Highly pathogenic avian influenza H5Nx in Poland in 2020/2021: a descriptive epidemiological study of a large-scale epidemic

Krzysztof Śmietanka[1][✉], Edyta Świętoń[1], Krzysztof Wyrostek[1], Edyta Kozak[1],
Karolina Tarasiuk[1], Natalia Styś-Fijoł[1], Kamila Dziadek[1], Krzysztof Niemczuk[2]

[1]Department of Poultry Diseases, [2]Director General
National Veterinary Research Institute, 24-100 Puławy, Poland
ksmiet@piwet.pulawy.pl

Abstract

Introduction: Highly pathogenic avian influenza (HPAI) outbreaks caused by the Gs/Gd lineage of H5Nx viruses occur in Poland with increased frequency. The article provides an update on the HPAI situation in the 2020/2021 season and studies the possible factors that caused the exceptionally fast spread of the virus. **Material and Methods:** Samples from poultry and wild birds delivered for HPAI diagnosis were tested by real-time RT-PCR and a representative number of detected viruses were submitted for partial or full-genome characterisation. Information yielded by veterinary inspection was used for descriptive analysis of the epidemiological situation. **Results:** The scale of the epidemic in the 2020/2021 season was unprecedented in terms of duration (November 2020–August 2021), number of outbreaks in poultry (n = 357), wild bird events (n = 92) and total number of affected domestic birds (approximately ~14 million). The major drivers of the virus spread were the harsh winter conditions in February 2020 followed by the introduction of the virus to high-density poultry areas in March 2021. All tested viruses belonged to H5 clade 2.3.4.4b with significant intra-clade diversity and in some cases clearly distinguished clusters. **Conclusion:** The HPAI epidemic in 2020/2021 in Poland struck with unprecedented force. The conventional control measures may have limited effectiveness to break the transmission chain in areas with high concentrations of poultry.

Keywords: highly pathogenic avian influenza, H5Nx, poultry, wild birds.

Introduction

Avian influenza virus (AIV) is an RNA virus that belongs to the *Orthomyxoviridae* family and *Influenzavirus A* genus. The classification of AIV into subtypes is based on the structure of the surface glycoproteins: haemagglutinin (HA) and neuraminidase (NA), encoded in subtype designations as H and N, respectively. So far, 16 HA subtypes and 9 NA subtypes have been identified in avian species and they form different combinations. Waterfowl and shorebirds are the natural reservoirs for AIV, in which low-pathogenic avian influenza (LPAI) viruses are sustained and cause subclinical infections (7). Upon transmission to domestic birds, LPAI viruses (LPAIV) of the H5 and H7 subtypes have the potential to mutate to highly pathogenic avian influenza (HPAI) viruses that cause serious illness with a high mortality rate and substantial losses to industry (15). The HPAI viruses (HPAIV)

usually infect wild birds as a result of "spill-back" events, *i.e.*, transmission of the virus from poultry to wild birds (21). The effectiveness of HPAIV spread by wild birds depends on genetic make-up of the virus and host-related factors (21).

In recent years, large-scale epidemics of HPAI have been mostly associated with the A/goose/Guangdong/1/1996 (Gs/GD) lineage of H5 viruses (hereafter referred to as H5 Gs/GD) that can have different NA subtypes (15). Since their first detection in China in the mid-90s, the HA genes of H5 Gs/GD viruses (initially existing as the H5N1 subtype) have evolved into multiple genetic clades, some of which (*e.g.* clades 2.2, 2.3.2.1 and 2.3.4.4) have had high propensity to infect wild birds (15, 21). In the meantime, the clade 2.3.4.4 H5 Gs/GD has shown a high frequency of genome exchange with LPAIV; the process is called "reassortment" and is responsible for the generation of different subtypes and genotypes. The analysis of the global situation with

respect to the occurrence of clade 2.3.4.4 H5 Gs/Gd in recent years proved that the H5N8 subtype has caused the highest number of outbreaks (2, 3, 8). However, as other NA subtypes have also been identified (*i.e.* N1, N2, N3, N4, N5, N6), the clade 2.3.4.4 H5 Gs/GD viruses are often described briefly as "H5Nx" viruses.

The H5 Gs/GD viruses have been introduced to Europe at least 10 times between 2005 and 2020 (21). During this period, Poland has experienced five incursions: in 2006 (clade 2.2 H5N1 subtype), 2007 (clade 2.2 H5N1 subtype), 2016 (clade 2.3.4.4b H5N8 and H5N5 subtypes), 2019 (clade 2.3.4/4.b H5N8 subtype) and 2020 (clade 2.3.4.4b H5N8, H5N5 and H5N1 subtypes) (16, 18–20). The size of outbreaks varied greatly but the 2020/2021 season was undoubtedly the largest and caused the highest economic losses to the poultry industry.

