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West Nile virus epizootic in Germany, 2018

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ABSTRACT

The summer of 2018 in Germany was the second hottest and driest on record. These generally extremely favorable climatic conditions most likely triggered the further expansion and the efficient propagation of the zoonotic arthropod-borne West Nile virus in many Southern/Southeastern and even Central European countries. WNV infections were detected for the first time in resident wild and aviary birds, such as common blackbirds, northern goshawks and great grey owls in Eastern and Southeastern Germany. The causative WNV strain belonged to the central European subclade II. Phylogeographic analysis indicated a single introduction event of WNV into Germany, most likely in 2016 from Czech Republic, and also a unique non-synonymous mutation in the NS3 gene. Extraordinary high temperatures in 2018 presumably led to decreased averaged extrinsic incubation period values for WNV in mosquitoes, leading to rapid virus amplification and greater transmission risk for vertebrates in Germany. Blood transfusion services and clinicians in Germany should be aware of these possible WNV infection risks in humans especially during late summer.

1. Background

Wild birds can be involved as amplifying hosts in the transmission of emerging zoonotic pathogens in Central Europe. Migratory birds may be an important spreader for (arbo)-viruses to new areas along their major flyways over Asia, Africa and Europe (Hubalek, 2004; Rappole et al., 2000; Komar 2003). Monitoring of infections in wild birds can, therefore, provide an early warning system for the introduction of viruses to new areas. For this purpose, we have set up a nationwide captive and wild bird surveillance network receiving up to 1000 bird samples annually to systematically monitor migratory and resident birds for zoonotic arboviruses including flaviviruses like West Nile virus (WNV) and Usutu virus (USUV). WNV is an important zoonotic arbovirus, which is maintained in an enzootic bird-mosquito-bird cycle, but can also be pathogenic for humans, equines and other mammals (Chancey et al., 2015; Ciota, 2017; Turell, 2012). Our earlier monitoring studies demonstrated neutralizing antibodies against WNV in migratory birds in Germany, but WNV specific RNA was never detected (Michel et al., 2018; Seidowski et al., 2010; Ziegler et al., 2012, 2015). *Culex pipiens* s.l./*Cx. torrentium* mosquitoes, which are the most potent WNV vectors, are inherently present in Germany and their susceptibility to the virus was demonstrated recently (Leggewie et al., 2016). The unusually hot climatic conditions in the summer 2018 all over Europe with an extremely long period of high temperatures over months may have provided conditions for the incursion and establishment of WNV into Germany as described in this report.

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Table 1
Epidemiological data of the WNV-infected birds detected in Germany.

Case	Date of confirmation	Bird species	Scientific name	Origin	City	Federal State	organ samples by RT-qPCR (range of ct-values)
1	28.08.18	great grey owl	*Strix nebulosa*	zoo	Halle	Saxony-Anhalt	14–20
2	10.09.18	northern goshawk	*Accipiter gentilis*	wild bird	Klein Weißandt	Saxony-Anhalt	17–34
3	12.09.18	great grey owl	*Strix nebulosa*	zoo	Poing	Bavaria	15–20
4	19.09.18	northern goshawk	*Accipiter gentilis*	private aviary	Bad Düben	Saxonia	17–35
5	21.09.18	common blackbird	*Turdus merula*	wild bird	Berlin-Mitte	Berlin	24 (mix pool)
6	26.09.18	common blackbird	*Turdus merula*	wild bird	Laage	Mecklenburg-Western Pomerania	24 (mix pool)

