SEROPREVALENCE OF SWINE INFLUENZA IN EUROPE AND INTERPRETATION OF SEROLOGICAL FINDINGS

K. Van Reeth, G. Labarque and M. Pensaert
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Introduction

In recent years the epidemiology of swine influenza (SI) in Europe has changed. The relatively novel H1N2 subtype appears to have become widespread and to circulate concurrently with H1N1 and H3N2 swine influenza viruses (SIVs). Until recently, there was limited knowledge of the current prevalence of each subtype in different regions of Europe. The circulation of 3 subtypes has also made the interpretation of SIV serology more challenging. In this paper, we first report the results of a SIV serosurvey that was undertaken during the EU concerted action «European Surveillance Network for Influenza in Pigs» (ESNIP). In the second part, we present our personal viewpoints on the use of SIV serology in practice.

Seroprevalence of different SIV subtypes in various areas of Europe

The major aim of the serosurvey was to determine the current prevalence of the H1N2 virus throughout Europe and to compare it with that of the «older» H1N1 and H3N2 virus subtypes. ESNIP partners from Belgium, the Czech Republic, France, Ireland, Italy and Poland, and 2 diagnostic laboratories from Germany and Spain participated in this study. The areas sampled included 12 out of the 19 main swine production regions in Europe. A total of 4377 sera (5 to 10 sera per farm) from 695 commercial swine farms (55 to 101 farms per country) were collected between 2001 and 2003. The sera were from adult fattening swine in France, from first parity sows in Italy and Poland, and from sows of various ages in the remaining countries. None of the pigs were vaccinated against influenza.

All sera were examined in haemagglutination inhibition (HI) tests against H1N2, H1N1 and H3N2 SIVs at a starting dilution of 1:20. The H1N2 strain (sw/Scotland/94) used was identical in all countries, but H1N1 and H3N2 strains differed. Experimental infection studies in pigs have demonstrated that there is no serological cross-reaction between subtypes in the HI test and that HI antibodies to a given subtype provide evidence of infection with that subtype (3). Thus, HI antibody titres Ó20 for a given subtype were considered indicative of an infection with that subtype.

Seroprevalence in sows: Table 1 shows seroprevalence rates in sows at the individual and at the herd level. In most herds only part of the animals tested positive for antibodies to a given subtype, so that seropositivity rates were higher for the herds than for the individual sows. The percent of positive reactions to the H1N2 virus was highest in Belgium, Germany and Spain, and somewhat lower in Italy. These 4 countries also had high seropositivity rates for H1N1 and H3N2 SIVs. In the Czech Republic and in Ireland, only few sera reacted with the H1N2 virus and seroprevalences of H1N1 and H3N2 were lower than in the previous countries. The sera from Poland were negative for H1N2 and H3N2 antibodies and the prevalence of H1N1 antibodies was lower than in any other country.

Table 2. Seroprevalence of different SIV subtypes in fatteners in France (Brittany) and Belgium (Flanders).

	% of seropositives					
	France		Belgium			
Sub-			2001-2002		2003	
type	pigs	herds	pigs	herds	pigs	herds
H1N2	25.7	41.8	17.9	25.0	50.6	67.9
H1N1	28.9	43.6	52.4	82.1	55.4	92.9
H3N2	0.0	0.0	39.3	57.1	15.5	32.1

Table 3. Prevalence of antibodies to multiple SIV subtypes in sows and fattening swine in Belgium.

		% of animals with antibodies to … subtypes			
		0	1	2	3
Sows		6.0	25.8	38.3	29.8
Fattening	2001-02	23.8	44.0	31.6	0.6
pigs	2003	16.7	47.0	33.3	3.0

Seroprevalence in fattening swine: The serological data from fattening swine in France are compared with those of similar examinations in Belgium (Table 2). The farms in Belgium were first sampled between June 2001 and May 2002 and resampled in March 2003. The pigs in France had antibodies to H1N1 and H3N2 SIVs, but not to H3N2. Though antibodies to all 3 subtypes were found in fatteners in Belgium, seropositivity rates for H1N2 and H3N2 varied significantly between the 2 sampling periods.

Incidence of infections with multiple SIV subtypes in sows and fattening swine: The serological data from Belgium were further analyzed so as to get an idea of the % of animals that had been infected with multiple SIV subtypes during their lifetime (Table 3). Most sows showed antibodies to a combination of 2 subtypes or to all 3 subtypes. Most fattening swine were seropositive for one subtype or for a combination of 2 subtypes, and few had antibodies to all 3 subtypes.

Table 1. Seroprevalence of H1N2, H1N1 and H3N2 viruses in different European countries.

SIV	% positive in …													
subtype	Belgium		Czech Rep.		Germany		Italy		Ireland		Poland		Spain	
	sows	herds	sows	herds	sows	herds	sows	herds	sows	herds	sows	herds	sows	herds
H1N2	57.8	89.0	3.2	15.4	32.5	71.3	13.8	35.5	0.6	2.0	0.0	0.0	52.8	87.0
H1N1	80.8	97.0	16.4	39.7	65.8	90.8	46.5	82.9	17.8	41.8	7.8	8.9	38.5	65.0
H3N2	53.8	86.0	0.8	5.1	57.7	87.4	41.7	68.4	4.2	16.3	0.0	0.0	38.0	67.0

Conclusion: All 3 SIV subtypes appear to be widespread in the main swine production regions in Europe, and they circulate concurrently. H1N1 is the dominant subtype in countries with lower SIV prevalences. Both sows and fattening swine may become infected with more than one subtype, but infections with all 3 subtypes are most frequent in sows. This can be explained by the fact that sows have a longer lifetime than fatteners and thus a higher chance to become exposed to multiple SIV subtypes.