The paper describes the major characteristics of the 2020/2021 HPAI epidemic in Poland and discusses possible factors that led to the unprecedented spread of the virus.

Material and Methods

Diagnosis of HPAI. The surveillance system for HPAI in poultry was composed of passive and active components. Passive surveillance had two elements: suspicion of HPAI (birds showing clinical signs and necropsy findings suggestive of HPAI) and "testing-to-exclude" (TTE) system (*i.e.*, samples from flocks where clinical signs or gross lesions were not specific but did not allow HPAI to be ruled out). Outbreak-related surveillance included testing of contact holdings (both with and without clinical signs). Active surveillance in poultry was based on testing clinically healthy flocks from restriction and/or surveillance zones before transport to the slaughterhouse or on testing ready-to-lay poultry before shipment to a designated holding. Additionally, healthy flocks also had active surveillance mandated approximately 3 weeks after re-population of positive holdings. The standard set of samples submitted for passive surveillance included samples of organs from at least 5 dead birds, 20 oropharyngeal and 20 cloacal swabs. For active surveillance (meat and ready-to-lay poultry), 60 oropharyngeal swabs were typically collected from each flock. As for the repopulated farms, organs or swabs from at least 10 carcasses were collected at the end of a 21-day period from the date of the re-population (if dead birds were not present, oropharyngeal swabs were collected from 20 healthy birds). The numbers of flocks per species and production category tested for AI are presented in Table 1. In total, samples from approximately 1,700 poultry holdings were examined between November 2020 and August 2021 (Table 1). As regards wild birds, samples of organs or oropharyngeal/cloacal swabs were tested. During the peak of the epidemic in wild birds, in order not to overwhelm laboratory capacity, swabs were taken and

brains were harvested from wild-bird carcasses submitted to the Polish National Reference Laboratory for Avian Influenza, *i.e.*, the National Veterinary Research Institute, without detailed necropsy. Samples were collected from dead birds (passive surveillance, n = 587) and healthy birds (active surveillance, n = 990). Wild-bird sampling for passive surveillance was coordinated by the veterinary inspectorate. Samples required in active surveillance were taken in collaboration with ornithologists from the University of Gdańsk and University of Łódź. The samples were tested in a real-time RT-PCR targeting the M gene (all influenza A subtypes) and identified for their HA and NA subtype. The diagnostic methods and reagents were described previously (18).

Table 1. Number and type of flocks per species and production category tested between 24 November 2020 (date of the first outbreak) to 9 August 2021 (date of the last outbreak)

Poultry category	Type of surveillance			Total
	Passive surveillance	Contact holdings	Active surveillance	
Laying hens	219	60	22	301
Broiler chickens	88	27	234	349
Broiler breeders	132	25	5	162
Dual-purpose chickens	32	15	6	53
Fattening turkeys	174	57	157	388
Turkey breeders	1	0	0	1
Fattening geese	37	4	24	65
Geese breeders	15	1	2	18
Fattening duck	109	11	203	323
Duck breeders	9	1	6	16
Pheasants	1	0	0	1
Guinea fowl	1	2	1	4
Multi-species	17	3	1	21
Total	835	206	661	1702

Sequencing and phylogenetic analysis. The virus sequences were obtained by Sanger sequencing of overlapping RT-PCR products or by high-throughput sequencing following amplification of all segments in an RT-PCR. The methods and the analysis pipeline were described previously (18, 20). Whole genome sequences were generated for 27 viruses and for another 50 strains only HA gene sequences were obtained. Additionally, 20 whole genome sequences of Polish strains produced by the European Union Reference Laboratory (IZSVe, Legnaro, Italy) and sequences from other countries available in the GISAID (Global Initiative on Sharing Avian Influenza Data) database were also included in the analysis. Phylogenetic trees were inferred in the MEGA X program (13) by using the maximum likelihood method and Hasegawa–Kishino–Yano model with 100 bootstrap replications.