2. Case reports and spatial and temporal distribution of the epizootic

Between August 28th and September 26th, 2018 six fatal WNV cases among wild birds and captive birds in zoological gardens (Table 1) were diagnosed by WNV-specific reverse transcription quantitative real-time polymerase chain reactions (RT-qPCR) that target the 5′NTR-region or the nonstructural protein 2A genes (Eiden et al., 2010). Usutu virus (USUV) infections were excluded by using a RT-qPCR specifically targeting the nonstructural protein 1 gene (Jost et al., 2011). All amplicons were sequenced eventually according to standard methods. The organ sample of the owl was prepared for a sequencing library according to a recently published protocol by (Wylezich et al., 2018). The resulting library was sequenced by applying the Ion Torrent S5 chemistry. The obtained full-length recovered genome sequence of the virus was submitted to GenBank (accession no. MH924836). The full-length genome sequences of the blackbirds were obtained using Sanger sequencing and primers described elsewhere (Cadar et al., 2014). Virus isolation was performed from the homogenized brain material of the first WNV infected bird (great grey owl), and Vero-cells showed a cytopathic effect after 4–5 days. The supernatant was positive for WNV by RT-qPCR (data not shown).

All WNV cases originated from eastern and southeastern Germany spanning almost the entire north-south expansion of the country, i.e. five different Federal States (Saxony-Anhalt, Saxony, Bavaria, Berlin and Mecklenburg-Western Pomerania) were reached. The two caged great grey owls died unexpectedly and without obvious clinical signs until to their last day, albeit virus concentrations in their organs (brain, kidney, spleen and heart) were extremely high. In contrast, two northern goshawks developed fatal neurological symptoms and carried comparably high virus loads in their organs. No case histories are available for the two blackbirds found dead and submitted by citizens in Berlin and Laage. Interestingly, virus concentrations in their organs were lowest compared to the goshawk and owl samples.

3. Genetic characterization and phylogeography of German WNV

The genetic characterization of German WNV was carried out as described in the supplement. Viral genome sequence identities of the German WNV strains ranged between 99.8 and 99.9% and 100% at the nucleotide (nt) and amino acid (aa) level, respectively. Compared with other members of the European clade of WNV lineage 2, identity matrices for the genome were 98.4–99.6 at nt and 98.5–99.9% at aa level. Bayesian maximum clade credibility (MCC) phylogenies of the complete polyprotein and partial NS5 gene revealed that all German WNV strains fell into a monophyletic group suggesting a single introduction event in 2016 (95% HPD, 2015–2018; posterior probability 0,96) (Figs. 1 and 2; Suppl. Fig.). Furthermore, all strains clustered together with a mosquito-related WNV strain from Czech Republic and a human strain from Austria forming the putative Central European subclade II (Figs. 1 and 2; Suppl. Fig.). The time scaled phylogeny indicates that the German WNV seems to be a descendant of an ancestor that probably existed in Czech Republic at least from 2011 (95% HPD, 2013-2010). The analysis of the polyprotein gene revealed one unique amino acid mutation located in NS3 protein ($K_{609}R$) of German WNV strains and a reversion of a codon mutation to an ancestral form ($K_{527}R$) in the NS5 gene which was only detected in the new putative central European subclade II and an ancestral WNV strain (Madagascar, 1978; DQ176636) (Fig. 2).

4. Risk of transmission

The risk of transmission depends on the environmental temperature as it modulates the mosquitos gonotrophic cycle and increases the blood feeding and oviposition rates (thereby favoring large biting vector populations) as well as determines the extrinsic incubation period (EIP), which gives the time between ingestion of a pathogen via blood meals and the vectors' ability to transmit the virus.

Daily EIP values (EIP_d) of WNV were calculated with the formula $-0.132 + 0.0092 \times$ temperature (Hartley et al., 2012). Day-to-day mean temperature data (July 1988–August 2018) were downloaded from http://www.ecad.eu (Haylock et al., 2008). Data analysis and visualization was conducted with the programme R (R Development Core Team., 2016) using the packages lubridate (Grolemund and Wickham, 2011) and raster (Hijmans, 2016). For risk assesment, EIP_d values for the subsequent days were summed up until the virus development was completed (= EIP). For each grid cell and year, EIP values were averaged for the period from 15th July to 14th August (= EIP_{ave}). The first WNV-positive bird was confirmed on 28.08.2018 but had already died on 16.08.2018 subtracted by an estimated intrinsic incubation period of two days. In order to compare the situation in 2018 with previous years, one map for EIP_{ave} values of 2018 was compared against the minimum annual EIP_{ave} per raster cell for the period 1988–2017 (Fig. 3a). Wide areas in Germany allowed very short EIP_{ave} values (< 14 days) in the summer 2018 (Fig. 3b).

