Communication

White-Tailed Sea Eagle (*Haliaeetus Albicilla*) Die-Off due to Infection with Highly Pathogenic Influenza Virus, Subtype H5N8, in Germany

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Abstract: In contrast to previous incursions of highly pathogenic H5 viruses, H5N8 clade 2.3.4.4b caused numerous lethal infections in white-tailed sea eagles (*Haliaeetus albicilla*) in Germany during the winter 2016/2017. Until April 2017, 17 HPAIV H5N8-positive white-tailed sea eagles had been detected (three alive and 14 dead). Mainly young eagles died (before reaching the adult plumage at 5 years), often with severe neurological symptoms, where histopathology revealed mild to moderate, oligo- to multifocal necrotizing polioencephalitis. Lethal lead (Pb) concentrations, proven as main mortality factor of the sea

eagles could be ruled out since values measured in liver or kidney tissue were all within background levels (< 1 ppm). Since the fall of 2016, the epizootic of HPAIV H5 clade 2.3.4.4b reportedly induced, for the first time, fatal disease in European white-tailed see eagles. The virus strain may become a new threat to a highly protected species across its distribution range in Eurasia. Positive cloacal swaps have proven that the eagles can spread the virus with their faeces.

Keywords: clade 2.3.4.4b, fatal infection, HPAIV H5N8, neurological symptoms, white-tailed sea eagle

Incursions of HPAI into Germany of subtype H5N8, clade 2.3.4.4, have been observed twice. In 2014/2015, a small number of cases in wild birds and of outbreaks in poultry and a zoo were caused by HPAIV H5N8 clade 2.3.4.4a (Conraths et al., 2016; Globig et al., 2016). The same clade was also detected in apparently healthy wild birds. In contrast, incursion of related HPAIV H5N8 clade 2.3.4.4b caused severe clinical signs and significant mortality in a large number of infected wild birds in 2016/2017. At Lake Plön in Schleswig-Holstein 94% (n=55) and at Lake Constance in southwest Germany, 89% (n=237) of the tufted ducks (*Aythya fuligula*) found dead, were tested positive for HPAIV H5N8 clade 2.3.4.4b (TSIS, https://tsis.fli.de/ accessed on 22.09.2017; Pohlmann et al., 2017). From November 9th to 21st, 2016, an obvious die-off with about 330 dead birds had been recorded on the shores of the islands of Ruden and Greifswalder Oie north of the isle of Usedom in the German part of the Baltic Sea, Mecklenburg-Western Pomerania the core breeding area of white-tailed sea eagles (among them appr. 150 scaups *Aythya marila*, 75 herring gulls *Larus argentatus* and 25 greater black-backed gulls *Larus marinus*; Verein Jordsand, pers. comm.). A sample of these birds, including scaups, herring gulls, a greater black-backed gull and a black-headed gull

