SEROPREVALENCE OF ANTIBODIES AGAINST SWINE INFLUENZA VIRUS IN PIGS OF DIFFERENT AGE

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Abstract
The prevalence of antibodies specific to swine influenza virus (SIV) among domestic pigs of different age, raised in large farms in Poland and Lithuania, was evidenced. Two thousand seven hundred and thirty five blood samples, taken from non-vaccinated animals were tested in HI assay against 3 SIV strains (H1N1, H1N2 and H3N2 subtypes). As a positive result the haemagglutinin titre ≥20 was estimated. Performed monitoring study showed that the most spread is H1N1 subtype of SIV. The occurrence of antibodies specific to SIV depended on the age of the tested animals. The first parity sows in Poland had low level of antibodies against H1N1 subtype (about 8%, at both - farm and individual levels) and no antibodies against H1N2 or human variant of SIV. In Lithuania, first parity sows presented the antibodies against H1N1 (27.3% of farms) and H1N2 (9.1% of farms) subtypes. The percentage of seroconversion among individual sows reached in this country 1.1 and 0.8 for H1N1 and H1N2 subtypes, respectively. It should be stressed that no antibodies against any of the tested subtypes of SIV were detected in boars and suckling piglets. The highest percentage of animals producing antibodies to all 3 antigens was detected, in both countries, among weaners and fatteners. In Poland it reached the level 11.1, 5.5, and 2.8 while in Lithuania - 18.2, 9.1, and 0, respectively against H1N1, H1N2 and H3N2 subtypes. The titre of the sera ranged from 20 to 160, but in the most samples it was low.

Key words: swine, swine influenza, serological monitoring, age.

Etiological agent of the disease is swine influenza virus (SIV) type A, belonging to the family Orthomyxoviridae, genus Influenzavirus (1, 5). Influenza viruses infect a large variety of species, including human (7, 18). Therefore, in addition to the economic impact there is the public health risk, posed by maintenance, evolution, and emergence of influenza viruses in swine (17). It should be stressed that pigs are an important host in virus ecology since they are susceptible to infection with both avian and human strains (20). They served as major reservoirs of H1N1 and H3N2 viruses and are often involved in interspecies transmission (19).

The course, nature, and severity of SI vary with the virus strain, but also with the age and immune status of the host (5, 15, 18).

Due to the fact that influenza viruses have the ability to agglutinate chicken and most mammals’ erythrocytes, the most common test used for serological evidence of specific influenza virus antibodies is haemagglutination inhibition assay (HI) (5).

The aim of the study was to determine the prevalence of antibodies specific to currently circulating swine influenza type A viruses among domestic pigs of different age, raised in large farms located in densely swine populated area in Poland and Lithuania. This data will provide a preliminary epidemiological picture useful for SI control.

Material and Methods

Serum samples. Two thousand eighty eight blood samples, taken from vena cava cranialis of non-vaccinated animals from different groups (sows and boars from reproduction units, as well as suckling piglets, weaners and fatteners), raised in 36 farms located in different provinces of Poland, as well as 647 blood samples from animals raised in 11 Lithuanian farms were tested. During the sampling, animals involved in the study were treated according to the established standards for the human care and use of animals. Samples of sera were stored at -20°C prior to testing.
Virus strains. Three reference SIV strains type A, of subtypes H1N1 (strain A/Sw/Bel/1/98, the titre EID$_{50}$ $10^{7.5}$/ml), H1N2 (strain A/Sw/Eng/96, the titre EID$_{50}$ $10^{6.8}$/ml) and H3N2 (strain A/Sw/FI/1/98, the titre EID$_{50}$ $10^{6.5}$/ml) were used in this study. HA titre was 64 for H1N1 strain and 256 for both H1N2 and H3N2 viruses. All three strains were provided by the University of Ghent.

The viruses were grown in the allantoic and amniotic cavities of 10-day-old embryonated SPF chicken eggs (Lohmann, Germany). After harvesting, the viruses were pooled, tested for haemagglutination activity using 1% (v/v) chicken red blood cells (RBC), aliquoted and stored at -70°C prior to use in serological assays.

Reference sera. In order to control the HI assay the reference hyperimmune sera for each subtype, prepared in chickens according to standard procedure, were included. The titre of control sera ranged from 640 (H1N1) to 1280 (H1N2 and H3N2 subtypes).

