Emergence of the pandemic H1N1 2009 influenza A virus in swine herds in Poland

Iwona Markowska-Daniel, Kinga Urbaniak, Marian Porowski¹, Paweł Karbowiak², Andrzej Kowalczyk, Edyta Kozak, Zygmunt Pejsak

Department of Swine Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland,¹ Veterinary Clinic, 62-010 Pobiedziska, Poland ²Vet-Com, 10-237 Olsztyn, Poland
iwonamd@piwet.pulawy.pl

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Abstract

The outbreaks of pandemic H1N1 influenza A virus (pdm-like H1N1 2009), detected for the first time in farrow-to-finish farms in Poland, were described. The nasal swabs and lung tissue collected from diseased/dead animals were tested using molecular techniques (RRT-PCR, MRT-PCR, RT-PCR, SSG-PCR, sequencing) and virus isolation. The amplification of the genetic material extracted from the tested samples confirmed the presence of the M1 gene sequence of type A influenza virus. Using MRT-PCRs no products characteristic for HA and NA of any swine influenza virus subtypes were obtained. Using SSG-PCR, products specific for pandemic HA and NA gene fragments were detected. Six new pdm-like H1N1 2009 strains were isolated and characterized. Phylogenetic analysis of the HA and NA genes revealed that they belong to one lineage with the pandemic strain A/California/04/2009 and other human strains, including human strains isolated in Poland in 2011.

Key words: pigs, swine influenza, pandemic H1N1 2009, symptoms, PCR, Poland.

Introduction

Influenza is a worldwide spread disease, caused by rapidly evolving RNA virus, which infects humans and a large variety of animal species. The new antigenic variants of influenza virus emerge constantly, causing rise to yearly epidemics and occasional pandemics (19). At the beginning of April 2009, a new influenza virus, genetically and antigenically unrelated to the seasonal human influenza viruses, emerged in Mexico. It rapidly spread around the globe. Within the following weeks, several laboratories confirmed human-to-human transmission of the virus and this was reported to WHO. This new human pandemic virus represented H1N1 subtype (pdm H1N1 2009) and it had a unique genome constellation. It was a quadruple reassortant possessing genes that originated from: Euro-Asiatic avian-like swine influenza virus (SIV), including the neuraminidase (NA) and matrix (M); from American lineages of triple reassortant SIV, including classical swine haemagglutinin (HA), and from avian, as well as from human influenza viruses (1). Due to the genetic composition of the virus, the disease was commonly referred to “swine flu”. The new pandemic virus predominantly infected humans. However, it also spread over to different animals species, including pigs, turkeys, ferrets, cats, dogs, and cheetahs (39, 40). The first outbreak of pdm-like H1N1 2009 in swine was confirmed on May 2, 2009 at a swine farm in Alberta, Canada (13).

In order to test the capability of the new virus to be established in swine population, an experimental infection and transmission studies were performed (3, 16). The obtained results clearly demonstrated that pdm H1N1 2009 virus is entirely capable of becoming established in global pig populations.

Since 2009, several incursions of pdm H1N1 2009 virus into domestic porcine populations have been reported from Europe, North and South America, Asia, and Africa (4, 5, 7, 9, 10, 12, 14, 18, 19, 21–24, 27, 29–31, 34, 37, 38, 41). Additionally serological studies conducted in several countries demonstrated the presence of antibodies specific to the virus in swine (4, 9, 32). Nowadays, this virus is considered an endemic SIV in some regions.
In Poland, the monitoring programme for swine influenza (SI) caused by H1N1 and H3N2 viruses has been initiated at the end of the 90s (17). It consists of serological, virological, and molecular studies. H1N2 and pdm-like H1N1 2009 viruses were included in the panel of reference reagents since 2002 and 2010, respectively. In serological monitoring of Polish herds, conducted between 2010 and 2012, the presence of antibodies specific to the pdm-like H1N1 2009 virus was confirmed. In 2010, they were evident in fatteners’ sera collected in slaughterhouses located in six out of 10 voivodeships. In 2011 and 2012, antibodies were detected in fatteners’ sera from all 16 provinces, indirectly indicating spreading of pdm-like H1N1 2009 virus among swine herds in Poland. However, until 2012, no pdm-like H1N1 2009 virus or its RNA were detected in samples of nasal swabs or lung tissues collected from pigs demonstrating influenza-like symptoms, submitted for testing to the Department of Swine Diseases at the National Veterinary Research Institute in Pulawy (NVRI).

