SEROLOGICAL EVIDENCE OF CO-CIRCULATION OF DIFFERENT SUBTYPES OF SWINE INFLUENZA VIRUS IN POLISH PIG HERDS

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Abstract

The aim of the study was to estimate the current epidemiological situation concerning swine influenza (SI) in Poland. The study was based on an annual passive survey of 11,770 fatteners’ sera from 584 herds, taken at slaughterhouses within the last 30 months (from January 2010 till June 2012), as well as, an active monitoring conducted in 2011 and 2012, in 25 farms, using 388 sera taken from life pigs of different age/technological groups. The analysis of simultaneous circulation of different swine influenza virus (SIV) subtypes was taken into a deep consideration. The wide spread of SIV in Poland, including the occurrence of multiple SIV infections was demonstrated. In 2010 and 2011, the domination of H1N1 subtype and the most frequently co-circulation of H1N1 and H1N2 viruses was evidenced, while in the first 6 months of 2012, the co-circulation of H1N1 and H3N2 viruses was detected more often. Based on the obtained results, it can be stated that the epidemiological situation concerning SI in Poland is dynamic and similar to that observed in other European regions with high pigs’ density; however, the prevalence of antibodies and the occurrence of mixed SIV infections is lower than in Western Europe.

Key words: pigs, swine influenza, swine influenza virus, co-infections, serosurveillance, Poland.

Swine influenza (SI) remains one of the most important and worldwide prevalent viral respiratory diseases of pigs, causing significant economic losses in swine industry. It is considered that swine influenza virus (SIV) may be responsible for up to 50% of acute respiratory disease outbreaks in pigs (12, 19).

It is known that three influenza A virus subtypes are currently circulating in swine worldwide: H1N1, H3N2, and H1N2. The origin, as well as, the antigenic and genetic characteristics of SIV subtypes varies in different continents or regions (12, 28). The first significant outbreaks of SI on the European mainland occurred in the late 1970s, after the transmission of H1N1 virus from wild ducks to pigs (14). This avian-like H1N1 virus has become established in the European pig population and became the dominant H1N1 SIV strain (3). Viruses of human origin, A/Hong Kong/68-like H3N2 formed a stable lineage in European swine since the early 1970s, but reassortant H3N2 viruses with human haemagglutinin (HA) and neuraminidase (NA) genes and avian-like swine H1N1 internal protein genes have become dominant since the mid 1980s (4, 5). Finally, triple reassortant H1N2 viruses have been isolated from pigs throughout Europe since the mid 1990s (2, 9, 11, 17, 23). These viruses contain an HA of human influenza virus origin, a NA of swine H3N2 virus origin, and internal protein genes of avian-like swine H1N1 virus origin (2). The HA of these H1N2 viruses demonstrates a low antigenic and genetic homology (only 70.4% amino acid identity in the HA1 region) with avian-like H1N1 viruses and there is no, or very rare, cross-reaction between H1N1 and H1N2 viruses in the haemagglutination inhibition (HI) test (23).

Currently, all three SIV subtypes are co-circulated within the global swine population, including Europe (3, 24, 28). In the most pig producing countries, SIVs are well adapted to pigs, therefore, infections caused by mentioned viruses are endemic. However, the prevalence and incidence of individual subtypes vary from one country to another and between different technological groups (8).

No harmonised control rules are laid down in the legislation for influenza in pigs. In some countries passive surveillance for direct or indirect detection of SIV circulating in pig population is conducted with the use of an HI test (8, 16, 25). In Poland, such passive serological surveillance programme for SI was initiated at the end of the 90s.

The aim of the paper is to present the current epidemiological situation concerning SI in Poland based on the results of the annual passive serosurvey conducted in slaughterhouses, and the results of active monitoring conducted in pig herds, using sera from
animals of different age/technological groups. The great attention was given to the analysis of simultaneous circulation of different SIV subtypes among Polish herds.

**Material and Methods**

**Samples for passive serosurvey.** Sera were collected from non-vaccinated fattening pigs in slaughterhouses located in all regions of Poland, during 30 months (January 2010 - June 2012). In total, 11,170 sera from 584 farms were tested.

**Samples for serological profiles (active monitoring).** Sera were collected from basic herds (sows and gilts), as well as, from 4, 6, 8, 10, 12, 16, and 20 weeks old pigs, raised in 25 farms located mostly in the North-East Poland. In total, 388 samples were analysed.

**Viruses.** Three reference SIV strains representing different subtypes: avian-like swine H1N1 (A/sw/Belgium/1/98), human-avian reassortant H3N2 (A/sw/Flanders/1/98), and reassortant H1N2 (A/sw/Gent/7625/99) were used. H1N2 virus has a human-like HA and it does not cross-react with avian-like H1N1 virus in cross-HI tests with post-infection swine sera.