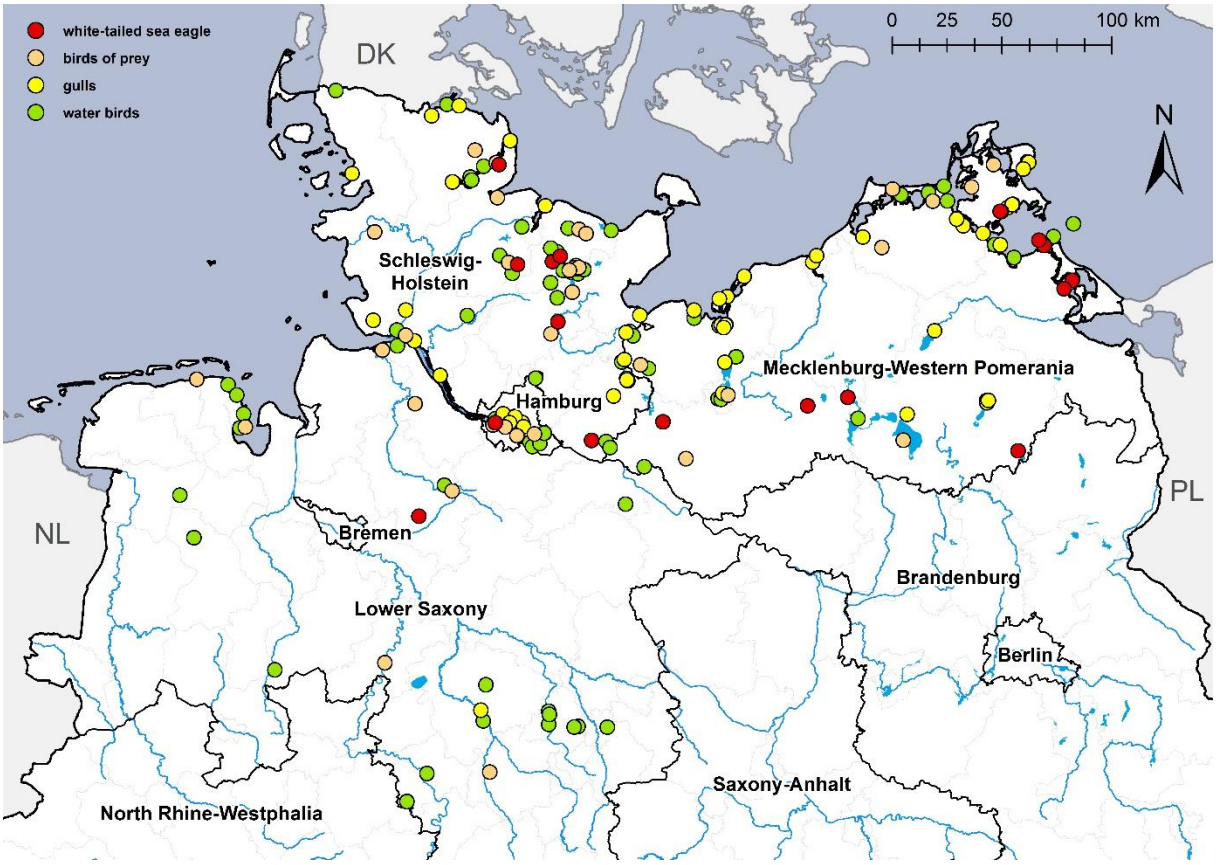
Chroicocephalus ridibundus, tested positive for HPAIV H5N8. Soon after the first HPAIV infections in wild birds had been reported, outbreaks in domestic poultry followed (Globig et al., 2018; Pohlmann et al. 2017, 2018). The first H5N8 positive white-tailed sea eagle was found in Schleswig-Holstein, Germany at the 15th November 2016. Simultaneous detection of cases in Hungary, and Poland suggested a rapid spread in wild bird populations.

The white-tailed sea eagle (WTSE) is a strictly protected bird species breeding in Germany. It is mainly found in the eastern and northern parts of the country, i.e. in the Federal States of Saxony, Lower Saxony, Schleswig-Holstein, Mecklenburg-Western Pomerania and Brandenburg. Breeding pairs in Mecklenburg-Pomerania, in Schleswig-Holstein, in Saxony and Lower Saxony are estimated at 311, 88, 69 and 37 in 2016, respectively (Anon., 2017). In 2015 143 breeding pairs were recorded in Brandenburg. While the adult breeding WTSE are more or less sedentary, some eagles migrate from Fennoscandian countries to the shallow lagoons of the southern Baltic Sea in Germany to spend their winter.

Cases were reported either by rangers of protected areas, hunters, ornithologists or citizens who subsequently notified the finding to the local veterinary authorities. Diseased HPAIV-infected WTSE that were still alive were euthanized. Swab samples were taken from the trachea and the cloaca and sent to State Veterinary Laboratories for rapid diagnosis by real-time reverse transcriptase polymerase chain reaction (RT-qPCR) for RNA of subtype M and H5. All H5-positive samples were sent to the National Reference Laboratory for Avian Influenza (NRL AI) at the Friedrich-Loeffler-Institute (FLI), Isle of Riems. Here, sub- and pathotyping was carried out as described by Pohlmann et al., 2017. Furthermore, necropsies were conducted under BSL-3 conditions at the FLI. Lead (Pb) values of the eagles were measured in liver and kidney tissue using the graphite furnace atomic absorption spectrometer (AAS) previously described by Krone et al., 2009. Immunohistologically, avidin-biotin-

complex method and polyclonal rabbit anti- influenza A FPV/Rostock/34-virus-nucleoprotein antiserum was used to detect Influenza-A antigen (Klopfleisch et al., 2003).

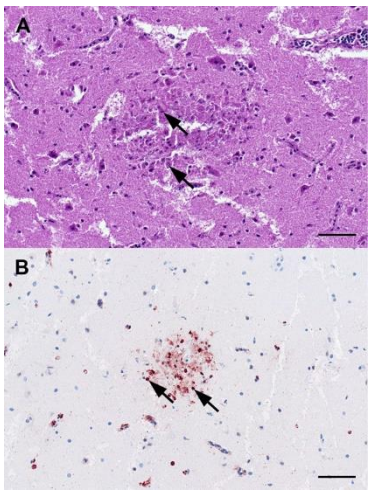
The HPAIV genome loads of the samples taken varied in RT-qPCR from low to very high (Table 1). In Germany, until April 3rd 2017, 17 HPAIV H5N8-positive WTSE had been detected (three alive and 14 dead). The last WTSE was found in early April in Hamburg (Figure 1, Table 1). In all cases, pathotyping revealed the high pathogenic strain (H5HP).