The aim of this paper is to describe the outbreaks of SI, caused by the pdm-like H1N1 2009 virus, which occurred in farrow-to-finish swine herds in Poland, and the laboratory procedures used for their diagnosis. According to the available information, this is the first direct confirmation of pdm-like H1N1 2009 outbreaks in Polish farms.

Material and Methods

Description of the farms. Two swine farms: “D” and “W” were included in this study.

Farm D. This was a large farrow-to-finish farm located in Dolnoslaskie province. The main herd in this farm consisted of 1800 sows and 600 gilts of PIC breeds. The batches of 80-90 sows were formed every week. The production was in all in–all out procedure with a thorough cleaning between batches. The piglets were weaned at 26-28 d of life. The mean number of piglets born per litter was 10.7, and mean number of piglets weaned per litter was 10.04. The farm was producing 2.38 litters per sow per year. The mean number of piglets born per sow per year was 25.5 and mean number of piglets weaned per sow per year was 23.9. The annual production of the farm reached over 50 000 fatteners.

The farm consisted of twelve buildings equipped with a mechanical ventilation system. There were 412 farrowing pens available in the units for delivery. Seven days before partum, the sows were moved to individual farrowing pens. Feed and water were dosed automatically. Pregnant and nursing sows were fed individually, according to the time of pregnancy or number of piglets. Weaners and fatteners were fed dry feed ad libitum.

A complete management and health data for the sows and their offspring were maintained. Laboratory examination confirmed that the herd was infected with *Mycoplasma hyopneumoniae* (Mhp), *Haemophilus parasuis* (Hps), *Streptococcus suis* (S. suis), and European type of porcine reproductive and respiratory syndrome virus (PRRSV).

The basic herd prophylactic programme consisted of vaccination against erysipelas, parvovirosis, colibacillosis, Glässer’ disease, and PRRS. The gilts were additionally vaccinated against circovirosis. The prophylactic programme for piglets consisted of vaccination against mycoplasmal pneumonia (MPS) and circovirosis.

Farm W. This was a small farrow-to-finish farm located in Mazowieckie province. The basic herd in this farm consisted of 80 sows of DanBred and Naima breeds. The batches of 10 sows were formed every 3 weeks. The all in–all out procedure was not applied in the production system. The biosecurity in this farm was low.

The piglets were weaned at about 28 d of life. The mean number of piglets born per litter was 13.9 and mean number of piglets weaned per litter was 11.9. The farm was producing 2.26 litters per sow per year. The mean number of piglets born per sow per year was 31.4 and mean number of piglets weaned per sow per year was 26.9. The annual production of the farm reached approximately 1230 fatteners and 680 weaners sold in the weight of 25 kg because of lack of the space.

The farm consisted of one building equipped with a mechanical ventilation system. There were 22 farrowing pens available in the units for delivery. Feed and water were dosed automatically. As in farm D, pregnant and nursing sows were fed individually, according to the time of pregnancy or number of piglets, while weaners and fatteners were fed dry feed ad libitum.

Laboratory examination confirmed that the herd was infected with European type of PRRSV, Mhp, *Pasteurella multocida* (Pm), *Bordetella bronchiseptica* (Bbr), and *Actinobacillus pleuropneumoniae* (App) - serotypes 2 and 9.

The basic herd prophylactic programme consisted of vaccination against PRRS, circovirosis, colibacillosis, erysipelas, parvovirosis, and atrophic rhinitis. The prophylactic programme for piglets consisted of vaccination against MPS, circovirosis, pleuropneumonia, and erysipelas.

Laboratory diagnosis. The nasal swabs were collected from affected animals in both farms. Additionally, the lung tissue samples were taken during post mortem (PM) examination of dead weaners in farm D. Both, the swabs and lung tissues were suspended in PBS buffer supplemented with penicillin (1000 U/mL), streptomycin (1 mg/mL), gentamicin (5 µg/mL), and FBS (5%). Tissue samples were then homogenised.

All samples were tested using molecular techniques (reverse transcription PCR - RT-PCR, real time reverse transcription PCR - RRT-PCR, multiplex
reverse transcription PCR - MRT-PCR, and Singleplex SYBR Green PCR - SSG-PCR). Simultaneously, virology methods (virus isolation and titration) were used. In each laboratory experiment, appropriate controls and reference materials were included.

