

Serological survey of the influenza A virus in Polish farrow-to-finish pig herds in 2011–2015

Ewelina Czyżewska-Dors, Arkadiusz Dors, Krzysztof Kwit,
Zygmunt Pejsak, Małgorzata Pomorska-Mól

Department of Swine Diseases,
National Veterinary Research Institute, 24-100 Pulawy, Poland
ewelina.czyzewska@piwet.pulawy.pl

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Abstract

Introduction: The aim of this study was to assess the seroprevalence of swine influenza A virus (SIV) in Polish farrow-to-finish pig herds. **Material and Methods:** Serum samples collected from 5,952 pigs, from 145 farrow-to-finish herds were tested for the presence of antibodies against H1N1, H1N1pdm09, H1N2, and H3N2 SIV subtypes using haemagglutination inhibition (HI) test. Samples with HI titres equal or higher than 20 were considered positive. **Results:** HI antibodies to at least one of the analysed SIV subtypes were detected in 129 (89%) herds and in 2,263 (38%) serum samples. Antibodies to multiple SIV subtypes were detected in 104 (71.7%) herds and in 996 (16.7%) serum samples. Concerning the seroprevalence rate, according to age category, the highest prevalence of the antibodies was detected in weaners, with regard to the H1N1, H1N2, and H3N2, and in sows, with regard to the H1N1pdm09. The lowest seroprevalence for all evaluated SIV subtypes was detected in finishers. **Conclusion:** The study indicates that antibodies against single and multiple SIV subtypes are circulating in Polish farrow-to-finish herds and highlights the importance of conducting a molecular surveillance programme in future studies.

Keywords: pigs, influenza virus, seroprevalence, co-seropositivity, Poland.

Introduction

Influenza A virus is an enveloped virus, with a segmented, single-stranded, negative RNA genome, belonging to the *Orthomyxoviridae* family and able to infect different species, including humans, birds, and pigs (25). In pigs, the virus causes respiratory disorders, manifested by dyspnoea, coughing, nasal discharge, fever, and anorexia; inducing the so-called swine influenza (SI) (16). SI is also linked to the porcine respiratory disease complex (PRDC), a term used to describe a multi-factorial respiratory infection responsible for economic losses in swine industry. So far, three subtypes (H1N1, H1N2, and H3N2) of the swine influenza A virus (SIV) have been identified among the worldwide swine population, with different origins and genetic characteristics in different continents and regions (26). In Poland, avian-like swine H1N1 (H1N1), human-like reassortant swine H1N2 (H1N2), human-like reassortant swine H3N2 (H3N2),

and pandemic H1N1 2009 (H1N1pdm09) subtypes have recently been documented (21). Pigs are animals of great importance since, beside their susceptibility to SIV, they are susceptible to infection with both avian and human influenza viruses, and other species may be infected by influenza viruses generated in swine (7, 27). Co-circulation in a herd with different SIV subtypes increases the chance of the emergence of a new, amalgamated virus, which may represent a zoonotic potential. Such phenomenon was observed in 2009, when a new influenza A virus (H1N1pdm09) emerged causing the first influenza pandemic of the 21st century (3). Due to their pathogenicity in pigs and their zoonotic capability, an SIV surveillance programme of the subtypes currently circulating in the domestic pig population is indispensable. A previous serological study conducted in Poland analysed the prevalence of antibodies against three SIV subtypes: H1N1, H1N2, and H3N2. Since 2010, H1N1pdm09 antibodies have been circulating in Polish swine herds.

However, detailed information on the proportion of seropositive herds and the seroprevalence at the animal level was not available (12).

The aim of this study was to assess the proportion of herds and pigs with antibodies against four SIV subtypes and to evaluate the circulation of the antibodies among pigs of different ages. The second objective was to provide information on co-seropositivity to more than one SIV subtype at herd and animal levels.

Material and Methods

Study design. The study was conducted between March 2011 and February 2015. The herds included in the study fulfilled the following requirements: a farrow-to-finish operation, with sow herd size over or equal to 20, and no vaccination used against SI. In each herd, blood samples were taken from 6 sows and 6–18 pigs of each age category (*i.e.* weaners, growers, and finishers). The number of samples per farm varied due to a different farm batch farrowing interval and ranged from 24 to 54 (41 ±6.8). In total, serum samples of 5,952 pigs originating from 145 farrow-to-finish herds were tested to determine the presence of antibodies against four different SIV subtypes: H1N1, H1N1pdm09, H1N2, and H3N2.