Birds found alive (n=3) demonstrated mild to severe neurological symptoms including torticollis, opisthotonus, limber neck, ataxia and movement in circles (Figure 2). Symptoms aggravated, when the birds were stressed, e.g. by handling. Perception and reaction seemed to be normal. In contrast to symptoms seen in lead-poisoned eagles such as depression, weakness, respiratory and coordination problems, birds infected with HPAIV were alert and overexcited. Eleven eagles examined were juvenile or immature, two were subadult and three adult (Table 1). Necropsy showed that gross lesions characteristic for HPAI were scarce or

absent. However, histopathological and immunohistological investigations revealed oligo- to multifocally necrotizing lesions in the cerebrum, cerebellum and brain stem.

Immunohistological and virological investigations confirmed influenza A virus nucleoprotein antigen, and / or H5N8-specific RNA in organ samples (Figure 3). In four cases separate pharyngeal and cloacal swabs were taken; two were negative, one was positive in the pharynx and another was positive in pharynx and cloaca, demonstrating that the white-tailed sea eagle can efficiently spread the virus in the faeces. Lead (Pb) values of liver and kidney tissue revealed values within the background level of < 1 ppm.



Reportedly, under normal conditions the most important mortality factor for WTSE during the winter months is lead poisoning, the second most frequent cause is collisions with trains (Krone et al., 2003; 2009). During the HPAIV H5N1 epidemic among wild birds in the same area in 2006, all sea eagles found dead (n=19) in the center of the outbreak, the Isle of Rügen (Mecklenburg Western- Pomerania), had always tested negative for AIV (Van den Brand et al., 2015). Obviously, species-specific differences regarding the susceptibility and vulnerability for HPAIV infection exist among birds of prey, as peregrine falcons (*Falco peregrinus*) and common buzzards (*Buteo buteo*) died due to infections with HPAIV H5N1 in 2006 (Van den Brand et al. 2015). In addition, hooded vultures (*Necrosyrtes monachus*) and a sparrowhawk (*Accipter nisus*) succumbed to HPAIV H5N1 infection in Western Africa in 2006 (Ducatez et al., 2007). In Europe, there were no reports of HPAIV-infected WTSE until 2016. Since the fall of 2016, the epidemic of HPAIV H5 clade 2.3.4.4b induced fatal disease in WTSE in the northern part of Europe.

WTSE are facultative scavengers, feeding on carrion especially during wintertime. If available, waterfowl is the main prey in autumn and winter (Nadjafzadeh et al., 2013). Obviously, diseased and handicapped waterfowl are an attractive prey for the eagles. In any case, a top predator such as the WTSE may become repeatedly exposed to a variety of pathogens including avian influenza viruses (AIV). Thus, individual birds may acquire immunity against such pathogens. The majority (two-thirds) of the affected WTSE during the current epizootic were juvenile birds, including those found alive (Table 1). We therefore hypothesise that juvenile and immature WTSE found dead and infected were immunologically naïve to the virus. Older and, presumably immunologically “experienced” eagles might have been cross-protected by heterologous immunity derived from previous influenza A infections. Only three eagles found were adult (older than four years of age).

Regarding the population integrity of the WTSE, a relatively rare and highly protected species, the relevance of the HPAIV-related death toll remains unclear. Fatal infections of

WTSE with HPAIV H5N8 were recently reported around the Baltic Sea from Finland (Isomursu et al., 2017) Denmark, and Sweden (OIE, 2017). In January 2018 a juvenile WTSE was reported to be infected with HPAIV H5N6 clade 2.3.4.4b in Ireland (ADNS, 2018).

Generally, infected WTSE act as sentinel birds to indicate HPAIV H5Nx clade 2.3.4.4b infections in water birds in their hunting areas. Since young WTSE are roaming through their range during dispersal up to their first four to five years of life, they concentrate where food is abundant. It is unknown whether 2.3.4.4b HPAIV infections in WTSE are invariably lethal or if eagles can overcome the disease and acquire immunity. HPAIV H5Nx of clade 2.3.4.4b may become a new threat to a highly protected wild species across its distribution range in Eurasia and may hamper reintroduction projects of WTSE when the virus is circulating in the local wild water bird population during winter time.