**RNA extraction.** Viral RNA was extracted from the tissue homogenate, swabs’ supernatant, and in case of the viral isolates, from embryonated SPF chicken eggs fluids with the use of commercial QIAamp Viral RNA Mini Kit (QIAGEN, Canada), according to the manufacturer’s instruction.

**PCR.** The viral RNA extracted from the samples was used for partial amplification of the M gene of type A influenza virus in RRT-PCR using a QuantiTect Probe RT-PCR Kit (QIAGEN, Canada). Sequences of primers and a probe and RT-PCR protocol were acquired from Slomka et al. (28).

In order to determine the virus subtype, the samples of RNA positive in RRT-PCR, obtained directly from clinical specimens and from the isolates, were simultaneously examined in two conventional MRT-PCRs for identification of HA and NA genes. The first one, with three sets of primers, was carried out to detect fragments of HA1 of human and avian origin and HA3 genes. The second MRT-PCR distinguished NA1 and NA2 genes. MRT-PCRs were performed using one step RT-PCR Kit (QIAGEN, Canada), according to Chiapponi et al. (6). Furthermore, these samples were amplified in RT-PCR using M-MLV reverse transcriptase (Invitrogen, Canada) and random primers (Invitrogen, Canada). The obtained cDNA was then examined in SSG-PCR with 2 sets of primers specific for pandemic HA and NA sequences obtained from Poon et al. (26), with use of QuantiTect SYBR Green PCR Kit (QIAGEN, Canada).

As positive control RNAs isolated from A/Duck/Bavaria/1977 (H1N1) (av-like H1N1) and A/California/2009 (H1N1) (pdm H1N1) were used.

Real-time PCRs and conventional PCRs were conducted with the use of Stratagene MX3005P (Agilent Technologies, USA) and T3 Thermocycler (Biometra, Germany), respectively.

**Virus isolation.** The samples positive in the RRT-PCR for M gene were used for virus isolation in the allantoic-amiotic cavity of the embryonated SPF chicken eggs, according to the classic method. Three to six passages were done. The TCID<sub>50</sub> titre of the isolates were estimated in MDCK cells, according to the standard procedure.

**Sequencing and phylogenetic analysis.** The amplified PCR products were sequenced in Genomed S.A. DNA analysis service (Poland). The nucleotide sequences were compared initially by the use of the clustal W alignment algorithm method. Phylogenetic trees of HA and NA genes were determined by the neighbour-Joining method using MEGA 4 software. Sequence distance was analysed with Megalign unit from Lasergene software.

### Results

**Description of SI outbreak in Farm D.** The first symptoms of respiratory tract disorders were observed in piglets in July 2011. They were preceded by the influenza-like illness of animal care takers employed in this farm; however, there was no medical or laboratory confirmation of the presence of pdm H1N1 2009 virus. At the beginning, the symptoms observed in piglets were mild, typical for endemic form of SI. Mostly, a cough and sneezing were observed between 10-14 d after weaning and symptoms had lasted about three weeks. Within the following few days, the symptoms were more acute and typical for viral-bacterial co-infection. Some weaners died, the losses were about 2-times higher (5%-6%), in comparison to the mortality index in weaning sector before the virus introduction (2.3%-2.5%).

In September, the symptoms of acute SI were observed in fatteners and in reproduction sector - in sows with piglets, and they had lasted for the following two weeks. In order to protect the remaining sows from the illness, the mass vaccination against SI with an inactivated trivalent vaccine (Gripovac3, Merial, France) was initiated, according to the producer’s recommendation. As a consequence, a significant improvement in the health status was observed and the immune status of the herd was stabilised. Due to the improved health status and difficult economic situation, the owner of the herd decided to postpone the next vaccination. Around half of March 2012, the symptoms typical for acute SI were observed again, mostly in weaners and fatteners. Some pigs died. During the PM examination local, diffuse, dark red inflammatory foci, with distinct demarcation line from healthy tissue, typical for acute SI, were visible in the lungs.

**Description of SI outbreak in Farm W.** In this farm, no influenza-like symptoms were observed in animal care takers employed. The first symptoms of respiratory tract disorders were observed in sows in October 2012. Mostly, fever, cough, and agalactiae were noted. Additionally, the fertility index and the number of dead-born piglets increased. In piglets, just cough and, as a consequence of MMA syndrome in sows, a diarrhoea were evidenced. Within the following few days, the symptoms of acute SI occurred in weaners and fatteners. No mortality was observed.