Haemagglutination inhibition test. H1N1, H1N1pdm09, H1N2, and H3N2 antibodies were determined by haemagglutination inhibition (HI) test (18). The test was performed according to standard procedures, using 0.5% chicken erythrocytes and 4 HA units of avian-like swine H1N1 (A/swine/Belgium/1/98), H1N2 (A/swine/England/96), H3N2 (A/Sw/Flanders/1/98), and A/H1N1 (A/swine/Poland/031951/12) subtypes. All sera were tested in serial two-fold dilutions, starting at 1:20. Samples with HI titres equal or higher than 20 were considered positive. A herd was considered seropositive when at least one of the tested serum samples showed a positive HI result.

Results

HI results revealed differences in seroprevalence among the analysed SIV subtypes, at both herd and animal level. In 129 (89%) herds, at least one seropositive sample to any of the four SIV subtypes was detected. In total, 112 (77.2%; CI 69.80% – 83.3%) herds were seropositive for H1N1, 86 herds (59.3%; CI 51.2 – 67.0%) for H3N2, 78 herds (53.8%; CI 45.7 – 61.7%) for H1N1pdm09, and 61 herds (42.1%; CI 34.3 – 50.2%) for H1N2. Antibodies to only one SIV subtype were detected in 25 (17.2%) herds. Thus, antibodies to multiple SIV subtypes were detected in 104 (71.7%) herds, wherein antibodies against 2, 3, or 4 different SIV subtypes were observed in 26 (17.9%), 52 (35.9%), and 26 (17.9%) herds respectively (Table 1).

Of the 5,952 serum samples tested, 2,263 (38%) had antibodies to at least one of the SIV subtype. In general, 1,212 pigs (20.4%; CI 19.4% – 21.4%) had antibodies against H1N1, 1,012 – against H3N2 (17.0%; CI 16.1 – 18.0%), 851 – against H1N2 (14.3%; CI 13.4 – 15.2%), and 572 were seropositive for H1N1pdm09 (9.6%; CI 8.9 – 10.4%). Among pigs with antibodies to at least one SIV subtype, 21.3% were seropositive to only one SIV subtype and 16.7% were seropositive to more than one SIV subtype, respectively (Table 2).

The distribution of antibodies against different SIV subtypes according to age group is shown in Table 3. Briefly, a similar pattern of antibody circulation in different age categories was noted with regard to all evaluated SIV subtypes. In relation to H1N1, H1N2, and H3N2, the highest prevalence of antibodies was detected in weaners. Concerning the distribution of H1N1pdm09 antibodies, the highest seroprevalence was observed in sows. The lowest seroprevalence for all evaluated SIV subtypes was detected in finishers (Table 3).

Table 1. Number and percentage of SIV (H1N1, H1N1pdm09, H1N2, and H3N2) seropositive herds (n = 145; CI 95%)

Antibodies against	Number of herds	%	95% CI
H1N1 only	20	13.8	9.1 – 20.3
H1N1pdm09 only	2	1.4	0.4 – 4.9
H1N2 only	3	2.1	0.7 – 5.9
H3N2 only	0	0.0	0.0 – 2.6
H1N1 + H1N2	2	1.4	0.4 – 4.9
H1N1 + H3N2	8	5.5	2.8 – 10.5
H1N1 + H1N1pdm09	12	8.3	4.8 – 13.9
H1N2 + H3N2	2	1.4	0.4 – 4.9
H1N2 + H1N1pdm09	2	1.4	0.4 – 4.9
H3N2 + H1N1pdm09	0	0.0	0.0 – 2.6
H1N1 + H1N2 + H3N2	16	11	6.9 – 17.2
H1N1 + H1N2 + H1N1pdm09	2	1.4	0.4 – 4.9
H1N1 + H3N2 + H1N1pdm09	26	17.9	12.5 – 25.0
H1N2 + H3N2 + H1N1pdm09	8	5.5	2.8 – 10.5
H1N1 + H1N2 + H3N2 + H1N1pdm09	26	17.9	12.5 – 25.0
Negative to 4 subtypes	16	11	6.9 – 17.2