Four of the here discussed six WNV-positive birds were found in these regions with highest transmission risk.

5. Discussion and conclusion

Understanding eco-epidemiological factors and evolutionary processes that contribute to the maintenance, emergence, and finally to the spread of arboviral diseases are critical to develop and implement surveillance strategies for their control. We have detected for the first time in Germany, the presence of WNV and genetically characterized by determining the complete genome sequence of three strains and for other 3 the partial NS5 gene from WNV infected birds and compared them with the members of the European clade of WNV lineage 2. Our phylogenies showed that the German WNV strains represent a new variant of a WNV strain from Czech Republic and together with an Austrian strain constitute a putative monophyletic Central European subclade (Fig. 1; Suppl. Fig.). The time to the most recent common ancestor (TMRCA) of the German WNV indicates a single introduction event during the mid-2016 with Czech Republic as a possible origin for the progenitor of the German epizootic (Fig. 1). The inferred spread of WNV indicates that Austria and Hungary were probably native to the

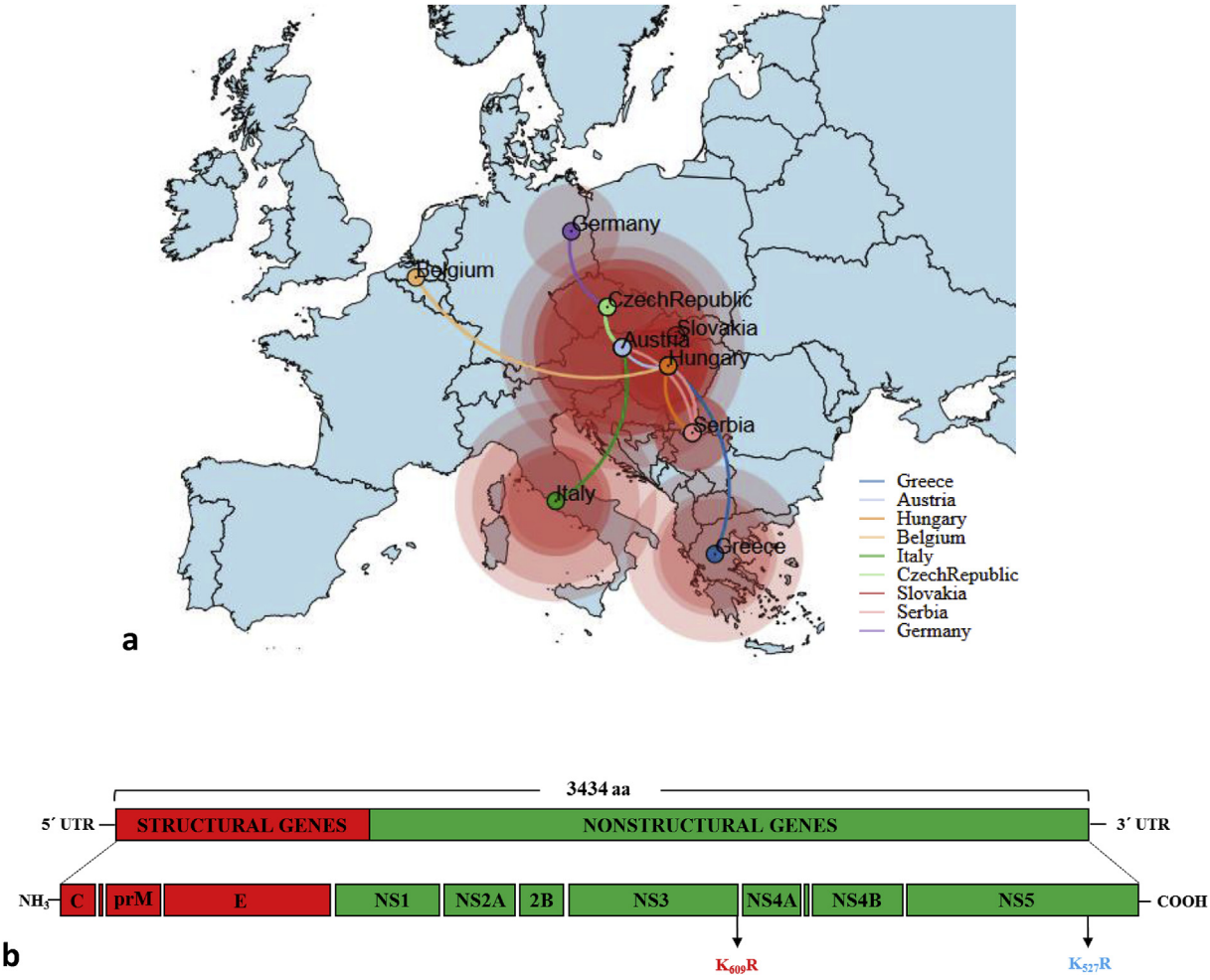

Fig. 1. Spatial dynamics of the European clade of WNV lineage 2 reconstructed from the MCC tree and a flexible demographic prior with location states and schematic representation of the specific amino acid replacements in the German WNV genomes. a. The directed lines between locations connect the sources and target countries (color coded) of viral strains and represent branches in the MCC tree along which the relevant location transition occurs. Location circle diameters are proportional to square root of the number of MCC branches maintaining a particular location state at each time-point. Discrete locations are geographic coordinates for capital cities of each European country. **b**. A number indicating the position of amino acid mutations in the gene and the single-letter amino acid codes are used to denote the country (Germany; red) and WNV subclade-specific (Germany, Austria, Czech Republic; blue) mutations along the polyprotein genes.