In November and December, the symptoms of App infection were evidenced, despite the fact that the herd was vaccinated against porcine pleuropneumonia. Nasal swabs from diseases sows, piglets, weaners, and fatteners from both farms, and the lung of the dead weaners from farm D, were collected and sent for testing to the NVRI. In farm D, an amplification of the genetic material extracted from nasal swabs taken from all age groups, except sows, and from the lungs confirmed the presence of the M1 gene sequence of type A influenza virus. Additionally, the laboratory tests confirmed the presence of *S. suis* and *Hps* in the
lung tissue samples tested. In case of farm W, the presence of the M1 gene sequence of type A influenza virus was confirmed in all nasal swabs tested.

Due to the positive signal in RRT-PCR, virus isolation was undertaken. Six new isolates of SIV - pdm-like H1N1 2009 - were received, two from material submitted by farm D (from nasal swabs and lung) and four from farm W, from nasal swabs of pigs. All these strains produced cytopathic effect in MDCK tissue culture. The TCID<sub>50</sub> titre of pdm-like H1N1 2009 isolates ranged from 10<sup>2.8</sup> to 10<sup>5.63</sup>/mL. The strains were named: A/swine/Poland/031951/2012, A/swine/Poland/031952/2012, A/swine/Poland/1562021/2012, A/swine/Poland/1562022/2012, A/swine/Poland/1562023/2012, and A/swine/Poland/1562025/2012.

The results of the RRT-PCR obtained from the isolates are presented in Fig. 1.

The new isolates were molecularly subtyped. Using MRT-PCRs, no products characteristic for HA and NA of any SIV subtypes were obtained (data not shown). Using SSG-PCR, the obtained cycle threshold values (Ct) ranged from 20.96 to 28.36 and from 17.46 to 30.00, for pandemic HA and NA gene fragments, respectively. The amplification plot is shown in Fig. 2, while the melting temperature (Tm) of each product in these reactions was shown in Table 1.

These were the first pdm-like H1N1 2009 viruses detected in laboratory examination of samples submitted from clinical outbreaks in Poland.

The HA1 and NA2 genes were sequenced and used for the phylogenetic analysis. The HA and NA genes of viruses isolated from both farms were closely related (97.6% and 96.6% nucleotide similarity, respectively).

Fig. 1. The thermal profile (A) and results of amplification of M1 gene of SIV using RRT-PCR A/swine/Poland/031951/2012, A/swine/Poland/031952/2012 (B), A/swine/Poland/1562021/2012, A/swine/Poland/1562022/2012, A/swine/Poland/1562023/2012, and A/swine/Poland/1562025/2012 (C). Both PCR reactions included positive and negative control (B, C).

Fig. 2. Amplification plot (fluorescence vs cycle number) and melting curve of pandemic HA (A, B) and NA (C, D) gene of SIV using SSG-PCR.
Phylogenetic analysis revealed that the described pdm-like H1N1 2009 viruses isolated from pigs in Polish herds can be segregated into one lineage, including the pandemic strain A/California/04/2009, and other human strains circulating since 2009 in Europe, Asia, and North America. A/swine/Poland/15620/12, isolated in farm W, had the highest similarity (99%) with human strain A/Warsaw/INS20/2011, while A/Swine/3195/2012, isolated in farm D, was more related to the earlier isolates of pandemic 2009 influenza viruses, with the highest nucleotide relation to A/Singapore/KK24/2010 (98.8%), and to the majority of human viruses isolated in Asia in 2009-2010 (data not shown, detailed analysis of all gene segments will be presented in another paper).

Discussion

After emergence of pdm H1N1 2009 virus in humans and its subsequent rapid spread across the world, several incursions into domestic pig herds have been reported. The human infected with the pandemic virus seem to be most likely a source for the spread of the infection to the pigs, however, the additional routes, like airborne transmission or transmission by vehicles cannot be excluded.

The presumed human-to-pig transmission was first reported in farrow-to-finish farm in Alberta, Canada (13). This herd was PRRS, Mhp, and App positive. Around April 20, 2009, pre-grower and grower pigs were clinically affected by respiratory disease. Mainly acute, deep non-productive cough with an abdominal effort, mild to moderate depression, and a decrease in feed consumption were noted. The symptoms had lasted about four weeks and a significant increase in mortality (from 0.43% to 0.87% in one building and from 0.19% to 2.04% in another one) were evidenced. At the beginning of May, the cough was noted in other production areas indicated, the virus had spread rapidly throughout the facility. In two buildings, only 5% of pigs presented influenza-like symptoms with no mortality. In nursery, only one sow and 10% of piglets were coughing, sneezing, depressed, and dehydrated; the cough was mildly productive suggesting a secondary bacterial infection. In PM examination, multifocal disseminated, dark red-purple inflammation focuses, sharply demarcated from unchanged regions were observed. The source of virus was determined to be an infected man.