Table 2. Seroprevalence of SIV (H1N1, H1N1pdm09, H1N2, and H3N2) in Polish pigs (n = 5,952; CI 95%)

Antibodies against	Number of animals	%	95% CI
H1N1 only	494	8.3	7.6 – 9.0
H1N1pdm09 only	194	3.3	2.8 – 3.7
H1N2 only	285	4.8	4.3 – 5.4
H3N2 only	294	4.9	4.4 – 5.5
H1N1 + H1N2	102	1.7	1.4 – 2.1
H1N1 + H3N2	186	3.1	2.7 – 3.6
H1N1 + H1N1pdm09	106	1.8	1.5 – 2.1
H1N2 + H3N2	142	2.4	2.0 – 2.8
H1N2 + H1N1pdm09	46	0.8	0.6 – 1.0
H3N2 + H1N1pdm09	60	1.0	0.8 – 1.3
H1N1 + H1N2 + H3N2	188	3.2	2.7 – 3.6
H1N1 + H1N2 + H1N1pdm09	24	0.4	0.3 – 0.6
H1N1 + H3N2 + H1N1pdm09	78	1.3	1.1 – 1.6
H1N2 + H3N2 + H1N1pdm09	30	0.5	0.4 – 0.7
H1N1 + H1N2 + H3N2 + H1N1pdm09	34	0.6	0.4 – 0.8
Negative to 4 subtypes	3,689	62.0	60.7 – 63.2

Table 3. Distribution of seropositive animals in all seropositive herds, according to age group (%; (CI 95%))

Age group	Subtypes			
	H1N1	H1N1pdm09	H1N2	H3N2
Sows	29.2	36.3	24.9	26.4
	(25.1 – 32.7)	(32.1 – 40.8)	(20.7 – 29.5)	(22.7 – 30.3)
Weaners	46.1	35.5	52.3	31.5
	(42.1 – 50.1)	(31.0 – 40.2)	(47.0 – 57.6)	(27.4 – 36.0)
Growers	27.3	15.2	33.7	28.8
	(25.1 – 29.5)	(13.2 – 17.4)	(30.7 – 36.7)	(26.4 – 31.4)
Finishers	17.9	7.3	24.8	26.1
	(16.2 – 19.7)	(6.0 – 8.9)	(22.3 – 27.4)	(23.8 – 28.4)

Discussion

Serological monitoring indicates a high occurrence of antibodies against SIV subtypes in Polish farrow-to-finish herds. Among the four analysed SIV subtypes, H1N1 and H3N2 antibodies were most commonly detected in investigated herds, with rates of 77.2% and 59.3%, respectively. Thus, H1N2 antibodies were the least likely detected (42.1%) in the investigated herds. Referring the current results to other countries, similar trends of SIV occurrence were observed in Italy and Spain. The rates of 77.2% of H1N1 and 59.3% of H3N2 seropositive herds detected in the present study were very similar to those found in Italy (75.6% and 60%). The main difference was observed with regard to the percentage of H1N2 seropositive herds, which was almost four-fold lower in Italy, compared to the current findings (14). However, in Spain the prevalences of seropositive herds to H1N1, H3N2, and H1N2 were higher than in the present study and reached 92.9%, 92.9%, and 64.3% respectively (22). Interestingly, a serosurvey conducted in 146 English farrow-to-finish herds, between 2008 and 2009, revealed that H1N2 was the most commonly observed SIV subtype (45%) followed by H1N1 (21%) (13). In the investigated English swine herds, no H3N2 antibodies were recorded. Similarly, no antibodies

against H3N2 were detected in swine investigated in France and Romania (6, 17).