The use of SIV serology in the veterinary practice

Though many infections remain subclinical, SIV is a major cause of acute respiratory disease outbreaks in swine (2) and an important contributor to more chronic, multifactorial respiratory problems (1). In the veterinary practice SIV serology is used for various purposes: to confirm the involvement of SIV in respiratory disease, but also to assess the SIV immune status on a farm or to optimize vaccination schedules. However, the interpretation of serological data is often frustrating and it has become increasingly complex with the emergence of a third SIV subtype. In this section, we will review some frequently asked questions about HI test results, as this remains the most used and best serologic test for SIV.

Question 1. How sensitive is the HI test? The HI test is relatively sensitive, provided that suitable strains are used as antigens in the laboratory. The test detects antibodies that bind to the receptor-binding site on the haemagglutinin protein of the virus, and this binding is subtype- and to a lesser degree strain-specific. Therefore, at least one representative of each of the 3 circulating SIV subtypes (H1N1, H3N2, H1N2) should be included in the test. It is best to use relatively recent strains that match antigenically with the strains circulating in the field, because this will increase the number of positive reactions and the antibody titres measured.

Question 2. How specific is the HI test, or can it distinguish between H1N1, H1N2 and H3N2 SIVs? As mentioned higher, the test is highly subtype-specific. In experimental studies, 97% out of 116 pigs that had been infected with one or with 2 different SIV subtypes only showed HI antibodies to the infecting subtype(s) (3 and unpublished). In the few pigs with serologic cross-reactions, antibody titres were very low (10-20) when compared to those against the infecting subtype (Ó160).

Question 3. What is the significance of low (1:10 and 1:20) HI antibody titres? Because these antibody titres may be due to non-specific inhibition of the haemagglutination, some labs set the cut-off value for the HI test at 1:40. On the other hand, even pigs with HI antibody titres of 1:10 or 1:20 may have been truly infected with SIV. After experimental infection of SIV seronegative pigs, serum HI antibodies to the infecting strain peak at 1:160-1:320 by 2-3 weeks PI, but they may have declined to 1:10-1:20 by 18 to 24 weeks. In the field, young pigs with residual maternal antibodies usually develop lower post-infection antibody titres compared to completely seronegative pigs. In the authors' lab, a farm is considered positive for a given SIV subtype when Ó2 out of a group of 10 animals have HI antibody titres Ó20.

Question 4. How to confirm an SIV infection by serology or which titres are indicative of an acute infection with SIV? Paired sera (an acute serum collected when disease is seen and a convalescent serum taken 2 to 3 weeks later) from approximately 10 pigs are required. If an infection with SIV occurred, antibody titres to the causative SIV subtype will be absent or negligible in the acute serum and rising or Ó4-fold higher in the convalescent serum. Convalescent sera alone are unreliable for the diagnosis of SIV. One complication is that serological responses to a given SIV subtype can be different in pigs with antibodies to other subtypes compared to those in fully seronegative pigs. For example, experimental H1N2 infection of previously H1N1-infected pigs may boost already existing H1N1 antibodies and vice versa (3). More examples will be presented during the symposium.

Question 5. How to evaluate the SIV immune status on a (non-vaccinated) farm? For a detailed picture of the SIV immune status on a farm, we recommend to sample pigs from 3 different age categories: 1) 10 adult fattening swine, which will show HI antibodies to the SIV subtypes that circulated during the previous 3-4 months; 2) 15 randomly selected sows, which may show antibodies to subtypes circulating more than 4 months ago; 3) 10 nursery pigs. These pigs may have maternal antibodies until 6-8 weeks of age. The presence of antibodies in 8-10 week old pigs may point to the continuous circulation of SIV on the farm. It should be stressed that the SIV serological picture is usually very heterogeneous.

Question 6. Will the HI antibody profile differ on vaccinated versus non-vaccinated farms? All of the above questions apply to the situation in non-vaccinated farms. The current SIV vaccines contain H1N1 and H3N2 SIV strains and they stimulate HI antibodies to those subtypes. Many vaccinated sows in the field have previously been infected with SIV, and vaccination has been shown to cause a dramatic booster of post-infection antibody titres under experimental conditions. This may explain why antibody titres in vaccinated sows in the field are significantly higher (frequently Ó1:160) than in unvaccinated sows and relatively uniform. Maternal antibodies in pigs from these sows may last until 14-16 weeks. Another point is that serologic cross-reactions with H1N2 seem to occur more readily in vaccinated than in unvaccinated pigs.

Conclusion: Serologic findings in a pig herd are usually not uniform and they need to be evaluated at the group level. Knowledge of the SIV vaccination status is essential for a correct interpretation of serologic data. If results of the HI test remain unclear, one can perform additional serologic tests such as the virus-neutralization (VN) test, which is more sensitive.

References

(1) Janke B Proceedings AD Leman Swine Conference 2000: 10-15.
(2) Loeffen W et al. Vet. Rec. 1999: 145, 175-180.
(3) Van Reeth et al. Virus Research 2004: 103, 115-124.

Acknowledgement

SIV research in the authors' lab was financially supported by the Belgian Ministry of Social Affairs and Public Health and by the Fund for Scientific Research - Flanders.