Reagents. Receptor destroying enzyme (RDE) of Vibrio comma (Sigma) was used for the elimination of non-specific inhibitors of haemagglutination.

Haemagglutination inhibition assay. Serum antibodies to SIV were detected by the HI test, according to standard procedure, established within the project “European surveillance network for influenza in pigs”. Essentially, sera were heat- treated at 56°C for 30 min. During the next step they were adsorbed with 50% (v/v) chicken erythrocytes for 1 h at 4°C. To remove non-specific haemagglutinins they were additionally treated with 100 U/ml RDE, overnight at 37°C, followed by the inactivation at 56°C for 30 min.

In each test the necessary serum, virus and RBC controls were included. HI microassays were done using 4 haemagglutinating units (HAU) of each virus and 0.5% (v/v) RBC. HI titre was taken as the reciprocal of the highest mean antibody dilution inhibiting 4 HAU of the virus. To facilitate reading exact end-points, the plates were slanted so that non-agglutinated cells flew freely as a teardrop. As a positive result the antihaemagglutinins titre ≥20 was estimated.

Results

Performed monitoring study showed that the spreading of antibodies specific to all the tested serotypes of currently circulating SIV strains, at both - farm and individual levels, is different in Poland and Lithuania. The most spread in both countries is H1N1 subtype of SIV. Detailed results are presented in Tables 1 and 2. In Poland seroconversion to this pathogen was evidenced in 4 out of 36 (11.1%) farms. The percentage of serologically positive pigs was similar and reached the level of 9.7 (Table 1). Much lower was the distribution of H1N2 antigenic variant in the tested farms. The percentage of Polish farms in which antibodies against this subtype were detected reached 5.5%. Among individual animals the percentage of seropositives was 2.9. Serosurvey for the subtype H3N2 demonstrated that the overall seroprevalence of specific antibodies was 2.8% and at individual level - 1.8%.

In contrast to the situation observed in Poland, in Lithuania significant differences in seroconversion to SIV were evidenced between farm and individual levels. The percentage of pigs seropositive to H1N1 subtype was 2.9 while antibodies specific to this virus were detected in 3 out of 11 farms (27.3%) (Table 2). One farm (9.1%) was positive in H1N2 test, while only 10 out of 647 pigs (1.5%) have antibodies against this pathogen. All the tested pigs were free from antibodies to H3N2 subtype.

The observed differences are probably the reason of relatively small number of positive farms in comparison to the total number of the tested farms.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number (%) of positive sera (herd/individual pig)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1N1</td>
</tr>
<tr>
<td>--------------------</td>
<td>------</td>
</tr>
<tr>
<td>sows</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>boars</td>
<td>61 (8.9)</td>
</tr>
<tr>
<td>neonatal piglets</td>
<td>0 (0)</td>
</tr>
<tr>
<td>weaners</td>
<td>0 (0)</td>
</tr>
<tr>
<td>fatteners</td>
<td>4 (11.1)</td>
</tr>
<tr>
<td>total</td>
<td>4 (11.1)</td>
</tr>
</tbody>
</table>

2088  204 (9.7) | 61 (2.9) | 37 (1.8)
Table 2  
Presence of antibodies against SIV in different age groups in Lithuania

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of sera (herd/individual pig)</th>
<th>Number (%) of positive sera (herd/individual pig)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H1N1</td>
</tr>
<tr>
<td>sows</td>
<td>11</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td></td>
<td>377</td>
<td>4 (1.1)</td>
</tr>
<tr>
<td>boars</td>
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<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>0 (0)</td>
</tr>
<tr>
<td>neonatal piglets</td>
<td>11</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>0 (0)</td>
</tr>
<tr>
<td>weaners</td>
<td>11</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td></td>
<td>116</td>
<td>10 (8.6)</td>
</tr>
<tr>
<td>fatteners</td>
<td>11</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>5 (5.8)</td>
</tr>
<tr>
<td>total</td>
<td>11</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td></td>
<td>647</td>
<td>19 (2.9)</td>
</tr>
</tbody>
</table>