The first temporal incursion of pdm H1N1 2009 virus into European pigs, described in Northern Ireland in about 5000 pig herd with 600 sows, was detected in September/October 2009 (37). The diagnosis of the case was made during routine submission of the samples for differential diagnosis of causative agents of respiratory diseases. The pigs in finishing and growing sectors experienced acute onset of respiratory signs. There was also a concurrent transient decrease in appetite noted in the lactating sows together with poor conception rates (up to 50% lower). The source of pigs' infection was not detected - no flu-like illness was reported by farmer, his family, farm workers or visitors. During mid-September two pigs, 10 and 16-week-old, from a second weaner-breeder herd died and were submitted for laboratory diagnosis. There was high morbidity in this herd (up to 100%), with mortality at 2%-3% in growers and 1%-2% in finishers. At PM examination there was slightly depressed, well-demarcated, pale purple consolidation in all lung lobes. In this farm, farmer had a respiratory illness before onset and development of pneumonia in the pigs. Subsequently, the pandemic virus was detected on the third premises, on detection followed the pre-export health certification of pigs with no apparent clinical signs. In this farm no recent history on respiratory disease in the farm staff or relatives was reported.

Consequently, the pandemic virus was discovered in Norway, England, Italy, Germany, Iceland, and Hungary (2, 9, 15, 19, 31, 38, 40). An interesting data concerning pdm-like H1N1 2009 virus in Norway was published by Grøntvedt et al. (9). Before the pandemic in 2009, an evidence for freedom from SI of Norwegian swine population was documented based on surveillance and control programmes that have been conducted since 1997. In 2009, all 118 Norwegian nucleus and multiplier herds, which all were farrow-to-finish herds, were tested for influenza A specific NP antibodies. About 43% of nucleus herds and 41% of multiplier herds were classified as positive for pdm H1N1 2009 virus. Around 40% of these herds displayed mild clinical signs of influenza-like sickness and/or increased reproductive disturbances in one or more age groups. Around 83% of these herds were in contact with people diagnosed with pandemic influenza or with people with influenza like symptoms. Mostly, pigs demonstrated typical SI symptoms and additionally symptoms such as abortions, stillbirths, reduced litter sizes, and returns to oestrus observed in sows (89% of farms). Sixty-three percent of the herds tested showed signs in two or more age groups of animals, 16% in all age group, and 32% only in one group (sows). The duration of the observed clinical signs varied between herds and between age groups.

Similar observations were reported by Williamson et al. (38) in England, where the surveillance

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<th>Table 1. Ct and Tm values obtained in SSG-PCR</th>
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programme for SI has been on-going since 1991. Sampling of the pigs was performed to give a 95% chance of detecting at least one pig with SIV, if infection prevalence was 5%. Mostly, it started around post-weaning period and usually lasted for three weeks. Nasal swabs and blood were collected. Fifteen percent of farms were tested positive in PCR, 47% of them were positive for pdm-like H1N1 2009 virus in PCR and virus isolation. The clinical symptoms were mostly visible in weaned pigs. In breeding herds either non-specific or no clinical signs were evidenced, symptoms typical for SI were observed occasionally in replacement gilts or suckling piglets. The English farms showed differences in disease presentation, and spread and duration of the infection. In some farms, pigs revealed rapidly spreading respiratory disease over a period of at least seven weeks with clinical disease being evident for 24 d but with minimal or any mortality. There were no abortions, premature farrowings, increased stillbirths, or weak piglets born to sows showing signs just prior to farrowing. Similarly to the situation observed in our study, pdm-like H1N1 2009 virus was detected concurrently with other pathogens, in particular S. suis, but no simultaneous infections with avian-like swine H1N1 or H1N2 strains were demonstrated virologically. Outbreaks of pdm-like H1N1 2009 virus were identified almost throughout the year, in 10 of 12 months following initial detection of the pandemic virus but in contrast to seasonal SI more outbreaks were evident in summer than in winter. Some farms were estimated as positive for pdm-like H1N1 2009 virus as part of the surveillance together with further on-farm epidemiological investigations undertaken.