Results

Description of the situation. The HPAI epidemic in the 2020/2021 season had three discernible but sometimes overlapping phases with different epidemiological dynamics (Fig. 1). The landmarks of the

phases were determined by the first detection of the virus at the end of November 2020 with subsequent outbreaks that followed in the next weeks until the beginning of February 2021 (phase I, Fig. 2a), severe weather conditions in February that triggered an epidemic in wild birds in the following weeks (phase II, Fig. 2b) and further escalation of the epidemic (from March 2021 onwards with the peak in April), mostly associated with the introduction of viruses into high-density poultry regions (phase III, Fig. 2c). In total, between 24 November 2020 and 9 August 2021, 357 HPAIV H5Nx outbreaks in poultry, 2 outbreaks in captive birds and 92 events in wild birds involving >150 birds were confirmed, with most of the poultry outbreaks localised in Mazowieckie (n = 132) and Wielkopolskie (n = 100) provinces (Fig. 2d). The highest number of outbreaks was found in chicken layers (both breeders and hens laying eggs for consumption, n = 118) followed by meat turkeys (n = 75), fattening ducks (n = 62), breeder geese (n = 9), fattening geese (n = 8), broiler chickens (n = 6) and breeder ducks (n = 5). Twenty-four positive commercial holdings were multispecies farms. The virus was also detected in 49 non-commercial, mostly backyard poultry holdings, where chickens were the predominant species. All outbreaks in poultry were caused by the HPAIV H5N8 subtype. The clinical signs observed in infected poultry did not substantially differ from those typically described for HPAI. The disease usually began with the drop in feed and/or water consumption that was accompanied (or quickly followed) by increased mortality and a drop in egg production (the latter not always observed in hens at the onset of clinical problems), depression, on some occasions shortness of breath, other respiratory signs (conjunctivitis, nasal discharge, sneezing) and diarrhoea. The neurological signs were very clearly seen in fattening ducks and entailed a broad spectrum of symptoms including: ataxia, tremors, lying on the back and pedalling with the legs, torticollis and paralysis. Breeder ducks usually did not display obvious clinical signs, and the drop in feed/water intake and decreased production of eggs (by approximately 50%) were the only observed abnormalities. As for wild birds, the vast majority of HPAIV detections were made in swans (mostly mute swans, *Cygnus olor*, 65 events with 160 positive birds) and the remaining birds belonged to the following species: common buzzard (*Buteo buteo*, n = 7), tundra bean goose (*Anser serrirostris*, n = 4), greylag goose (*Anser anser*, n = 3), white stork (*Ciconia ciconia*, n = 3), garganey (*Spatula querquedula*, n = 2), wild goose (species unidentified, n = 1), tufted duck (*Aythya fuligula*, n = 1), great cormorant (*Phalacrocorax carbo*, n = 1), Eurasian coot (*Fulica atra*, n = 1), white-tailed eagle (*Haliaeetus albicilla*, n = 1), goshawk (*Accipiter gentilis*, n = 1), magpie (*Pica pica*, n = 1), and house sparrow (*Passer domesticus*, n = 1). Most detected viruses were classified to the H5N8 subtype but single infections with the H5N1 subtype (white stork) and H5N5 subtype (tufted duck) were also identified. The highest number of wild-bird cases was reported for Gdańsk Bay in northern Poland.

Phylogenetic and molecular investigations. A total of 97 HA sequences of Polish strains were analysed. All tested viruses belonged to the 2.3.4.4b clade and they grouped together with other HPAIV H5Nx which have circulated in Europe since autumn 2020. A significant intra-clade variation was found with spatio-temporal clusters that were identified for viruses detected in high-density poultry areas (Fig. 3). The analysis of fully-sequenced HPAIV H5Nx strains from Poland (n = 47) revealed the existence of one genotype of H5N8 subtype (genotype A). Conformations of genome segments of Polish H5N8 and H5N1 viruses were similar to those found in other European countries. In contrast, the H5N5 isolate showed a unique gene constellation as its PB2, PB1, NP and NA clustered with Eurasian LPAIV strains, whereas the remaining segments were highly similar to the 2020/21 H5N8 viruses. In two HPAIV H5N8 strains found in domestic ducks and geese at the beginning of May 2021, single mutations (PB2 D701N) associated with increased polymerase activity and replication in mammalian cell lines were detected (9).
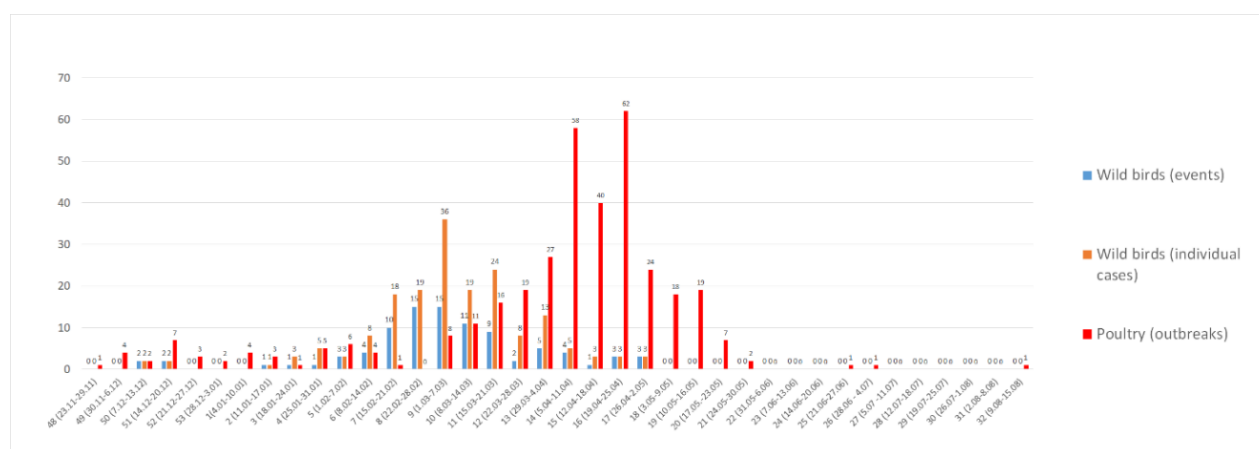

