Outbreak of Highly Pathogenic Avian Influenza H5N8 in November 2016 in Wild Birds in Switzerland

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Since November 2016, Switzerland is affected by a major outbreak of avian influenza (AI) in wild birds. The Avian Influenza A virus belongs to the family *Orthomyxoviridae* and is a single stranded, segmented, negative-sense RNA virus. Single point mutations (antigenic *drift*) occur frequently, and exchange of entire genome segments between two different influenza viruses co-infecting a host cell simultaneously (antigenic *shift*) may lead to significant changes in virus characteristics and are key factors for onset of new pandemics. Haemagglutinin (HA) and neuraminidase (N) are the two major surface glycoproteins. Highly pathogenic avian influenza (HPAI) viruses contain a multi basic cleavage site within the HA, exhibit an intravenous pathogenicity index > 1.2 in 6-week-old inoculated chickens or cause more than 75% mortality within 10 days in 4 to 6-week-old intravenously inoculated chickens (OIE, 2015). The recent AI strain, HPAI H5N8 2016/17, belongs to clade 2.3.4.4b, which is different from the HPAI H5N8 clade 2.3.4.4a virus, that was introduced into Europe in February 2014, causing outbreaks in commercial chicken holdings in Hungary, Germany, Italy, the Netherlands and the United Kingdom (Verhagen et al., 2015). Clinical symptoms of AI vary from subclinical to severe respiratory or enteric disease depending on the virus strain, the virus pathogenicity and the host. Wild ducks as the natural reservoir usually exhibit no or mild, mostly enteric symptoms. In contrast, HPAI in chickens and turkeys may lead to 100% mortality within a few days (Swayne et al., 2013).

Chronology of the outbreak and measures

In September 2016, the FAO empres watch (FAO, 2016) published an alert on the detection of a "Goose/Guangdong/96 lineage H5N8 HPAI of clade 2.3.4.4" in migratory water birds at Lake Ubsu-Nur in the Russian Federation (Lee et al., 2017). Hungary reported the first outbreak in wild birds on October 19, 2016 (OIE, 2016) and the first outbreak in poultry on November 1, 2016 (OIE, 2016). In Switzerland, on November 4, 2016, several dead tufted ducks were found in the yacht harbour of Kreuzlingen (Lake Constance). Three carcasses were selected by the veterinary service and submitted to the National Reference Centre for Poultry and Rabbit Diseases (NRGK) in Zurich. The suspicious necropsy findings and the subsequent positive M-gene real-time reverse transcriptase PCR ((rtRT-PCR), (Dalessi et al., 2007)) confirmed the detection of AIV, further corroborated by a positive H5 rtRT-PCR (Slomka et al., 2007). For pathotyping and determination of the neuraminidase subtype, organ samples (brain and kidney) were submitted to the Institute of Virology and Immunology (IVI) in Mittelhäusern. HPAI H5N8 could be confirmed by PCR and sequencing (Gall et al., 2008) on November 9, 2016 with an immediate report to the OIE (OIE, 2016). Within one week a fast spread across the Swiss Central Plateau from the north-east (Lake Constance) to the south-west (Lake Geneva) occurred. During the second week of the outbreak, a newly designed N8 rtRT-PCR was implemented at the NRGK: forward primer "Flu_N8_IVI_F": 5'-GYTCCATTGTRATGTGTGGA-3', reverse primer "Flu_N8_IVI_R": 5'-ACRTCTTRTCGATGTCAAARG-3' and probe "FLU_N8_IVI_P": 5'-FAMTGGWCRTGGCACGATGGAGCTATYC-BHQ-1-3'. Altogether until the end of March 2017, 119 of 387 tested samples were positive for H5N8 over the course of 21 weeks. The number of samples and the positive detections changed over time. In the first week 81% (17 out of 21), in the second week 67% (60 out of 89), and in the third week 39% (29 out of 74) of samples yielded positive results. From the fourth week on until the end of the year 11 out of 93 (12%) submissions were tested positive. In 2017 (January - March), 2 out of 106 (2%) were positive. In total, 14 wild bird species were affected in Switzerland (Table 1) but no introductions into commercial poultry or backyard flocks occurred. The last positive case of the winter season was found on

January 1, 2017 at Lake Geneva. The Federal Food Safety and Veterinary Office (FSVO) enacted a regulation for risk areas (BLV, 2016) on November 11, 2016. It was extended nationwide on November 15 (BLV, 2016) and suspended on March 18, 2017 (BLV, 2017).

