Exposure of sero-positive gilts to swine influenza virus may cause a few stillbirths per litter

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Abstract

Six pregnant gilts were purchased from a high health herd and were found to be serologically positive for swine influenza virus (SIV) subtype H3N2. Three of the gilts, at 80 to 82 days of gestation, were experimentally exposed a second time to the same SIV subtype — H3N2. No clinical signs resulted from the second exposure to SIV and hemagglutination-inhibition (HI) titers for SIV at 4 weeks postexposure were unchanged suggesting that the gilts had not been reinfected. However, the second exposure to SIV affected the number of pigs born alive. Each of the 3 litters from the twice exposed gilts suffered 2 or 3 stillborn piglets per litter. In contrast the 3 matched, sero-positive gilts that were not exposed to SIV (controls) had no stillborn piglets. These differences were statistically significant using a *t*-test for unequal variances ($P = 0.0086$). Sera from 2 of the stillborn piglets were negative for HI antibodies and there was no indication from the pigs born alive that the H3N2 virus had crossed the placenta.

Résumé

Six cochettes en gestation ont été achetées d'un troupeau à statut sanitaire élevé et se sont révélées sérologiquement positives pour le virus de l'influenza porcin (SIV) de type H3N2. Au jour 80 à 82 de gestation trois de ces cochettes ont été exposées expérimentalement pour une deuxième fois au même type de SIV (H3N2). Suite à cette deuxième exposition au virus, aucun signe clinique ne fut noté et les titres d'inhibition de l'hémagglutination anti-SIV étaient inchangés 4 semaines suivant la ré-exposition, suggérant ainsi qu'il n'y avait pas eu de ré-infection. Toutefois, la deuxième exposition au SIV influença le nombre de porcelets nés vivants. Pour chacune des portées provenant des trois cochettes exposées deux fois au SIC il y eu 2 ou 3 mort-nés par portée. Par opposition, chez les trois autres cochettes qui n'ont pas été ré-exposées au SIV aucune n'a eu de porcelet mort-né. Ces différences n'étaient pas statistiquement significatives par test de T pour variances inégales (P = 0,0086). Les sérums de 2 des porcelets mort-nés étaient négatifs pour les anticorps HI et il n'y avait pas d'indication chez les porcelets nés vivants que le virus H3N2 avait traversé la barrière placentaire.

(Traduit par Docteur Serge Messier)

Swine influenza virus (SIV) causes an acute respiratory disease in young and adult pigs. Some reports also suggest that reproductive failure, farrowing difficulties, and neonatal problems can be associated with swine influenza outbreaks (1–3). The reproductive problems include infertility; abortions; stillbirths; and weak, poor-doing piglets (4). Early investigations with SIV in the United States (US) indicated that pigs born alive from dams inoculated during the first stages of gestation with live virus had higher mortality rates and lower weaning weights than those from control dams (5). Moreover, sporadic abortions late in pregnancy and increased stillbirths have also been reported during swine influenza outbreaks in Europe and in North America (6–8). After one severe swine influenza outbreak in France, only 3 of 13 sows in their 1st wk of pregnancy completed gestation and farrowed pigs, in 2 of 8 sows that were 1 mo into gestation total embryonic resorption occurred, and in the 18 sows that were more than 45 d into gestation a single abortion occurred (9).

In North America, since August 1998, the situation with swine influenza disease has changed and many swine producers and veterinarians report severe respiratory disease and some abortions in herds caused by a new SIV subtype. Significant respiratory disease continues even in vaccinated herds. The signs of acute influenza are high fever (40.0 to 41.5°C), coughing, labored breathing, abortions, and a low percentage of deaths in sows and in boar studs (10). The cause of the more severe influenza disease has been the introduction of a new H3N2 swine influenza subtype that has become widespread in North America (11). Since then, another influenza virus subtype, H1N2, also has been reported in the US and has been isolated from herds experiencing outbreaks of severe respiratory disease and abortions (8,12).

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Received June 9, 2003. Accepted November 17, 2003.

The purpose of this report is to further characterize reproductive problems associated with exposure to the new North American H3N2 subtype. The experiment was conducted to obtain piglets with high levels of passive H3N2 antibody. Naturally infected gilts were exposed a second time to H3N2 SIV near the beginning of their 3rd trimester. Reproductive failure (stillbirths) occurred only in the litters from gilts that were exposed a second time to the H3N2 virus during gestation.

Six pregnant gilts (sero-negative for porcine reproductive and respiratory syndrome virus [PRRSV]) were purchased from a high health herd that vaccinates only for parvovirus and for leptospirosis. Upon arrival at the National Animal Disease Center (NADC), the gilts were found to have hemagglutination inhibition (HI) titers to SIV indicating that the gilts had been naturally infected with SIV subtype H3N2 at some previous time on the farm (the gilts were negative for subtype H1N1). Their HI titers ranged from 80 to 320.

Three of the gilts with HI titers of 80, 160, and 160, respectively, were housed in a single isolation room at the NADC. In an attempt to increase their serum antibody titers, these gilts were boosted intranasally with 1.5 mL per nostril using a syringe and tightly fitting intranasal tip with fully virulent, live H3N2 virus with a titer of 6.3×10^5 $CCID_{50}$/mL. The other 3 gilts with initial HI titers of 160, 320, and 320, respectively, were not exposed to virus and were housed separately in another identical isolation room.