Fig. 1. Number of HPAI outbreaks in poultry and wild birds in Poland in 2020/2021 by week of detection; an epidemiological event in wild birds can involve more than one positive bird found at the same site and time
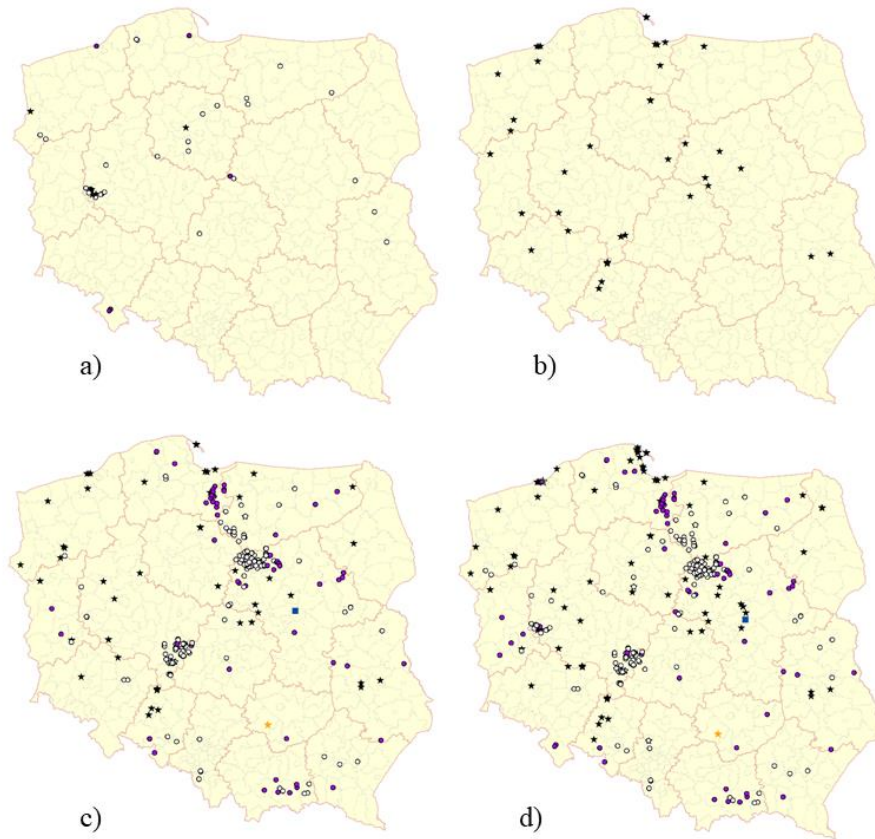
Fig. 2. Distribution of HPAI outbreaks in Poland in the 2020/2021 season. a) HPAI detections in poultry and wild birds during the first phase of the epidemic (end of November 2020–beginning of February 2021); b) HPAI in wild birds during the peak of detections in February–March 2021; c) detections in poultry, wild and captive birds since 1 March 2021; d) detections during the whole 2020/2021 season (24 November 2020–9 August 2021). White dots – outbreaks in commercial poultry; purple dots – outbreaks in non-commercial poultry holdings; black asterisks – H5N8 events in wild birds; yellow asterisks – H5N1 detections in wild birds; blue squares – detections in captive birds
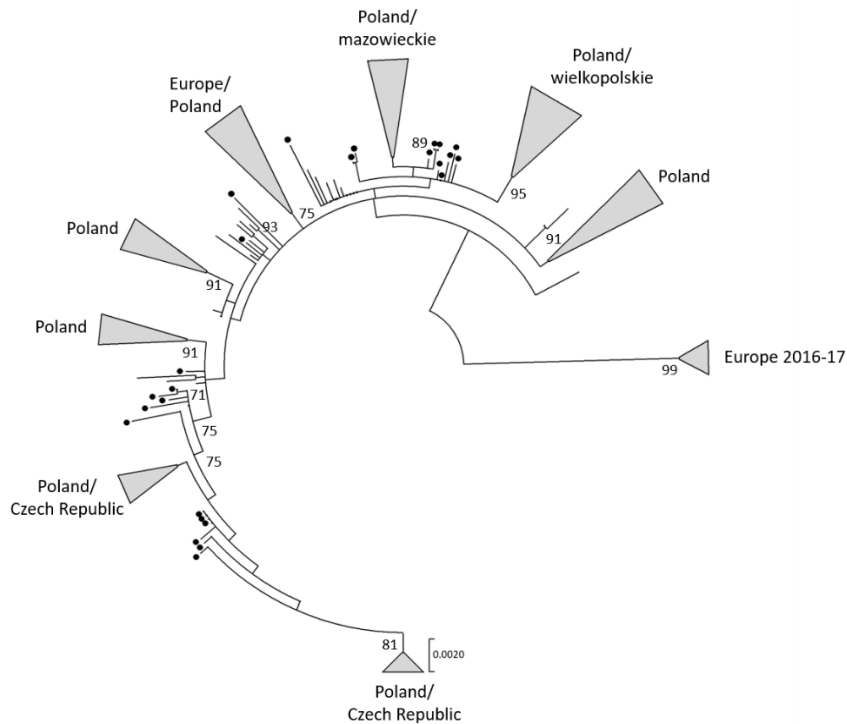