progenitor of the Central and Southern European outbreaks (Figs. 1 and 2). However, further studies on possible sources and introduction route (e.g., infected mosquitoes, resident or short-distance migratory birds) of WNV in affected areas in Germany are needed to verify this assumption. The unique non-synonymous mutation detected in the C-terminal portion of NS3 genes of German WNV strains may suggest an adaptive evolution. It is known that the NS3 C-terminal portion contains RNA triphosphatase (NS3RTPase) and RNA helicase (NS3Hel) activities involved in capping and viral RNA synthesis (Bollati et al., 2010; Brault et al., 2007). In addition, a reversible mutation specific for the member of the new putative Central European subclade II was found in the C-terminal RNA-dependent RNA polymerase (NS5RdRp) domain. Whether these mutations cause an increase in WNV fitness in regards to establishment and spatial diffusion requires further analysis. The adaptation of WNV as in the case of USUV to naïve vector and vertebrate host populations can be considered key determinants in the establishment and spatial diffusion of WNV (Cadar et al., 2017).

The WNV occurrence in Germany is most likely a consequence of unusual climatic conditions in Europe, which were characterized by an early start of a hot and rainless season in April/May 2018 that persisted at least until the beginning of September. Similar climatic conditions provided conditions suitable for high numbers of human WNV cases and several new WNV hot spot areas in Southern European countries, e.g. Croatia, Italy, Slovenia, Romania and Greece (https://ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/disease-data-ecdc). The number of human WNV infections in 2018 already exceeds the total number of the previous five years, illustrating the high WNV activity and the high probability of further virus spread. In addition, the introduced WNV found highly favorable temperature conditions in Germany, allowing autochthonous transmissions. At present, there is a large USUV epizootic ongoing in Germany and elsewhere in Europe probably causing death of hundreds of thousands of common blackbirds, owls and birds of prey (Luhken et al., 2017), which was also triggered by the extremely favorable climatic conditions. This USUV bird die-off and the accompanied surveillance program implemented was possibly helpful to discover the here described WNV cases as it has raised the publics' attention and led to sharply rising number of diagnostic sample submissions. It was surprising to note that the cases are widely spread over almost 900 km (Munich to Rostock) in Eastern/Southeastern Germany (Fig. 3b).