At animal level, the H1N1 subtype also dominates, followed by H3N2, H1N2, and H1N1pdm09, with seroprevalence rates of 20.4%, 17.0%, 14.3%, and 9.6%, respectively. A similar situation, with respect to the occurrence of dominant SIV subtype, was recorded in a previous study examining SIV seroprevalence in Poland, where fattener serum samples, collected from January 2010 to June 2012, were used (11). Despite of detected seroprevalence of 27.3% for H1N1, 20.3% for H3N2, and 16.2% for H1N2 (*i.e.* higher compared to current findings), it seems that the situation within the last five years has been rather stable. In contrast, in Spain and Belgium, the prevalence of antibodies against H1N1, H1N2, and H3N2 was higher. However, the same trend of the occurrence of dominant SIV subtype was recorded in Spain, while in Belgium, the H3N2 was most prevalent (15, 22). In Italy, the seroprevalence of H1N1 (38%) and H3N2 (30.9%) was also higher, compared to the present results, while the seroprevalence of H1N2 (4%) was more than three-fold lower (14). The differences in the seroprevalence rate among countries might result from a different study design, or other factors; *e.g.* herd size and management system, stocking and pig farming density, geographic

location, and the frequency of movement of non-farm workers between farms, which were postulated as risk factors for SIV circulation and seropositivity (9, 13, 23).

With regard to the novel SIV H1N1pdm09, antibodies were detected in 53.8% of the investigated herds. However, a seroprevalence of 9.6% was the lowest, compared to other subtypes. A comparable seroprevalence rate of 13.9% was observed in Greek pigs (8) whereas in Norway, approximately 25% of sampled pigs had antibodies against H1N1pdm09 (5). One reason for the observed differences between H1N1pdm09 seroprevalence rates might be associated with a lack of cross-protection against viruses of the same HA and/or NA subtypes, since the Norwegian pig population was free from all SIV subtypes, prior to the emergence of H1N1pdm09 in October 2009 (5). According to previous studies, immunity promoted by the avian-like swine H1N1 infection could protect pigs against the H1N1pdm09 infection (1, 4, 19).

The results of the present study provide insight into the prevalence of antibodies against one or more SIV subtypes in investigated herds and pigs. Antibodies against more than one SIV subtype were detected in 71.7% of the investigated herds. The proportion of animals with antibodies to more than one SIV subtype was lower than the proportion of pigs with antibodies to only one of them (16.7% vs. 21.3%). In previous study, it was demonstrated that the percentage of pigs with antibodies against only one SIV subtype was also more frequent than co-seropositivity to more than one SIV subtype (11). Similar results were also observed by Kyriakis *et al.* (8), who found that 27.9% of pigs had antibodies against one SIV subtype and 20.6% against multiple SIV subtypes. By contrast, in the Spanish pig population the occurrence of co-seropositivity was higher compared to the prevalence of antibodies against only one SIV subtype (22). It should be stressed that concurrent circulation in a herd with more than one SIV subtype increases the chance of co-infection of a single pig with several different SIV subtypes. The infection of a single host by more than one SIV subtype, at the same time point, provides the opportunity for reassortment events, which may affect the host and tissue specificity of the new reassortant virus, making them potentially lethal to humans (7, 27).

The dynamics of antibody circulation against all investigated SIV subtypes, in pigs of various age groups, showed a similar pattern. Referring to all the evaluated SIV subtypes, the proportion of seropositive pigs in different age groups was rather low and decreased gradually with age. In a farrow-to-finish herd, due to maternal immunity, this can persist for approximately 12 weeks. The detection of antibodies in finishers is considered as an indicator of recent SIV infection (10, 13, 24). In the present study, only 17.9%, 7.3%, 24.8%, and 26.1% of finishers had antibodies against H1N1, H1N1pdm09, H1N2, and H3N2 respectively. This suggests that the occurrence rate of recent SIV infection was rather low in the investigated

herds. However, this approach would not detect herds with recent infection among sows and weaners, and could lead to bias in favour of the proportion of herds with true SIV circulation. It should be pointed out that maternal immunity partially protects piglets against clinical signs but not against infection, and piglets with maternal immunity may become infected and shed the virus through their natural secretions (2, 20). Taking this into account, it may be assumed that serology results alone are insufficient to estimate the true epidemiological situation concerning SIV and should be extended to include a molecular approach.

In conclusion, the current study confirmed that antibodies against four SIV subtypes are widespread in Polish farrow-to-finish herds, but with moderate levels of seroprevalence rates. Due to the association of SIV with economic losses in pig production and their importance in the generation of novel virus with potential public health concerns, continuous serological monitoring of SIV is indispensable. However, to improve knowledge concerning the SIV epidemiology situation in Polish pig population and to ameliorate the effectiveness of control strategies concomitant to serological monitoring, future studies should involve molecular surveillance of SIV circulation.

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