Discussion

Europe was affected by two waves of HPAI H5N8. The first wave began in November 2016 and lasted until the end of the year (FLI, 2017). Switzerland was only affected during this period. A second wave with remarkably more introductions into poultry, especially in France and Germany, began in February 2017 and is still ongoing in several European countries (Plateforme ESA, 2017). Clusters of outbreaks in high dense poultry producing regions were observed (FLI, 2017). The number of introductions into poultry, the variety of affected wild birds and the geographical distribution of reporting countries (e.g. Great Britain, Finland, Portugal, Uganda), reveal the far-reaching impact of HPAI H5N8 (FAO, 2017).

Lee and co-workers (2015) mapped the main flyways of migratory birds from the breeding sites in Siberia towards either Asia, Europe or North America. The European flyway subdivides into three routes. Lake Constance and Lake Geneva are located along the south-western route and are important stopover sites, harbouring up to 500'000 migratory birds each winter, whereof approximately 105'000 tufted ducks (Strebel, 2016). Tufted ducks were the most affected wild aquatic bird species in Switzerland, followed by Caspian gulls and mute swans (Table 1). Tufted ducks belong to the subfamily *Aythyinae*, populate fresh water lakes and feed on bivalves, snails and invertebrates mainly by diving (Keller, 2011). They are water-bound as well as many of the other affected species (Table 1) - this might lower the risk of virus transmission into poultry holdings.

Table 1: List of wild bird species in Switzerland which were tested positive for avian influenza virus H5N8 until the end of March 2017. Number of positive samples compared to total of submitted samples of respective species, numeric and expressed as a percentage. The right column shows selected sequencing results of the cleavage site within the haemagglutinin HA (marked in bold).

Species English (*Latin*) German/French	Positive/Tested/%	Sequence HA
Tufted duck (*Aythya fuligula*) Reiherente/Fuligule morillon	54/62 (87.1%)	PLRE**KRRKR**/GLF
Caspian gull (*Larus cachinnans*) Weisskopfmöwe/Goéland leucophée	17/19 (89.5%)	n.a.
Mute swan (*Cygnus olor*) Höckerschwan/Cygne tuberculé	10/37 (27.0%)	PLRE**KRRKR**/GLF
Black-headed gull (*Chroicocephalus ridibundus*) Lachmöwe/Mouette rieuse	8/23 (12.5%)	PLRE**KRRKR**/GLF
Common pochard (*Aythya ferina*) Tafelente/Fuligule milouin	6/8 (75.0%)	n.a.
Great crested grebe (*Podiceps cristatus*) Haubentaucher/Grèbe huppé	5/26 (19.2%)	n.a.
Mallard (*Anas platyrhynchos*) Stockente/Canard colvert	4/13 (30.8%)	PLRE**KRRKR**/GLF
Little grebe (*Tachybaptus rubicollis*) Zwergtaucher/Grèbe castagneux	3/3 (100.0%)	n.a.
Gull (*Laridae*) Möwe/Mouette	3/7 (42.9%)	n.a.
Red-crested pochard (*Netta rufina*) Kolbenente/Nette rousse	2/8 (25.0%)	n.a.
Black coot (*Fulica atra*) Blässhuhn/Foulque macroule	1/9 (11.1%)	n.a.
Common moorhen (*Gallinula chloropus*) Teichralle/Gallinule poule-d'eau	1/2 (50.0%)	n.a.
Peregrine (*Falco peregrinus*) Wanderfalke/Faucon pèlerin	1/1 (100.0%)	n.a.
Common buzzard (*Buteo buteo*) Mäusebussard/Buse variable	1/30 (3.3%)	n.a.
Species not classified	3/3 (100.0%)	n.a.