Author Contribution: O.K. and A.G. designed the study; O.K., R.U. and S.G. performed the necropsy of the eagles; R.U., J.S. and S.G. analysed the histological samples; T.H. analysed, sequenced and pathotyped the virus; A.G., C.H., F.C. and M.B. contributed essential data and reviewed the manuscript; O.K. wrote the paper.

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Conflict of interest: The authors declare no conflict of interest.

References

ADNS [Animal Disease Notification System] (2018). Overview report. Retrieved from: https://ec.europa.eu/food/animals/animal-diseases/not-system_en

Anonymous. Agency for Environment, Nature Conservation, and Geology M-WP (2017). unpublished

Arbeitsgemeinschaft Adlerschutz Niedersachsen (2016). Bericht der Arbeitsgemeinschaft Adlerschutz in Niedersachsen über den Brutverlauf bei See- und Fischadler im Jahr 2016. Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (NLWKN), Staatliche Vogelschutzwarte, 12p.

Conraths, F. J., Sauter-Louis, C., Globig, A., Dietze, K., Pannwitz, G., Albrecht, K., Höreth-Böntgen, D., Beer, M., Staubach, C. & Homeier-Bachmann, T. (2016). Highly Pathogenic Avian Influenza H5N8 in Germany: Outbreak Investigations. Transboundary and Emerging Diseases, 63, 10-13. *doi*:10.1111/tbed.12443

Ducatez, M. F., Tarnagda, Z., Tahita, M. C., Sow, A., de Landtsheer,S., Londt, B. Z., Brown, I. H., Osterhaus, A. D. M. E., Fouchier, R. A. M., Ouedraogo, J.B. & Muller, C. P. (2007). Genetic characterization of HPAI (H5N1) viruses from poultry and wild vultures, Burkina Faso. Emerging Infectious Diseases, 13, 611-613.

Globig, A., Starick, E., Homeier, T., Pohlmann, A., Grund, C., Wolf, P., Zimmermann, A., Wolf, C., Heim, D., Schlößer, H., Zander, S., Beer, M., Conraths, F. J. & Harder T. C. (2016). Epidemiological and Molecular Analysis of an Outbreak of Highly Pathogenic Avian Influenza H5N8 clade 2.3.4.4 in a German Zoo: Effective Disease Control with Minimal Culling. Transboundary and Emerging Diseases, 15. *doi*: 10.1111/tbed.12570.

Globig, A., Staubach, C., Sauter-Louis, C., Dietze, K., Homeier-Bachmann, T., Probst, C., Gethmann, J., Depner, K.R., Grund, C., Harder, T.C., Starick, E., Pohlmann, A., Höper, D., Beer, M., Mettenleiter, T.C. & Conraths, J.F. (2018). Highly Pathogenic Avian Influenza H5N8 Clade 2.3.4.4b in Germany in 2016/2017. Frontiers in Veterinary Science, 4, 240. | https://doi.org/10.3389/fvets.2017.00240

Isomursu, M., Tammiranta, N. & Venäläinen E.-R. (2017). Outbreak of highly pathogenic avian influenza in Finnish white-tailed eagles. The collection of Abstracts and Short Notes of the SEAEAGLE 2017 conference, Roosta, Estonia, pp. 48-49.

Krone, O., Langgemach, T., Sömmer, P. & Kenntner, N. (2003). Causes of mortality in white-tailed sea eagles from Germany. In: Sea Eagle 2000. Proc. Swedish Soc. For Nat. Conserv./SNF, Helander B, Marquiss M, Bowerman W (eds), Stockholm, Sweden, 211-218.

Krone, O., Kenntner, N., Trinogga, A., Nadjafzadeh, N., Scholz, F., Sulawa, J., Totschek, K., Schuck-Wersig, P. & Zieschank, R. (2009). Lead poisoning in white-tailed sea eagles: Causes and approaches to solutions in Germany. In Watson RT, Fuller M, Pokras A and Hunt WG (eds.) Ingestion of lead from spent ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, Idaho, USA, 289-301.

Nadjafzadeh, M., Hofer, H. & Krone, O. (2013). The link between feeding ecology and lead poisoning in white-tailed eagles. Journal of Wildlife Management, 77, 48-57.

OIE (World Organisation for Animal Health, 2016 [cited 2017 Sept 1]). Update on highly pathogenic avian influenza in animals (type H5 and H7). Retrieved from: http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2016/

Pohlmann, A., Starick, E., Harder, T., Höper, D., Globig, A., Staubach, C., Dietze, K., Strebelow, G., Ulrich R.G., Schinköthe, J, Teifke, JP, Conraths F.J., Mettenleiter, T.C. & Beer, M. (2017). Outbreaks among Wild Birds and Domestic Poultry Caused by Reassorted

Influenza A(H5N8) Clade 2.3.4.4 Viruses, Germany, 2016. Emerging Infectious Diseases, 23 (4), 633-636. *doi*: 10.3201/eid2304.161949.