It was also demonstrated that the occurrence of antibodies depends on the age of animals. During the testing of the prevalence of antibodies against SIV among first parity sows in Poland it was noted that they have low level of antibodies against H1N1 subtype. The percentage of positive farms was 8.3 and the percentage of individual animals reacting with this antigen reached 8.9. All the serum samples were negative in HI tests with H1N2 and H3N2 subtypes of SIV (Table 1). In Lithuania in primiparous sows the antibodies against H1N1 and H1N2 subtypes of SIV were demonstrated. As before, significant difference was observed between farm and individual level. Three out of 11 farms (27.3%) were positive for H1N1 antigen and 1 farm was positive for H1N2 virus. The percentage of seroconversion among sows reached 1.1 and 0.8 for H1N1 and H1N2 subtypes, respectively (Table 2). No prevalence of antibodies against H3N2 subtype was evidenced. It should be stressed that no antibodies against any of the tested subtype of SIV were detected in boars as well as in suckling piglets, either in Poland and Lithuania (Tables 1 and 2). The highest percentage of positive animals, presenting antibodies to all 3 antigens, were detected in group of weaners and fatteners, in both countries. In the group of weaning piglets, aged about 2 months, antibodies specific to H1N1 antigen were detected in 4 out of 36 Polish farms (11.1%) and in 75 out of 604 (12.4%) animals (Table 1). Survey with H1N2 antigen demonstrated the presence of seropositives in 2 farms (5.5%) and in 34 weaners sera (5.6%). In Lithuania the percentage of positive farms was 9.1 while the percentage of weaners reacting with this antigen was lower and reached 2.6 (Table 2). The lowest percentage of seropositives was detected against H3N2 subtype. Comparison of seroconversion to all evaluated SIV subtypes demonstrated the same percentage of the farms in which specific to SIV antibodies were detected among fatteners and weaners. Such situation occurs in both countries (Tables 1 and 2). Analysis of the results obtained with the sera of fatteners and weaners tested with H1N1 antigen proved that at the herd level the situation was exactly the same while at the individual level the percentage of seroconverted fatteners in comparison to weaners was higher in Polish farms and lower in Lithuanian farms. The results of the test with H1N2 antigen demonstrated slightly higher prevalence of antibodies among fatteners than among weaners, in both countries. This indicates that infection of pigs with SIV occurs mainly during the mixing of animals from different litters and with different immune status, which take place just after weaning. The obtained results indicated that the titre of the tested sera was generally low, it ranged from 20 to 160 but the highest number of sera reacted at the titre 20. The titre >160 against A/Sw/Bel/1/98 strain was evidenced only in 2 sera. The titre >160 against the strain A/Sw/Eng/96 was noted in 22 sera (0.8%) and against the strain A/Sw/F1/1/98 in 14 sera (0.5%). Such results suggest earliest contact of the tested animals with SIV. Active infection of animals with this pathogen was not clinically evidenced during the testing and was confirmed by presented serosurvey.

Discussion

Infections of domestic swine with SIV were detected in most countries intensively producing pigs but there are only few papers published, concerning the distribution of infection in animals of different age. It is well known that maternal immunity can complicate the diagnosis in suckling or weaning piglets from immunised or possessing post-infection antibodies sows. Colostrum antibodies against SIV persist for 2-4 months, depending on the initial level (2, 5, 12, 18). It should be stressed that weaning pigs with high titer of maternal antibodies may be protected against fatal
illness, but not against infection and replication of the virus (10). Those animals may shed the virus (6, 10). It was evidenced that also after depletion of maternal antibodies pigs may be infected, shed virus, have signs of the disease, and have a typical primary antibody response (5, 10). The rate of virus recovery and severity of signs of the disease are inversely related to level of passive immunity (3, 4, 5, 18). The titer of serum antibody in the convalescent phase may be lower than in the acute phase of the disease, even when diagnosis was confirmed by virus isolation. This situation results from the inhibition of active antibody production by passive antibodies (3, 14, 15, 16, 18). Furthermore, maternal antibodies seem to prevent the reaction with immunocompetent cells of the host by masking the antigenic determinants of the virus (4, 6, 16, 18). When infection occurs in the presence of low concentration of passive antibodies, which takes place usually in feeder pigs, clinical illness and incomplete immunological response may be evidenced (10). Such animals re-exposed to the virus show mild disease and shortened time of its duration (4, 10).

Groene Beilage et al. (6) performed the trial aimed at the evaluation of the influence of maternal antibodies on the frequency, severity and serology of SIV infection using 3894 pigs, raised in 960 farms. They demonstrated that in North-West of Germany about 50% of non-vaccinated sows and about 80% of vaccinated sows show positive reaction with H1N1 and/or H3N2 SIV strains. They also suggest that animals between 8 to 12 weeks of age are the most often exposed to the infection with SIV. The appearance of influenza was usually at the beginning of fattening and was clearly associated with the replacement of feeder pigs.