n.a. = not analyzed

Other key factors for the absence of HPAI H5N8 outbreaks in Swiss poultry holdings are speculated to be: 1) duck meat production and geese fattening is not common in Switzerland; 2) turkey meat production is a niche production accounting for 1.8% of total poultry production in Switzerland (Agristat, 2015); 3) storage of feed and bedding is usually indoor or in silos which limits accessibility for wild birds; 4) mostly use of expanded feed; 5) low maximum livestock density per flock and square meter restricted by law (TSchV, 2008); 6) poultry farms are more evenly spread across the country, with no densely populated poultry areas like e.g. in Lower Saxony in Germany; 7) biosecurity standards are high and generally well maintained; 8) population of overwintering wild aquatic birds in Switzerland is apparently stable, continuous arrival of new individuals is not common along the flyway (after the main migration event in November, a rapid spread among the wild bird population occurred mostly without drastic population decline). Despite these advantages, the following risk factors must be considered: 1) Switzerland is located along the 0°C isotherm line in winter. Migrating aquatic birds stay close to this line to keep access to non-frozen water bodies while limiting energy waste and increase the possibility to be among the first birds back in Siberia to occupy the best breeding sites (Reperant et al., 2010); 2) in 2015 91% of Swiss livestock poultry was kept in BTS (particularly animal-friendly housing) system with a protected outdoor area (BLW, 2016) allowing close contact to wildlife. Considering the above, Switzerland may have to face future AI outbreaks, potentially also in livestock poultry population and must thus be prepared. Hence, experiences gained since November 2016 were analyzed and discussed by cantonal authorities, reference laboratories and different stakeholders (among others: poultry industry, fancy breed association) in an AI-debriefing meeting at the FSVO in June 2017. Positive points were the rapid decisions of authorities in cooperation with the official veterinary service, reference laboratories and the industry as well as exchange of information with neighbouring states. This allowed the implementation of accepted regulations by all parties. Further, the centralized and generally well-orchestrated communication strategy of the FSVO dealing with media requests and regular updates of the FSVO website led to a uniformity of public information. Amendable and discussed points were: 1) sampling guidelines were still based on the HPAI H5N1 outbreak in 2005/06 and had to be updated shortly after the outbreak. Suitable sampling material was not at hand in all cantons; 2) the exact number of affected birds in Switzerland is not known. In clusters of dead birds only few individuals were randomly selected for analysis and if a species at a particular location had already been tested positive it was not retested. This economic resource management resulted in sufficient diagnostic and personnel capacities in reference laboratories at all time and upscaling was not necessary. However, awareness has to remain high that an upcoming larger or potentially zoonotic outbreak could occur and would increase the number of samples extraordinarily. Stakeholders and politicians need to know, that such a crisis can only be handled by well-trained personnel and that cooperation plans between laboratories and authorities need to be ready before the onset of a crisis to be able to process high amounts of samples within a short time; 3) the decision to extend the regulation nationwide was reasonable, as H5N8 was detected at all major lakes of the Central Plateau, and as Switzerland's water-rich topography complicates zoning of every single detection in wild birds and would exhaust resources. The prohibition of exhibitions might have been drastic for fancy breeders, but was important for risk reduction of a virus spread across cantonal boundaries. Especially as an AI infection may be undetected in backyard poultry or fancy breeds due to smaller flocks with a different age-composition, possibly resulting in non-uniform and less dramatic clinical signs or fewer deaths (C. Bothmann, LAVES, Oldenburg, personal communication).

Although H5N8 kills water birds, it is maintained to some extent in healthy water fowl and circulates within the local population, as shown by virus detection in juvenile mute swans and mallards found dead at Lake Neuchâtel and Lake Geneva in August 2017. Thus, such a virus does not miraculously disappear after an outbreak event, and constant vigilance is necessary.

Conclusion

Reviewing the history of AI reveals a tendency to larger and more frequent outbreaks over time. The virus itself seems to develop towards optimal adaptation in its natural host, which is reflected in moderate mortality, but high levels of virus shedding. This should encourage to reconsider and improve biosecurity within the different poultry housing systems. The disease awareness of the poultry industry and the public should be upheld, especially before and during the critical time of the year. To prevent the emergence of novel human pathogenic strains induced by antigenic shift, seasonal flu vaccination is recommended in humans. With onset of a new outbreak, measures and sampling guidelines should be immediately available to contain the spread as fast as possible. Furthermore, choanal and cloacal swabs are the sample materials of choice as submitting carcasses for analysis increases the risk of virus spread and ambiguous sampling methods affect statistics. The required swabs, tubes, protective clothing and further materials must be promptly available in every canton ("Seuchenkoffer" / "trousse d'urgence"). Passive surveillance and

reporting of dead wild birds are key factors in early detection, because AI is not eradicable and future outbreaks must be expected.

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