Persistence of antibodies after natural infection with swine influenza virus and epidemiology of the infection in a herd previously considered influenza-negative

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Summary

A herd previously considered influenza-negative became infected with swine influenza virus (SIV). After virus circulation had apparently stopped in the herd, antibodies to SIV could still be demonstrated in sows 28 months postinfection. The source of infection was not determined, and various possibilities are discussed.

Keywords: swine, swine influenza virus, antibodies, epidemiology

S erology is one of the most useful diag-nostic tools in swine medicine. Understanding the limits of that tool is important for interpretation of results. Hemagglutination inhibition (HI) has been the serologic test most widely used for detection of antibodies to swine influenza virus (SIV), although some diagnostic laboratories are now offering several different ELISA tests.

Results obtained using the HI test may vary, depending on the SIV strain used as antigen.[1] Rossow et al[2] showed that after an outbreak associated with an H3N2 strain of SIV, the HI test, using as antigen the regular prototype of H3N2 virus routinely used in diagnostic laboratories, gave low or no detectable titers. When the strain isolated from the case was used as antigen,

convalescent sera had expected titers (range of reciprocal titers, 20 to 320). These results demonstrate the value of testing convalescent sera with homologous virus when serological test results are unexpectedly negative or low.

The half-life of maternal antibodies was estimated by Loeffen et al[3] to be about 12 days for both H1 and H3 influenza viruses. Depending on the initial titer of the dam, piglets may thus remain seropositive for as long as 3 to 4 months.

Another issue concerning serological diagnosis of SIV is persistence of detectable antibodies after vaccination or natural infection. Erickson et al[4] reported that following the use of one commercially available vaccine, antibodies to SIV could be detected only for a short time. Pigs had their peak titers 2 weeks after the second vaccination, and were seronegative 8 weeks later, when tested both by HI and an ELISA.

The literature is surprisingly scarce on long-term persistence of SIV antibodies after natural infection. Knowing how long these antibodies last is important, since it may help determine when contact with the organism occurred. If antibodies last only a few months, high antibody titers in nonvaccinated animals should mean that infection is recent. This would be true for all pigs except piglets that have recently consumed colostrum containing SIV antibodies. Renshaw[5] found that some experi-

mentally-infected pigs, tested using the HI test, were still seropositive on day 441 after infection. However, only four pigs were infected and followed serologically, and the antigen used in the test was the strain used for inoculation, which might have positively affected the persistence of the antibodies detected.

It may be difficult to obtain data on persistence of antibodies over a long time period because, in a field situation, it is often not possible to know whether a seronegative animal that initially becomes infected with the pathogen of interest later comes in contact with the same pathogen. If this were the case, re-infected pigs might mount a secondary immune response, which would falsely suggest that antibodies were persisting a long time. The ideal situation occurs when initial infection of a previously negative herd is detected, and the organism is then eliminated from that herd. Since the organism is no longer circulating in the herd and the date of the initial infection is known, it is possible to determine how long antibodies to that pathogen may persist after natural infection.

The means by which swine herds become infected with SIV are not always identified. The present case investigates both the persistence of antibodies following natural infection of a herd, and means by which the herd might have become infected.

Case description

A small farrow-to-finish herd of 160 sows, maintained for the purpose of selling breeding stock, was populated in 1999. The herd, which was housed within a single building divided into rooms, was considered negative for SIV and many other significant pathogens of swine, including porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, and *Actinobacillus*

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pleuropneumoniae, on the basis of lack of clinical signs, the origin of the herd (populated using medicated and segregated early weaning), serological testing, and slaughter checks. Serological tests that were performed on a regular basis included HI for swine influenza (Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec, Saint-Hyacinthe, Québec, Canada); Idexx ELISA for PRRSV (Idexx Laboratories, Westbrook, Maine); DAKO ELISA for *Mycoplasma hyopneumoniae* (DAKO, Glostrup, Denmark); and long-chain LPS ELISA for *Actinobacillus pleuropneumoniae* (Faculté de Médecine Vétérinaire, Saint-Hyacinthe, Québec, Canada). The usual procedure was to test finishing pigs in the rooms containing the oldest animals, with testing at least four times annually for most agents, and less frequently for SIV. Three to five pigs per room, in a minimum of two rooms, were normally sampled. For example, 20 pigs were tested on April 2, 2001 (Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec), and all were seronegative to SIV.

At the end of May 2001, finishing pigs suddenly began to cough. According to the owner, the clinical signs started on May 24, peaked on May 27, and were almost gone on June 4. Coughing was particularly evident in pigs in the finishing unit, although it was also observed to a lesser extent in nursery pigs. Only one finishing pig died after showing respiratory signs, and sows were virtually unaffected clinically. This sudden onset and cessation of clinical signs, in a herd that was thought to be previously negative, strongly suggested the involvement of SIV. Fifteen finishing pigs sampled on June 4 were all seropositive (Biovet Inc, Saint-Hyacinthe, Québec, Canada). The HI test was performed in a different laboratory in June than in April for practical reasons, but both diagnostic laboratories used the same H1N1 strain of SIV as antigen (A/Sw/Quebec/91). The 15

pigs sampled in June were tested serologically for porcine respiratory coronavirus (Svanova Biotech, Uppsala, Sweden) and PRRSV, and found negative. Table 1 shows the SIV serological results for finishing pigs tested at that time, as well as results of testing previous and subsequent serum samples obtained from pigs of the same age in that herd.