**Haemagglutination-inhibition (HI) test.** Sera were tested in HI test performed according to a standard procedure. All sera were treated with receptor-destroying enzyme from *Vibrio cholerae* and adsorbed with chicken red blood cells (RBC) to remove non-specific inhibitors of agglutination and natural serum agglutinins, respectively.

Two-fold serum dilutions were tested, starting at a dilution 1:20. HI tests were performed with 4 haemagglutinating units of the virus and 0.5% chicken RBC. All necessary reference sera, virus, and RBC controls were included in each test. The sample was considered seropositive when the antibody titre was ≥20 to a given subtype.

**Results**

The wide spread of SIV in Poland within the last 30 months was demonstrated. From a total number of 11,170 tested sera as much as 7,127 (63.8%) gave positive result in HI test. Detailed data concerning the number and percentage of seropositive animals against three SIV subtypes among sera taken from fattening pigs in slaughterhouses are presented in Table 1.

Our data clearly present that H1N1 subtype was dominant in 2010 and 2011. In 2010, the antibodies against H1N1 reference strain of titre ≥20 were detected in 1,583 sera (30.1%) and it was about 2 times higher when compared to the two remaining subtypes. In 2011, the distribution of antibodies against all SIV subtypes was rather equal but a slight domination of H1N1 was still visible, while in 2012 it was slightly lower in comparison to H3N2 virus.

When analysing the situation in 2011, the prevalence of antibodies against H1N2 and H3N2 was clearly about 2 times higher, in comparison to the situation noted in 2010.

In the first six months of 2012, the seroconversion against H3N2 virus was also very high and reached the level similar to that observed during 12 months of 2011. It is possible that this subtype would be dominant among Polish herds in the future.

The antibody titre in tested sera was usually low, which confirms the endemic circulation of the mentioned viruses among Polish herds.

The individual serological data were further analysed to determine the percentage of animals that had been infected with multiple SIV subtypes. Summarised indirect data concerning the co-circulation of the viruses, estimated by using the HI test, are presented in Table 2.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of tested sera</th>
<th>H1N1% of positive sera</th>
<th>H1N2% of positive sera</th>
<th>H3N2% of positive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>5,250</td>
<td>583/30.1</td>
<td>626/11.9</td>
<td>815/15.5</td>
</tr>
<tr>
<td>2011</td>
<td>3,379</td>
<td>996/29.5</td>
<td>862/25.5</td>
<td>875/25.9</td>
</tr>
<tr>
<td>2012</td>
<td>2,541</td>
<td>465/18.3</td>
<td>322/12.7</td>
<td>583/22.9</td>
</tr>
</tbody>
</table>

Table 1

Prevalence of antibodies to H1N1, H1N2, and H3N2 swine influenza viruses in fattening pigs’ sera

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of positive sera analysed</th>
<th>H1N1+H1N2% of positive sera</th>
<th>H1N1+H3N2% of positive sera</th>
<th>H1N2+H3N2% of positive sera</th>
<th>H1N1+H1N2+H3N2% of positive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>3,236</td>
<td>237/7.3</td>
<td>221/6.8</td>
<td>150/4.6</td>
<td>285/8.8</td>
</tr>
<tr>
<td>2011</td>
<td>2,603</td>
<td>217/8.3</td>
<td>104/4.5</td>
<td>139/5.3</td>
<td>231/8.9</td>
</tr>
<tr>
<td>2012</td>
<td>1,370</td>
<td>90.65</td>
<td>115/8.4</td>
<td>61/4.5</td>
<td>168/12.3</td>
</tr>
</tbody>
</table>

Table 2

Co-circulation of different subtypes of swine influenza viruses in fattening pigs’ sera (based on serological examination)
As it is shown in Table 2, the infections with multiple SIV subtypes also occur in Polish herds. The percentage of pigs with simultaneous presence of antibodies against two or three different subtypes was lower than the percentage of pigs with antibodies against only one subtype. The sera with antibodies against all SIV subtypes exceed the percentage of sera with antibodies against different combination of two subtypes in every year. When antibodies against two serotypes were only detected in 2010 and 2011, co-circulation of H1N1 and H1N2 viruses were most frequently observed, while in the first 6 months of 2012, co-circulation of H1N1 and H3N2 viruses was more often evidenced, which might be correlated with increasing percentage of seropositivity against H3N2 in fatteners’ sera.