Fig. 3. Phylogenetic tree of the HA gene of Polish H5Nx viruses. Selected sequences from Europe and Asia were also included in the analysis. For clarity, several clusters were compressed and the label indicates the origin of within-cluster sequences. Polish sequences located outside the compressed groups are marked with dots. Bootstrap percentages >70% are shown next to the branches

Discussion

The first detection of H5 Gs/GD viruses in the 2020/2021 season in Europe was confirmed in mid-October 2020 in the Netherlands and was followed by notifications from the United Kingdom, Germany, Denmark, Ireland, Belgium, France and Sweden (1). The detections were made in poultry, wild and captive birds. Early in the season, the barnacle goose (*Branta leucopsis*) was the most frequently HPAIV-positive bird species but the virus was detected in more than 20 species of wild birds. The genetic analysis demonstrated that the virus belonged to the 2.3.4.4b clade but clustered independently from viruses present in Europe in previous seasons, even the directly preceding one (2). High genetic diversity was already seen in the early phase of the epidemic: the detected viruses belonged to three subtypes (H5N8, H5N1 and H5N5) and four genotypes originating from the reassortment events with LPAI viruses. In the subsequent weeks, the virus spread across Europe and diversified even further with more subtypes (H5N2, H5N3 and H5N4) and genotypes reported from multiple countries (2, 14).

The first confirmation of HPAI H5Nx in Poland was achieved quite late (24th November) in comparison to other European countries and the 2020/2021 HPAI season in Poland with its three major phases began with a rather gradual escalation. The first phase (between November and the start of February) was characterised by a small number of outbreaks in poultry (weekly median value = 3) and a very small number of wild bird cases, which on several occasions were detected only in active searches for carcasses in close proximity to virus-positive poultry holdings. With the exception of a small region in western Poland (Wolsztyn and adjacent counties in Wielkopolskie province), where to some extent a secondary spread was observed, the existing evidence coming from epizootic investigations and phylogenetic studies suggests that most of the HPAIV-positive poultry holdings were primary outbreaks that arose as a result of direct or indirect contact with wild birds. Interestingly, a lot of positive holdings were big commercial farms. In total, by the end of January 2021, 32 outbreaks in poultry and 13 detections in wild birds (in 7 locations) were confirmed. The situation was not advantageous but was stable.

In February 2021, a significant drop in temperatures (below −15℃) coincided with the field observation of moderate and mass mortalities among wild birds across a large area of Poland and marked the second phase of the HPAI season. It was particularly noticeable around the Gdańsk Bay in northern Poland. Samples collected from wild birds, mostly mute swans but also other waterfowl and birds of prey, on many occasions tested positive for an H5 virus that in the overwhelming majority of cases was subtyped as H5N8 virus. The sudden aggravation of the epidemiological situation resembled that in February 2006, when the peak of HPAIV H5N1 detections in wild birds in Europe occurred during harsh winter conditions (11). Possibly, gatherings of wild birds coming from different regions to places where the chances of survival were higher (*e.g.* unfrozen water bodies) created high population densities that favoured extensive spread of the virus. It should be noted that mass wild-bird mortalities during severe winters are not unexpected as such because they are triggered by food and water shortages. It was shown that the percentage of dead birds which are HPAIV-positive may be low even in the epicentre of infections (10), but mass die-offs of wild birds, especially in urban areas, increase the effectiveness of passive surveillance and raise the detection rate of HPAIV.