In Italy, the outbreaks of pdm-like H1N1 2009 virus were described in Lombardia (the region with the highest density of pig population in Italy) but also in Sicily (10, 19). As in Polish herds, the SIV co-circulate with PRRSV. The Italian isolate from pigs has shown three unique amino-acid changes in PB2, PB1, and PA. Similarly, in Hungary two new porcine H1N1 influenza viruses were obtained from influenza-like illness that had unique amino acid changes in PB2, HA, and NA genes, compared to the H1N1 viruses of pig origin (2).

The incursion of pdm H1N1 2009 virus into pig herds was also reported repeatedly from Australia, Asia (Singapore, Japan, Thailand, South Korea, Vietnam,), North America (The United States and Canada), South America (Argentina and Brazil), and Africa (Cameroon, Reunion Island) (4, 5, 7, 9, 10, 12–14, 18, 19, 21–24, 27, 29–31, 34, 37, 38, 40, 41).

From the data presented above, a wide spread of new virus in pig population is clearly visible. Moreover, in some countries the reassorted pdm H1N1 2009 virus was isolated from pigs (11, 20, 25, 33, 35). In Germany, pig herds were investigated in greater details since 2009. Swabs and lung tissue from pigs with respiratory symptoms were examined for the presence of influenza A virus genome by modified generic real-time RT-PCR detecting part of the M gene, and positive samples were further characterised by conventional RT-PCR assays, and finally subjected to sequence analysis of full-length genome segments. Influenza RNA-positive samples were also processed for virus isolation and the isolates were antigenically characterised by HI assays. Out of 20 H1 viruses isolated from seven German Federal States, including Lower Saxony (a region with the highest density of pig population in Germany), 13 were characterised as H1N1, three as pdm-like H1N1 2009, and four as H1N2. The HA and NA sequences of the pdm-like H1N1 2009 viruses showed the highest identity scores (about 99.5% on the nucleotide level) to human pandemic virus; however, one isolate carried the HA and six further genome segments of the human pandemic virus, but NA sequence was characterised by phylogenetic analysis as a typical representative of the European porcine N lineage. This demonstrates reassortment compatibility and activity between the pandemic strain and other swine H1N1 viruses. In Italy, the reassorted virus was also derived from pandemic human virus but carried an NA gene similar to that of H1N2 SIV (20). Similar situation occurred in 2010 in Canada, where new swine H3N2 virus reassorted with pdm H1N1 2009 virus emerged in swine and mink (33). The natural co-infection involving pandemic strain and seasonal H1N1 viruses has been also reported in human (8).

Generally, influenza A viruses are endemic within European pig population, with high levels of seroprevalence to H1N1, H1N2, and H3N2 strains. The clinical picture of SI caused by the pdm-like H1N1 2009 might vary between the herds but the clinical findings described in most countries were similar to those seen in our study, and following infection with endemic SIV circulating in swine populations. The clinical picture of natural infection caused by pandemic virus also correlated with the observations from experimental infections of pigs with pdm H1N1 2009 virus.

Weingartl et al. (36) compared the properties of pdm H1N1 2009 strain from human with swine pdm-like H1N1 2009 Canadian isolate by experimental infection of pigs. They observed differences in virus recovery from the lower respiratory tract. Human virus was consistently isolated from the lung of the infected pigs, while only one pig infected with virus of porcine origin yielded live virus from the lung, despite comparable amounts of viral RNA and antigen in both group. Virus antigen detection and viral-RNA clearance from the lungs were also prolonged.

Serological examinations for SI have been conducted in Poland since 1998 (17). Based on the results of our long-term studies it can be stated that epidemiological situation concerning SI is similar to that observed in other European countries. From the results of monitoring studies conducted between 2010 and 2012, it is clearly evident that pdm-like H1N1 2009
virus was prevalent in Polish herds much earlier before its first isolation. It should be stressed that in some cases no clinical symptoms were seen in the pigs infected with pdm-like H1N1 2009 virus, as a consequence the disease might go unnoticed. Such situation has potential implications for virus spread, as infection may pass from apparently healthy herds to susceptible herds through normal network structures. It might explain why the pdm-like H1N1 2009 virus was not discovered in Polish herds earlier.

The continued circulation of pdm H1N1 2009 virus in human and its transmissibility and spread within pig population, together with the somewhat irregular pathobiological characteristics observed in experimentally infected pigs suggest an on-going evolution. This emphasises the need for a continued active serological and virological surveillance programme to monitor the status of pig populations and analyse the porcine influenza viruses. It also shows the importance of close cooperation between different authorities from veterinary and public health.

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