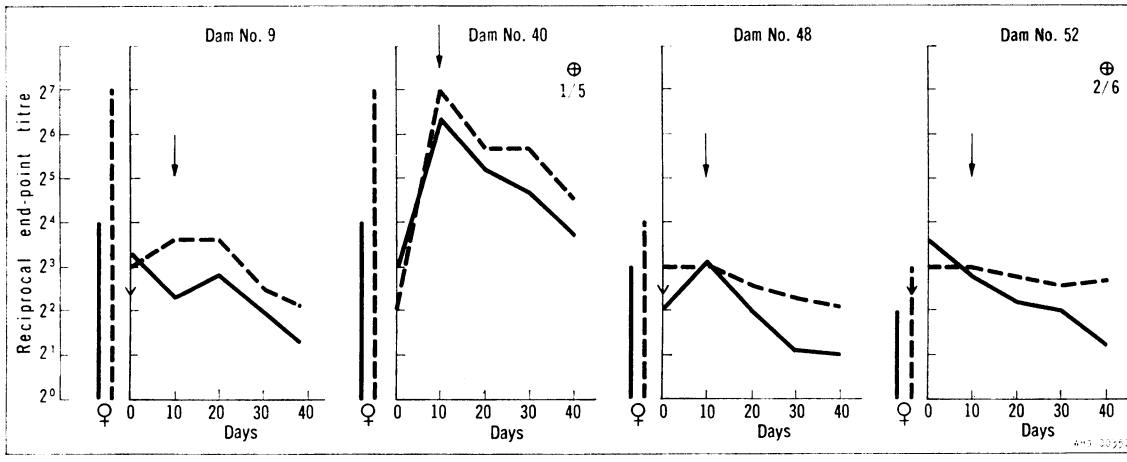
No. of animal	Days after delivery				
	0	10	20	30	38
Dam 9	128 16	ND			
Piglet 1	<8 8	E			
2	<8 8				
3	<8 8	→ 16 4	16 4	16 4	<8 2
4	<8 8	→ 16 4	8 8	8 4	<8 2
5	<8 16	→ 32 4	16 16	8 4	<8 2
6	<8 8	→ 8 4	16 8	<8 4	<8 2
7	<8 16	→ 16 8	16 8	<8 4	<8 2
8	<8 8	→ 8 8	8 4	<8 4	8 8

No. of animal	Days after delivery				
	0	10	20	30	40
Dam 52	<8 4	ND			
Piglet 1	4 8	E			
2	4 4				
3	8 8	→ 4 4	8 4	8 4	8 2
4	8 8	→ 8 4	8 4	8 4	8 2
5	8 16	→ 8 4	4 4	4 4	Died
6	16 16	→ 32 16	8 4	4 4	8 2
7	8 16	→ 4 16	Died		
8	4 16	→ 8 8	8 8	8 4	4 4

[a] In each cell the *upper* value represents the VN antibody titres and the *lower* value the HI antibody titre. ND = not done. E = exsanguinated immediately after delivery or within 12 hours. → = challenged intranasally on 10th day after delivery with 10^7 EID_{50} of swine influenza virus. **C** = uninfected controls in the same litter.

FIG. 2

GEOMETRICAL MEAN VN AND HI TITRES AMONG COLOSTRUM-FED PIGLETS OF SOWS ORIGINALLY INFECTED AS WEANLINGS WITH SWINE INFLUENZA VIRUS



Time of challenge of piglets with swine influenza virus. For other symbols, see legend to Fig. 1.

When piglets farrowed by dams with humoral antibodies and then colostrum-fed were challenged with virulent virus (on the 10th or 18th day after delivery, when they had a high level of passive serum antibodies), no result of infection with the virus was observed. The results suggest that the infection cannot be interpreted as a booster dose because no rise in antibody titre was observed in any of the challenged animals. This is in accordance with previous observations that an antigenic stimulus in newborn animals with passively acquired antibodies does not provoke a rise of antibodies (Miller et al., 1962; Bögel & Liebelt, 1964; Šterzl et al., 1960; Mensik, 1966).

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RÉSUMÉ

INFECTION EXPÉRIMENTALE DE PORCS RÉCEMMENT SEVRÉS PAR UN VIRUS GRIPPAL PORCIN DE TYPE A : 3. IMMUNITÉ CHEZ LES PORCELETS ISSUS DE MÈRES PORTEUSES D'ANTICORPS INFECTÉES EXPÉRIMENTALEMENT UN AN AUPARAVANT

De jeunes truies porteuses d'anticorps sériques spécifiques à la suite d'une infection grippale provoquée ont mis bas un an environ après l'inoculation expérimentale. Les tentatives d'isolement du virus effectuées à ce moment à partir des placentas et de matériel (suspensions de tissu pulmonaire, sécrétions nasales, liquide péricardique) prélevé chez les porcelets nouveau-nés sont restées infructeuses.

Des anticorps antigrippaux spécifiques ont été trouvés dans le sérum de porcelets saignés immédiatement après la naissance, ce qui semble indiquer la possibilité d'une transmission des anticorps par voie placentaire, bien que l'éventualité de l'ingestion par les jeunes animaux d'une certaine quantité de colostrum ne puisse être totalement exclue. Les titres d'anticorps étaient en général plus élevés chez les mères que dans la descendance.

Les porcelets, nés de mères sérologiquement positives, qui ont ingéré du colostrum ont été trouvés porteurs d'anticorps. Les titres se sont accrus vers le 10e jour suivant la naissance, se sont maintenus à ce niveau jusqu'au 20e jour, puis ont diminué progressivement jusqu'au 30e jour. Ces porcelets n'ont présenté aucun signe pathologique lorsqu'ils ont été soumis à une infection d'épreuve par une souche virulente de virus grippal porcin et aucune réponse immunitaire dans les 30 jours qui ont suivi.

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