In March, as temperatures started to rise, wild birds dispersed and an increasing number of outbreaks in poultry was noted shortly afterwards. Disturbingly, the H5N8 virus spread into regions with high concentrations of poultry production. First it struck in the Wielkopolskie province, where between March and May, 81 outbreaks in poultry were confirmed. The region is abundant with domestic waterfowl production and indeed, most of the HPAI H5N8 outbreaks were detected in ducks and geese. It is noteworthy that approximately 20% of positive holdings (mostly fattening Pekin ducks) were detected through testing of healthy meat flocks before shipment to the slaughterhouse. Infection with H5 clade 2.3.4.4 (especially early viruses – or "group A" – present in Europe in 2014/15) can be asymptomatic in Pekin ducks, but the representatives of the more recent H5N8 of this clade ("group B") are usually virulent in this species (6, 12). Additionally, based on the feedback received from veterinary inspection, re-visits of the farms following positive laboratory results led to the observation that at least some of the birds in the flock had already started to sicken. Thus, the positive results in asymptomatic flocks of waterfowl were more likely associated with the detection of infection during the incubation period rather than the possibility of subclinical infection. Field observations in the same region confirmed that in non-adult domestic waterfowl, especially Pekin ducks, the disease was severe with a range of neurological signs and high mortality. Nonetheless, the obtained results highlight the indispensability of laboratory testing of apparently healthy meat duck and geese flocks from areas under restriction before movement to slaughterhouses.

The virus caused the most devastating impact in the northern part of Mazowieckie province, particularly in Żuromin and Mława counties. For many years, this region has been associated with intensive poultry farming (mostly laying hens, broiler breeders and broilers) but in the past 15 years the total production has almost tripled. Before the 2020/21 season, the presence of HPAI (caused by H5N1 clade 2.2.2 virus) was detected in the region once in 2007 (17) but the outbreaks were confined to a few commercial layer holdings and the situation was quickly contained. The

first outbreak in the current season was confirmed on 23 March 2021, but this time the attempts to stop the spread of the virus were unsuccessful and altogether >100 outbreaks in the two named counties were confirmed, most of them on large industrial farms. In order to stop the fast spread of the virus in some high-density areas, preventive culling in a 1-km radius was applied to supplement conventional control measures foreseen by the legislation. The total number of birds that died or were destroyed in outbreaks, contact holdings and other holdings located in the 1-km buffer zone exceeded 11 million. Undoubtedly, the high concentration of poultry farms located in close proximity to one another was the major drivers that favoured the rapid spread of the virus. The pathogen was dispersed by as yet not specifically identified human activities and possibly by the wind (this only over short distances). Interestingly, the statistical analysis using linear and non-linear models on data from the 2007, 2016/2017 and 2019/2020 epidemics in Poland showed that greater chicken population density did not increase the probability of HPAI outbreak occurrence (logistic regression showed even a negative impact) (10). Indeed, before the 2020/2021 season, HPAI epidemics rarely affected chickens and had the highest impact on turkeys, geese and ducks. Also in other European countries (*e.g.* France and Hungary), rapid and large-scale dissemination of the virus during this and previous epidemics caused by the H5 Gs/GD viruses had usually occurred in waterfowl and not in chickens (4, 8). The 2020/2021 season urges epidemiologists to revise previous knowledge about the risk factors related to the occurrence of outbreaks.

Although the Mazowieckie province was hardest hit during the spring of the 2020/2021 epidemic, positive results were being obtained from other regions of Poland. The virus was detected in poultry in 15 out of the 16 provinces of the country. In addition to primary outbreaks originating from wild birds, at least two human-mediated modes of virus spread were identified: intra-EU trade with infected ducklings (healthy at the time of transport) and illegal trade of infected pullets to non-commercial holdings (5). The overall direct losses of the HPAI in the 2020/2021 season exceeded 14 million birds, but as pointed out above, about 80% of the losses were concentrated in Mazowieckie province (5).