Similar observations were published by Madec et al. (12) who examined the persistence of the activity of H1N1 subtype in 16 pig breeding units in Brittany. They noted that influenza passive antibodies rapidly decreased in piglets and after 3 months of age the conversion was evidenced only in about 10% of the investigated animals. In another trial conducted by Madec et al. (13) in growing-finishing pigs, raised in 15 farms in France, it was evidenced that clinical symptoms of SI occurred in 11 out of 15 (73.3%) tested farms. Seroconversion to H1N1 strain of SIV was noted in growing-finishing pigs in 3 farms (20%) while antibodies to H1N2 subtype were detected in 7 herds (46.7%). In 2 farms (13.3%) simultaneous seroconversion to both mentioned subtypes occurred, while none of the pigs reacted against H3N2 virus at the post-weaning stage.

Looft et al. (11) performed long-lasting study of 32 breeding herds in the Netherlands, in order to estimate the incidence of influenza virus infections, caused by H1 and H3 subtypes, in piglets before the start of the finishing period. Blood samples, taken in each farm from 4-5 and 8-9 week-old piglets, each two weeks within 5 months, were tested for antibodies against SIV in HI test and ELISA. It was evidenced that 80% of piglets at the age of 4-5 weeks had no maternal antibodies and they were fully sensitive to infection with SIV. About 50% of the tested herds were suspected of

virus circulation during the weaning period because the observed seroprevalence was significantly higher than expected. The mentioned authors suggest that the most commonly infection with SIV occurs at the age of about 10 weeks.

Jeong et al. (9) tested by ELISA 501 pig sera from different technological groups. The overall seroprevalence of the SIV specific antibodies was 39.1%. The prevalence of antibody according to the age were 47.2%, 19.6%, 36.3%, 36% and 66.7% for suckling, weaned, growing and finishing pigs and sows, respectively. In another study these authors evidenced that SIV occurs often together with PRRS. The overall dual seroprevalence of SIV and PRRSV antibodies, evidenced in this study, was 25.9%. The highest prevalence of dual antibodies was observed among sows (42.3%) and finishing pigs (34%), while the lowest among weaned piglets (9.8%) (8). It was also demonstrated that the serological results were influenced by the herd size, the highest prevalence of SIV specific antibodies were noted in the herd with 1001-2000 pigs (31.6%) while the lowest in the herd with less than 1000 pigs (18.6%).

Candotti et al. (2) analysed the time when maternal immunity for different antigenic variants of SIV disappears. They tested 1000 piglets and 298 dams (gILts and sows of the first to fourth parturition) from 21 farms of closed breeding system. In the study performed by the mentioned authors blood samples were taken from piglets of 3, 6, 9, 12 and 15 weeks of age. Only 1 out of 21 farms, located in South Italy was completely seronegative. The half life of the antibodies was between 6.3 and 7.5 weeks (6.6 for H1N1, 6.3 for H1N2 and 7.5 for H3N2). It was demonstrated that in suckling piglets maternal antibodies specific to H1N2 subtype of SIV significantly decreased from 9 weeks of age while in case of classical H1N1 strains as well as human variant of H3N2 colostral antibody concentration decreased at 15 weeks of age. The expected decline of H1 antibodies occurs during 15 weeks for subtype H1N1 and 23 weeks for H3N2. The statistically significant higher prevalence of specific antibodies to all the tested subtypes of SIV (H1N1, H1N2 and H3N2) was observed in piglets between 3 and 6 weeks of age in comparison with the other age groups. For example for the antigenic variant of H1N1 it reached the level of 36% among 3-week-old piglets and over the time the percentage of positive animals decreased to 14%, 6.8%, 3.9% and 0% in piglets from the groups of 6-15 weeks of age, respectively. These results are not in agreement with our findings because we observed the highest prevalence of specific to SIV antibodies among weaning pigs from 4-6 weeks of age up to the fattening period. The difference results probably from the fact that in Italy the vaccination against influenza is common while in Poland and Lithuania the prophylaxis against SIV is not applied.

Summarising, our results provide useful information for the evaluation of criteria for the vaccination of pigs against SIV and preliminary epidemiological picture for the control of the disease.
References