Starting in January 2002, SIV serological results for finishing pigs were negative, and have remained negative since then. Fifteen blood samples obtained from sows in November 2002 and September 2003 were tested for antibodies using an HI test (Biovet Inc) and, for the November 2002 samples, a blocking ELISA test (Biovet Inc) (Table 2). The A/Sw/Quebec/91 strain of SIV was used as antigen in both tests. All sows born in the herd in October 2001 or later have remained negative to SIV. All 10 of the sows tested that were present in the herd in May 2001 (ie, during the SIV outbreak) were still seropositive in November 2002. Five of these 10 sows were tested again in September 2003 (approximately 28 months postinfection), and four were still seropositive, one of them (Sow #1) at the highest dilution tested. These results in sows, coupled with the serological results in finishing pigs and with the lack of clinical signs, suggest that by October 2001, SIV had stopped circulating in the herd, and that antibodies to SIV, detected by both tests, persisted in the sows for a long time after natural infection.

The source of infection for this herd, where strict biosecurity measures were observed, was not identified. As the herd had been totally closed since its population in 1999, and used an internal replacement system, introduction of asymptomatic carrier pigs was not responsible for the infection. The herd is not in a hog-dense area and is 4 km away from the nearest farm. It is not known if that closest herd was infected with SIV or not. The producer reported

that 3 or 4 days before clinical signs were noticed, a strong swine manure odor, not coming from his own barn, was perceptible at his farm site, which was something that had never happened before. Swine slurry from one or a few farms had apparently been dispersed on the land closer to his farm than in the past.

Discussion

Attempts to isolate the SIV virus were not made because finishing pigs, which in May 2001 showed classical signs of swine influenza, were all seronegative by HI in April 2001 and all seropositive in June 2001. Although the HI test was performed in different laboratories in April and June, the same SIV strain was used as antigen in both tests, and results presumably were comparable. Furthermore, samples obtained from the herd in July 2002 and tested by HI in the second laboratory were all negative, as were samples tested in May and October 2003 by the first laboratory.

Although these data do not necessarily mean that antibodies to SIV produced after natural infection will always persist for a long time, the present case suggests that in some situations, they may persist for more than 28 months. These results for naturally infected pigs are in agreement with those obtained by Renshaw[5] for experimentally-infected pigs.

Another point of interest concerns the relationship between the time of infection and the titers obtained. It is normally expected that serological titers will reach their peak a few weeks or months after infection with a given organism, then gradually decline over time. Results obtained in this study show that detection of high SIV antibody titers is not limited to cases where a recent infection occurred.

It is also relevant to question whether the virus totally disappeared from the herd after the clinical outbreak, or if some animals might have remained carriers of the virus

Table 1: Finishing pigs seropositive by the hemagglutination inhibition test for swine influenza virus (SIV) in a herd previously considered negative for SIV but infected with an H1N1 strain in May 2001

	October 2000	April 2001	June 2001	January 2002	April 2002	July 2002	May 2003	October 2003
No. tested	10	20	15	10	10	8	15	10
No. positive	0	0	15[1]	0	0	0	0	0

[1] Range in reciprocal serum titers of 10 to 160, with titers ≥ 10 considered positive.

Table 2: Birth dates and reciprocal hemagglutination inhibition (HI) titers of sows in November 2002 and September 2003 in a herd that had a swine influenza outbreak in May 2001 and no evidence of virus circulation later

Sow ID	Birth date[1]	HI titer[2]	
		November 2002[3]	September 2003
1	August 28, 1999	≥ 640	≥ 640
2	August 29, 1999	160	ND[4]
3	August 29, 1999	80	80
4	December 23, 1999	320	ND
5	August 12, 2000	20	ND
6	August 14, 2000	≥ 640	320
7	October 4, 2000	160	160
8	October 4, 2000	≥ 640	ND
9	October 15, 2000	320	ND
10	November 5, 2000	160	ND
11	July 15, 2001	< 10	< 10
12	August 21, 2001	10	< 10
13	September 27, 2001	< 10	< 10
14	October 23, 2001	< 10	ND
15	November 24, 2001	< 10	< 10
16	October 7, 2002	ND	< 10
17	October 19, 2002	ND	< 10
18	October 20, 2002	ND	< 10
19	October 21, 2002	ND	< 10
20	October 23, 2002	ND	< 10
21	October 30, 2002	ND	< 10
22	November 4, 2002	ND	< 10

1   Sows #1 through #10 were in the herd at the time of the clinical outbreak in May 2001.

2   Reciprocal titers ≥ 10 considered positive. Highest serum dilution tested was 1:640.

3   Sows #1 through #15 also tested by blocking ELISA in November 2002, with positive results (≥ 30% inhibition) in all except Sows #14 and #15. The lowest positive titer was observed in Sow #13 (35.9% inhibition).