Phylogenetic studies confirmed the extensive evolution of the viruses expressed by significant variation of the HA gene. The analysis of the tree topology supports the hypothesis about wild birds as the source of primary outbreaks both in terrestrial and aquatic poultry, but on the other hand, the clusters revealed in the Wielkopolskie and Mazowieckie provinces demonstrate that secondary spread also played a role in high-density regions. Investigation of the genome segment constellation revealed the existence of only one genotype of H5N8 (genotype A) in Poland out of seven that have been identified in Europe and Central Asia (2). However, it should be stressed that only a small fraction of viruses detected in Poland have been sequenced and the circulation of other genotypes cannot be ruled out. Interestingly, the Polish H5N5 isolate from a tufted duck was the only representative of a unique genotype not present in other European countries. This virus probably emerged by reassortment of H5N8 HPAIV and low-pathogenic strains circulating in wild birds. In some cases, the observation of genome changes over time provided evidence of a slow evolution towards the selection of molecular markers characteristic of increased adaptation to mammals (*e.g.* PB2 D701N mutation). It should be highlighted that such changes are the effect of natural genetic variation of influenza viruses and do not intrinsically increase viruses' zoonotic potential, particularly if they occur sporadically. However, the conclusions of such findings should regularly be laid before decision-makers to appraise them of the potential risk that HPAIV may pose to public health.

To summarise, the HPAI H5Nx epidemic in 2020/2021 was unquestionably the largest ever recorded in Poland in terms of duration, number of affected counties, number of positive holdings and affected birds. The principal control measures foreseen in the legislation such as culling and movement restrictions, although effective in areas with low- and medium poultry concentrations, seemed to have minimal impact on the virus spread in regions with very high poultry density. Additional measures should be considered in such areas to minimise the huge costs of an epidemic, *e.g.* emergency vaccinations. It should be noted, that a decision to vaccinate birds against HPAI, especially in the face of dynamically changing AIV and possible problems that would arise for export, is a compromise that in the long run may not pay off. A substantive debate on the possible use of emergency vaccination as a short-term supplementary measure targeted at high-risk areas and species (or categories) of poultry should start, taking into account cost-benefit analysis and the effectiveness of the licensed vaccines against currently circulating HPAI virus strains. At the same time, a continuous, non-retrospective genetic monitoring of circulating strains could enrich the outcomes of epizootic investigations (and in consequence lead to the better management of outbreaks) as well as improve early warning systems for public health, especially in the light of the observed dynamic evolution of viruses towards the acquisition of genetic signatures associated with increased zoonotic potential. Invariably, biosecurity remains the major preventive measure that minimises the risk of HPAI virus introduction and circulation within domestic birds.

Conflict of Interests Statement: The authors declare there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: Not applicable.

References

1. Adlhoch C., Fusaro A., Gonzales J.L., Kuiken T., Marangon S., Niqueux É., Staubach C., Śmietanka K., Terregino C., Van der Stede Y., Aznar I., Baldinelli F.: Scientific report: Avian influenza overview – update on 19 November 2020, EU/EEA and the UK. EFSA J 2020, 18, 6341, doi: 10.2903/j.efsa.2020.6341.

2. Adlhoch C., Fusaro A., Gonzales J.L., KuikenT., Marangon S., Niqueux É., Staubach C., Terregino C., Muñoz Guajardo I., Lima E., Baldinelli F.: Scientific report: Avian influenza overview December 2020–February 2021. EFSA J2021, 19, 6497, doi: 10.2903/j.efsa.2021.6497.

3. Adlhoch C., Fusaro A., Kuiken T., Niqueux É., Staubach C., Terregino C., Muñoz Guajardo I., Baldinelli F.: Scientific report: Avian influenza overview November 2019–February 2020. EFSA J 2020, 18, 6096, doi: 10.2903/j.efsa.2020.6096.

4. Adlhoch C., Fusaro A., Kuiken T., Niqueux É., Staubach C., Terregino C., Muñoz Guajardo I., Baldinelli F.: Scientific report: Avian influenza overview February–May 2020. EFSA J 2020, 18, 6194, doi: 10.2903/j.efsa.2020.6194.

5. Adlhoch C., Fusaro A., Gonzales J.L., Kuiken T., Marangon S., Niqueux É., Staubach C., Terregino C., Aznar I., Muñoz Guajardo I., Lima E., Baldinelli F.: Scientific report: Avian influenza overview February 2021– May 2021. EFSA J 2021, 19, 6951, doi: 10.2903/j.efsa.2021.6951.

6. Beerens N., Germeraad E.A., Venema S., Verheij E., Pritz-Verschuren S.B.E., Gonzales J.L.: Comparative pathogenicity and environmental transmission of recent highly pathogenic avian influenza H5 viruses. Emerg Microbes Infec 2021, 10, 97–108, doi: 10.1080/22221751.2020.1868274.

7. Bodewes R., Kuiken T.: Changing role of wild birds in the epidemiology of avian influenza A viruses. Adv Virus Res 2018, 100, 279–307, doi:10.1016/bs.aivir.2017.10.007.