The next analysis concerns the prevalence of antibodies against all subtypes of SIV in pigs from different age/technological groups. The detailed data from the study are summarised in Table 3.

When comparing the data from basic herd, it can be concluded that SIV has circulated to a higher extent among young gilts than among sows of different parity. It is more visible when analysing the prevalence of antibodies to H1N1 and H1N2 SIVs.

Table 3
Prevalence of antibodies to H1N1, H1N2, and H3N2 swine influenza viruses in pigs from different age/technological groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of tested sera</th>
<th>H1N1</th>
<th>H1N2</th>
<th>H3N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>sows</td>
<td>124</td>
<td>40/32.2</td>
<td>38/30.6</td>
<td>40/32.2</td>
</tr>
<tr>
<td>gilts</td>
<td>30</td>
<td>14/46.7</td>
<td>13/43.4</td>
<td>10/33.4</td>
</tr>
<tr>
<td>piglets</td>
<td>78</td>
<td>68/30.3</td>
<td>56/25</td>
<td>113/50.4</td>
</tr>
<tr>
<td>weaners</td>
<td>78</td>
<td>58/23.4</td>
<td>37/14.9</td>
<td>125/50.4</td>
</tr>
<tr>
<td>fatteners</td>
<td>78</td>
<td>53/18.6</td>
<td>54/18.9</td>
<td>111/38.9</td>
</tr>
</tbody>
</table>

The survey for H1N2 subtype demonstrated the lowest number% of seropositive samples in comparison to H1N1 and H3N2. It also varied over time, ranging from 11.9% to 25.5%; however, over 2-fold increase was evidenced from 2010-2011.

In serological investigation of the pig sera performed in the study, it was clearly demonstrated that H1N1 virus dominated in Poland in 2010 and 2011 and its seroprevalence at farm level was the highest and reached 30.1% and 29.5% of tested fatteners’ sera, respectively. It was consistent with prevalence in basic herd population. In sows and gilts, it reached 32.2% and 46.7%, respectively. These results confirm the epidemiological status of Polish herds evidenced in the previous study (10). However, during the first 6 months of 2012, it was lower, which might result from a significant increase in the seroprevalence of two other subtypes.

The epidemiological situation concerning SI in Poland is similar to that observed in other European regions containing the highest density of pigs’ population; however, the prevalence of SIV antibodies differ significantly between countries and it is still higher in Western Europe than in Poland and other Central and Eastern European countries. According to Van Reeth et al. (24) within the last decade seroprevalence rates for H1N1 and H3N2 higher than 50% were noted in Belgium (80.8% and 53.8%, respectively) and in Germany (70.8% and 58.6%, respectively), and slightly lower than 50% in Italy (46.4% and 41.7%, respectively) and Spain (38.5% and 38.0%, respectively). The seroprevalence rate for H1N2 emerged in mid 1990s, novel H1N2 reassortants comprising an HA of avian-like swine H1N1 virus origin have been detected (7, 11, 21). This second generation of H1N2 viruses is antigenically unrelated to the older H1N2 viruses from the 90s. The prevalence of this new antigenic variant of H1N2 virus remains unknown. Additionally, novel H1N1 reassortants with a human-like HA have been reported occasionally (7, 11).

The next analysis concerns the prevalence of antibodies against all subtypes of SIV in pigs from different age/technological groups. The detailed data from the study are summarised in Table 3.

When comparing the data from basic herd, it can be concluded that SIV has circulated to a higher extent among young gilts than among sows of different parity. It is more visible when analysing the prevalence of antibodies to H1N1 and H1N2 SIVs.

Analysing the data from serological profiles, obtained after the testing of sera taken from piglets, weaners and fatteners, it can be assumed that in Poland, similarly as in some other countries (20), piglets constitute a very important age group for continuing circulation of SIV in the tested herds. The percentage of sera with positive result ranged from 25% to 50.4%, depending on the virus subtype, with the highest seropositivity to H3N2 SIV. The high level of seropositivity to H3N2 subtype was evidenced also among weaning piglets and fatteners, which confirms the tendency of a wider spread of this subtype in Poland within the last months.

Discussion

The epidemiology of SI and the interpretation of HI test results for SIV have become increasingly complex over the last twenty years, because, beside H1N1 and H3N2 strains, which were the only SIVs present in European swine populations, new reassortants of H1N2 occur. More importantly, apart from the triple reassortants H1N2 containing an HA from human H1N1 viruses circulating during the early 1980s, which
was higher than 50% in Belgium and Spain (57.8 and 52.8%, respectively), 32.1% in Germany, but lower than 20% in Italy (13.8%). In Belgium, Germany, and Spain, SIV seronegative farms were rare (1%, 2.2%, and 4%, respectively), while 8% of the Italian farms tested were SIV seronegative. On the other hand, in the Czech Republic and Ireland, where small farms and backyard pig production is predominant, seroprevalence rates for H1N1 were clearly lower than in the countries mentioned above (20.7% and 17.8%, respectively); moreover, H3N2 and H1N2 antibodies were found occasionally (15). Additionally, most sows were negative for antibodies to all SIVs tested in these two countries.