4   Not determined.

but stopped shedding it. It is generally believed that pigs infected with SIV remain carriers and shed the virus only for short periods of time.[6-8] For example, Clavijo et al[8] showed that 3 and 5 days postinfection, the virus could be isolated from nasal swabs from all 30 of 30 pigs, but 11 days postinfection, virus could not be isolated from any of 15 pigs. Furthermore, the virus could not be isolated from any of the 73 tissue samples (ie, tracheobronchial lymph nodes, lung, tonsils) tested from pigs euthanized 14 days after infection. In the present case, the lack of seroconversion in all pigs born from October 2001 onwards suggests that the virus stopped circulating in the herd after that date, and was seemingly not present in the herd anymore.

On an epidemiological basis, SIV is not considered to be transmissible through artificial insemination, which was used in this herd for genetic renewal.[9] Easterday and Van Reeth[10] reported that in densely swine-populated regions, airborne spread may contribute to explosive epidemics over large geographic areas. Tofts[11] described an outbreak of swine influenza in which one of the infected herds had no known contacts with other infected herds, but was 4 km downwind from more than 13,000 affected pigs on other farms. He concluded that transmission of the virus appeared to be by direct contact and local aerial transmission.

There is little information on the possible presence of SIV in the intestines or feces of pigs. Kawaoka et al[12] experimentally in-

fected pigs and ferrets by intranasal inoculation with strains of swine, human, equine, and avian influenza virus, and examined different sites from which virus could be recovered. More than half of the avian, porcine, and equine strains of influenza virus replicated in the intestines of ferrets, proving that intestinal replication of influenza viruses is not limited to the avian species. The human influenza strain, but not the other strains of virus tested, replicated in the intestinal tracts of pigs. Slobodeniuk et al,[13] using electronic microscopy, identified influenza virus in the small intestines of piglets with diarrhea.

Even if SIV were present in the manure or slurry from pigs, it would also need to remain infectious long enough for these to become potential sources of infection. Bøtner[14] investigated the inactivation period for some swine viruses in slurry kept at different temperatures. The inactivation time for SIV was 9 weeks at 5°C, 2 weeks at 20°C, > 24 hours at 35°C, > 24 hours at 40°C, > 2 hours and 30 minutes at 50°C, and 1 hour at 55°C. Therefore, the virus apparently can survive in slurry for a significant period of time, particularly at cool temperatures. When slurry is sprayed on the land of farms, with infectious virus present in sufficient quantities to infect pigs, aerosol or possibly insects might serve as potential carriers to introduce the virus into nearby swine herds. In the early '80s, Madec et al[15] described how the virus spread in Brittany, an area that was previously virtually negative for SIV. Dissemination was very rapid in hog-dense areas and, apart from the possibility of aerosol for herds where the introduction of infected pigs could not have been the source of infection, spraying of manure in the neighborhood of the farm was included in the list of potential causes that might have played a role in transmission of the virus. Although the chronology of events and circumstances would seem to favor the hypothesis of slurry spraying as the source of infection in the present case, it might also be coincidental. At this time, although there is no scientific confirmation that SIV may be transmitted from one farm to another by spraying contaminated slurry or manure, more research is needed before this possibility may be removed from the list of potential methods of transmission.

People might have been a source of infection as well, since transmission of SIV from

pigs to humans and from humans to pigs has been reported.[10] Because of the very high health status of this herd, few people were allowed access into the building, and those admitted were required to observe a down-time period of at least 24 hours without pig contacts, take a shower, and wear barn-designated clothes and boots. Finally, other possible means of transmission, such as birds, insects, contaminated fomites, or trucks, may also have been involved.

According to the authors' experience, SIV is one of the organisms, along with PRRS virus, *M hyopneumoniae,* and porcine respiratory corona virus, that are particularly difficult to keep out of pig barns. Adhering to strict biosecurity protocols is not as effective in preventing introduction of these pathogens, compared to others like *Sarcoptes scabiei, Brachyspira hyodysenteriae,* and toxigenic *Pasteurella multocida*. This is particularly true in areas of high swine density. Canada is free from other pathogens that might also be included in the list of organisms often transmitted from one herd to another by means other than introduced animals, for example, foot-and-mouth disease, African swine fever, classical swine fever (hog cholera), and pseudorabies viruses. More efforts are needed to determine and quantify the causes, other than introduction of asymptomatic carrier pigs, that are responsible for infection of herds previously negative for SIV.

Implications

- Antibodies detected after natural infection with SIV may persist more than 28 months.
- High antibody titers to SIV do not necessarily suggest that the animals had recent contact with the virus.

- SIV may be introduced in herds by means other than asymptomatic carrier pigs.
- More efforts are needed to determine and quantify the causes, other than introduction of infected pigs, that are responsible for infection of herds previously negative for SIV.

Acknowledgements

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