8. Brown I., Mulatti P., Śmietanka K., Staubach C., Willeberg P., Adlhoch C., Candiani D., Fabris C., Zancanaro G., Morgado J., Verdonck F.: Scientific report: avian influenza overview October 2016–August 2017. EFSA J 2017, 15, 5018, doi: 10.2903/j.efsa.2017.5018.

9. Gabriel G., Abram M., Keiner B., Wagner R., Klenk H.-D., Stech J.: Differential polymerase activity in avian and mammalian cells determines host range of influenza virus. J Virol 2007, 81, 9601–9604, doi: 10.1128/JVI.00666-07.

10. Gierak A., Śmietanka K.: The impact of selected risk factors on the occurrence of highly pathogenic avian influenza in commercial poultry flocks in Poland. J Vet Res 2021, 65, 45–52, doi: 10.2478/jvetres-2021-0013.

11. Globig A., Staubach C., Beer M., Köppen U., Fiedler W., Nieburg M., Wilking H., Starick E., Teifke J.P., Werner O., Unger F., Grund C., Wolf C., Roost H., Feldhusen F., Conraths F.J., Mettenleiter T.C., Harder T. C.: Epidemiological and Ornithological Aspects of Outbreaks of Highly Pathogenic Avian Influenza Virus H5N1 of Asian Lineage in Wild Birds in Germany, 2006 and 2007. Transbound Emerg Dis 2009, 56, 57–72, doi: 10.1111/j.1865-1682.2008.01061.x.

12. Grund C., Hoffmann D., Ulrich R., Naguib M., Schinköthe J., Hoffmann B., Harder T., Saenger S., Zscheppang K., Tönnies M., Hippenstiel S., Hocke A., Wolff T., Beer M.: A novel European H5N8 influenza A virus has increased virulence in ducks but low zoonotic potential. Emerg Microbes Infec 2018, 7, 132, doi: 10.1038/s41426-018-0130-1.

13. Kumar S., Stecher G., Li M., Knyaz C., Tamura K.: MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 2018, 35, 1547–1549, doi: 10.1093/molbev/msy096.

14. Lewis N.S., Banyard A.C., Whittard E., Karibayev T., Al Kafagi T., Chvala I., Byrne A., Meruyert S., King J., Harder T., Grund C., Essen S., Reid S.M., Brouwer A., Zinyakov N.G., Tegzhanov A., Irza V., Pohlmann A., Beer M., Fouchier R.A.M., Akhmetzhan S., Brown I.H.: Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. Emerg Microbes Infec 2021, 10, 148–151, doi: 10.1080/22221751.2021.1872355.

15. Lycett S.J., Duchatel F., Digard P.: A brief history of bird flu. Philos Trans R Soc Lond B Biol Sci 2019, 374, 20180257, doi: 10.1098/rstb.2018.0257.

16. Śmietanka K., Fusaro A., Domańska-Blicharz K., Salviato A., Monne I., Dundon W.G., Cattoli G., Minta Z.: Full-length genome sequencing of the Polish HPAI H5N1 viruses suggests separate introductions in 2006 and 2007. Avian Dis 2010, 54, 335–339, doi: 10.1637/8782-040109-ResNote.1.

17. Śmietanka K., Minta Z.: Avian influenza in Poland. Acta Biochim Pol 2014, 61, 453–457.

18. Śmietanka K., Świętoń E., Kozak E., Wyrostek K., Tarasiuk K., Tomczyk G., Konopka B., Welz M., Domańska-Blicharz K., Niemczuk K.: Highly pathogenic avian influenza H5N8 in Poland in 2019–2020. J Vet Res 2020, 64, 469–476, doi: 10.2478/jvetres-2020-0078.

19. Świętoń E., Fusaro A., Shittu I., Niemczuk K., Zecchin B., Joannis T., Bonfante F., Śmietanka K., Terregino C.: Sub-saharan Africa and Eurasia ancestry of reassortant highly pathogenic avian influenza A(H5N8) virus, Europe, December 2019. Emerg Infect Dis 2020, 26, 1557–1561, doi: 10.3201/eid2607.200165.

20. Świętoń E., Śmietanka K.: Phylogenetic and molecular analysis of highly pathogenic avian influenza H5N8 and H5N5 viruses detected in Poland in 2016–2017. Transbound Emerg Dis 2018, 65, 1664–1670, doi: 10.1111/tbed.12924.

21. Verhagen J.H., FouchierR.A.M., Lewis N.: Highly pathogenic avian influenza viruses at the wild–domestic bird interface in Europe: future directions for research and surveillance. Viruses 2021, 13, 212, doi: 10.3390/v13020212.