At 27 d postinoculation, no dramatic HI titer increase occurred in the 3 inoculated gilts. For these 3 inoculated gilts, HI titers were unchanged from their arrival day titers 40 d earlier. In contrast, the group of 3 non-boosted gilts had titers of 160 (2 gilts had 2-fold lower HI titers and one gilt's titer was unchanged from its arrival day titer). The serological data indicated that the experimentally infected gilts did not develop either an anamnestic or a heterologous increase in HI antibody titer. However, collectively their HI titers did not decline as rapidly as those of the 3 control gilts that were not exposed a second time to SIV.

During farrowing the gilts were monitored closely, as prescribed by the institutional animal care committee guidelines. The maximal interval between observations was 4 h. No unusual difficulties during labor (straining) were recorded for any of the gilts. However, differences in the number of stillborn piglets between the 2 groups of animals were statistically significant and quite striking. The results are shown in Table I. For the gilts that were not experimentally exposed a second time to SIV, all 38 piglets were born healthy and no stillbirths occurred. In contrast, for the treated gilts, there were 2 or 3 stillborn piglets per litter. Often these stillborns were observed at delivery or soon after they were born. Stillborn piglets occurred both early and late in the litter.

To compare the percentage of stillborn piglets from each group of gilts, a *t*-test for unequal variances was performed. Another *t*-test for equal variance was conducted on the total number of piglets in a litter for SIV inoculated versus control gilts. The SIV inoculated gilts had a significantly higher percentage of stillbirths than the non-inoculated, control gilts ($P = 0.0086$). The SIV inoculated gilts had stillbirths occurring in all 3 litters (mean = 21.67% stillbirths per litter) compared with the control gilts where no stillbirths occurred in any of the 3 litters. Total piglets per litter were not statistically different between the 2 groups although the control gilts had a mean number of piglets per litter equal to 12.67 compared to 10.67 for the SIV inoculated group.

Table I. Number of stillbirths per total number of piglets in a litter

	SIV exposed gilts	Non-exposed gilts
	3/12	0/11
	2/11	0/13
	2[a]/9	0/14
Total[b]	7/32	0/38

SIV — swine influenza virus

[a] Cardiac blood sample — the stillborn pigs were negative for HI antibody

[b] Significantly different ($P = 0.0086$) using the *t*-test for unequal variances

Cardiac blood was drawn from 2 stillborn piglets in the litter with 9 total piglets (Table I). Sera from these 2 stillborns were negative for HI antibodies. Additionally, the colostrum-deprived piglets born alive ($n = 25$) from the SIV inoculated gilts were bled at 1 wk of age and these sera were also negative for HI antibody.

Six naturally infected, pregnant gilts, sero-positive for SIV subtype H3N2, and perhaps, infected around breeding time, were used in this experiment. Near the beginning of their 3rd trimester at 80 to 82 d of gestation, 3 of the gilts were experimentally exposed to SIV subtype H3N2. No loss of appetite or signs of respiratory disease resulted from the experimental exposure to the H3N2 virus, but body temperatures were not determined. Stable HI titers of the SIV exposed gilts at day 27 postexposure suggested that the gilts had solid immunity to the H3N2 virus used for inoculation. However, observations at farrowing indicated that the experimental exposure to SIV affected the number of pigs born alive. Each of the 3 litters from the re-exposed gilts suffered 2 or 3 stillborn piglets per litter whereas the matched, sero-positive control gilts had no stillborn piglets. These differences were statistically significant using a *t*-test for unequal variances ($P = 0.0086$). Sera from 2 of the stillborns were negative for HI antibodies and there was no indication from the pigs born alive that the H3N2 virus had crossed the placenta.

The total time for farrowing and the intervals between the delivery of piglets did not appear to be different for the boosted or the non-boosted gilts. Stillbirths probably result from hypoxia during the farrowing process and contributing factors include short or lengthy gestation periods; ambient temperature extremes; or pyrexia due to the viral infection, nutritional factors, housing, and exercising of the dams (13). The latter factors, nutrition, environmental conditions, housing, and exercise, were controlled and the same for both the boosted and non-boosted gilts. One of the SIV twice exposed gilts farrowed at 117 d of gestation, whereas the other 5 gilts farrowed on day 114 or 116 of gestation. Also, stillborn piglets were delivered early and late in the farrowing process. The actual mechanism for SIV to cause fetal hypoxia and stillbirths is not clear. Because of the lack of sero-conversion, no virus isolation was attempted from stillborn piglets or placentas.

In summary, these findings suggest that intranasal SIV exposure of sero-positive dams during pregnancy may result in a few stillborn piglets per litter. The results are subtle (only a few stillbirths per

litter) and would be overlooked unless similarly matched, unexposed control gilts were used. Although subtle, these intrapartum deaths will have significant economic impact for swine herds where SIV is circulating.

Acknowledgments

The author thanks Deb Palmquist for the statistical analysis. Also, technical assistance and animal care provided by David Michael and Andrew Gibson were appreciated.

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