Recently, we detected more WNV positive birds in Germany, like a second great grey owl in the wild park near by Munich, the partner bird from the third case, which died one week later on the infection, two snowy owls in an aviary in Berlin and two more northern goshawks with clinical symptoms (data not shown). Moreover, two equine cases (one fatal and one with recovery) were also diagnosed meanwhile.

Fig. 2. Bayesian maximum clade credibility (MCC) tree representing the time scale phylogeny reconstruction of the European clade of WNV lineage 2 including the German WNV strains from this study. The colored branches of MCC tree represent the most probable geographic location of their descendant nodes (see color codes). Bayesian posterior probabilities (> 90%) are indicated at the nodes. Time is reported in the axis below the tree and represents the year before the last sampling time (2018). The estimated TMRCA (time to the most recent common ancestor) of German WNV strains from is shown with 95% posterior time intervals in parentheses.

It is particularly noteworthy that a 31-year-old veterinarian, who performed the necropsy of the bird from case 3 (great grey owl), developed flu-like symptoms 3 days after and revealed one month after the necropsy specific WNV-IgM antibodies (ProMed-Mail archive number 20181006.6074497, accessed 06.10.2018).

Therefore, it must be assumed that there is a larger epizootic ongoing in wildlife animals over this wide area and the few confirmed submitted captive and wildlife bird cases represent only the outmost tip of an iceberg.

The future will show how the WNV strain lineage 2 will establish and further spread in Germany and how high the risk of further human infections will be.

Declaration of interest

None.

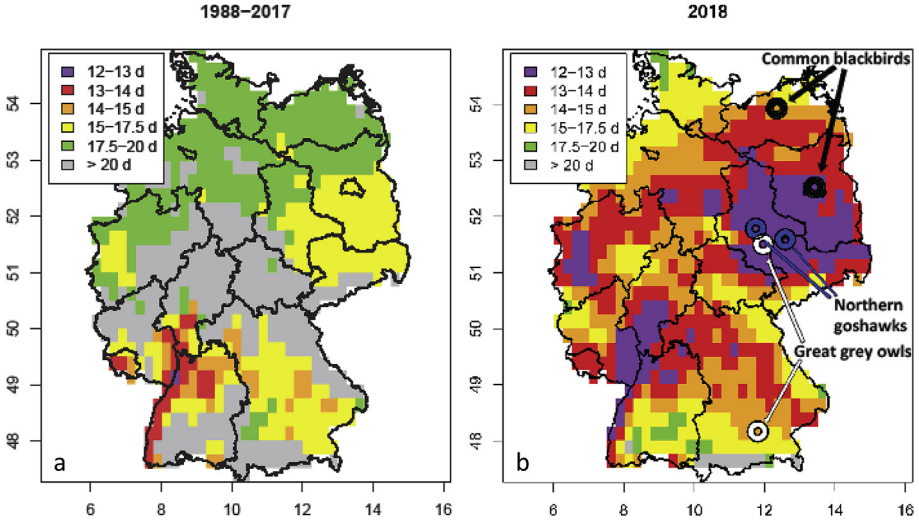

Fig. 3. Average extrinsic incubation period from 1988 to 14th August 2018. (a) Minimum annual average extrinsic incubation period between 15th July to 14th August for the years 1988–2017, (b) Average extrinsic incubation period between 15th July to 14th August 2018 and distribution of West-Nile virus positive birds (circles).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2018.12.005.

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