In general, as previously mentioned, the H1N1 subtype is dominant in Europe; however, it seems that the H3N2 virus disappeared from some regions, whereas the H1N2 virus is becoming one of the most prevalent subtypes in others (3, 7, 9, 17, 21). The data available in the latest period (November 2010 - March 2012) from the study conducted in the frame of European Surveillance Network for Influenza in Pigs 3 indicate that 49% of SIV isolates belong to the enzootic avian-like swine H1N1 lineage that emerged in 1979, 21% belong to the enzootic human-like reassortant swine H1N2 lineage that emerged in 1994 and they are the most prevalent in Denmark, France, Germany, Italy, and Spain. Ten percent belong to the enzootic human-like reassortant swine H3N2 lineage that emerged in 1984, and H3N2 viruses are still highly frequent in Belgium, the Netherlands, Germany, Hungary, Italy, and Spain with tendency to disappear in the UK, Denmark, and France, whereas around 6% are viruses coming from reassortment between enzootic SIVs, for example a novel enzootic H1N2 strain in Denmark (18). Furthermore, new reassortant viruses, not only between the three endemic SIV subtypes, but also between SIV and seasonal human influenza viruses, have occasionally been detected during the last years, for example the pandemic pH1N12009 strain, which has become endemic in several pig herds in the world, including Poland, within the last 2 years.

It should be emphasised that although the epidemiology of SI differ between European countries, it is entirely different between continents (28). In the USA, H1N1, H3N2, and H1N2 SIVs are also enzootic (seroprevalence about 66.3%-100%), but their origin and evolution differ from that of the European viruses and several different genotypic variants of reassortant viruses have been isolated from pigs in the North America (6, 12, 13, 22, 26).

The prevalence of SIV specific antibodies in Asia is smaller (about 45%) than that observed in the USA and different genetic variants were isolated from this geographic region (1, 27). The differences indicated above can be partly explained by the different geographic location, lower pig density and/or differences in the structure of the swine industry, as well as, economic situation. It is well known that for SIV, as for other respiratory viruses, high pig density, increasing herd size, short distance between herds, and large size of neighbouring herds increase the risk of infection with SIV (19). The extensive circulation of SIV in swine dense regions and the occurrence of co-infections are facilitated by the continuous availability of young non-immune pigs. The structure of the swine industry may also explain why SIV may continue to circulate in the population year-round, while human influenza has a seasonal occurrence. Indeed, the vast majority of pigs are slaughtered at the age of 6 months and the feeder and fattening pig population has a rapid turnover, so that susceptible individuals are continuously available.

As regards the co-infection of pigs with different SIV subtypes the study has demonstrated that mixed infection with SIV subtypes exists in Poland. This is emphasised by the fact that 4%-8.4% of the pigs simultaneously had antibodies against two subtypes, and 8.8% to 12.3% were positive to all subtypes. Thus, H1N1, H1N2, and H3N2 viruses are not only co-circulating concurrently, but they also frequently cause consecutive or mixed infections. This provides an opportunity for genetic reassortment to occur between these viruses (11, 21). While antigenic drift within a given virus subtype is less important in pigs than in humans (3), more influenza virus genotypes are apparently circulating in pigs, compared to other mammalian species.

Data concerning the circulation of multiple SIV subtypes, obtained in the study, also demonstrated a lower prevalence of co-infections, when compared to the situation observed in other European countries, with a high seroprevalence of SIV. In the present investigation, the percentage of sera with multiple antibodies was found lower than the percentage of fatteners positive to single SIV subtype, while in most European pig producing countries the percentage of pigs with simultaneous presence of antibodies against two or three different subtypes exceeded the percentage of fatteners with antibodies against only a single subtype. This might result from a significantly lower prevalence of SIV in Poland and different herd structure or management systems.

Summarising, the epidemiological situation concerning SI in Polish pig herds is similar to that observed in European countries, but the prevalence of co-infections with more than one SIV subtype is smaller.

Since pigs infected with SIV are considered as an important factor for public health concern, therefore, the regular global scale monitoring of the epidemiological situation concerning SI is necessary for providing new data on epidemiology of the disease, as well as, for human health protection.

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References