Pohlmann, A., Starick, E., Grund, C., Höper, D., Strebelow, G., Globig, A., Staubach, C., Conraths, F.J., Mettenleiter, T.C., Harder, T. & Beer, M. (2018). Swarm incursions of reassortants of highly pathogenic avian influenza virus strains H5N8 and H5N5, clade 2.3.4.4b, Germany, winter 2016/17. Scientific reports 8 (1):15. doi: 10.1038/s41598-017-16936-8.

Projektgruppe Seeadlerschutz Schleswig-Holstein (2017). Großvogelschutz im Wald. Jahresbericht 2016. Lithographische Werkstätten Kiel, 36p.

Van den Brand, J. M. A., Krone, O., Wolf, P. U., van de Bildt, M. W. G. , van Amerongen, G., Osterhaus, A. D. M. E. & Kuiken, T. (2015). Host-specific exposure and fatal neurologic disease in wild raptors from highly pathogenic avian influenza virus H5N1 during the 2006 outbreak in Germany. Veterinary Research, 46, 24.

Table 1. White-tailed sea eagles infected with HPAIV H5N8 in Germany between November 2016 and April 2017 (MWP, Mecklenburg-Western Pomerania; LS, Lower Saxony; SH, Schleswig-Holstein; HH, Hamburg). Four carcasses were destroyed before a necropsy could be performed, so that their sex could not be determined (marked with n.d. = not determined).

date carcass found	**age**	**sex**	**federal state**	**H5**	**N8**	**HPH5**
15.11.2016	subadult	u	SH	+	+	+
21.11.2016	juvenile	female	MWP	++	++	++
27.11.2016	adult	male	SH	+++	+++	+++
28.11.2016	juvenile	female	SH	+++	+++	+
28.11.2016	juvenile*	male	MWP	+	+	+
01.12.2016	juvenile	u	LS	+++	+++	+++
02.12.2016	juvenile	male	MWP	+++	+++	+++
03.12.2016	immature	female	SH	++++	++++	++++
03.12.2016	immature	female	SH	++++	++++	++++

05.12.2016	juvenile	male	MWP	++	+++	+++
10.12.2016	immature	male	MWP	++++	++++	++++
29.12.2016	juvenile	male	MWP	++	+++	++
29.12.2016	adult	female	MWP	+++	+++	+++
29.12.2016	juvenile*	male	MWP	+	+	++
09.01.2017	juvenile	female	MWP	+	+	+
29.01.2017	juvenile*	male	MWP	++	++	++
03.04.2017	adult	female	HH	++	++	++

Age classes are defined as juvenile eagles up to 1 year, immature eagle between 2 and 3 years, subadult eagle in their fourth year and adult eagles older than five years of age; sex was not determined (n.d.) in four cases; results q RT PCR (TC: threshold cycle): ++++ = TC<20; +++ = TC 20-24; ++ = 25-29; + = 30-35; (+) = 36-39; asterisk indicates eagles found alive, but have been euthanized

Figure 1. Cases of HPAIV H5N8 detected in wild birds in the federal states of Germany Lower Saxony, Schleswig-Holstein and Mecklenburg-Western Pomerania. Water birds (green dot) comprise ducks, geese, swans, herons, coots, cormorants. Birds of prey (orange dot) comprise buzzards, falcons and one owl, gulls are shown by a yellow and white-tailed sea eagles by a red dot.

Figure 2. Juvenile, male white-tailed sea eagle tested positive for HP AIV H5N8 found 8th February 2017 (see table 1) displaying torticollis and coordination problems. The wings are dropped and the eagle is crouching on its intertarsal joints.

Figure 3. Light microscopic findings in the cerebrum of white-tailed sea eagles. **A.)** Mild, acute, oligo- or multifocal, necrotizing polioencephalitis characterized by condensed, hypereosinophilic neuroglial cells with pyknotic and karyorrhectic nuclei (arrows) represents a typical lesion. Hematoxylin eosin; Bar = 50 µm. **B.)** Immunohistochemistry confirmed the presence of influenza A virus nucleoprotein antigen within neuroglial nuclei (arrows) within the lesions. Polyclonal rabbit anti- influenza A FPV/Rostock/34-virus-nucleoprotein

antiserum; avidin-biotin-complex method; 3-amino-9-ethyl-carbazol chromogen (red-brown); hematoxylin counterstain (blue); Bar = 50 µm.