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Research article

Emergence and establishment of Usutu virus infection in wild and captive avian species in and around Zurich, Switzerland—Genomic and pathologic comparison to other central European outbreaks

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ABSTRACT

In late summer 2006 considerable mortality in wild and captive Passeriformes and Strigiformes was observed in Zurich, Switzerland. All animals were found in a range of 2 km². Observed clinical signs involved depression, ruffled plumage, incoordination, seizures and peracute death. Nutritional status was generally moderate to poor in wild birds, and variable in captive animals. Necropsy showed marked splenomegaly, a mild hepatomegaly, and pulmonary hyperemia in most animals. Histopathologic lesions were very discrete and consisted mainly of neuronal necrosis, leucocytolysis in and around the brain blood vessels, and miliary liver necrosis. The diagnosis Usutu virus (USUV) infection was established by USUV-specific immunohistochemistry and reverse transcription-polymerase chain reaction. Partial nucleotide sequence comparisons revealed >99% identity between the viruses that emerged in Zurich in 2006, in Vienna in 2001, and in Budapest in 2005. Since 2008 a significantly lower mortality was observed in wild Passeriformes, but USUV infection was confirmed for the first time beyond Zurich city limits. Indoor housing and regular treatment against ectoparasites are likely to have prevented acute USUV disease in captive Strigiformes. USUV is a mosquito-borne flavivirus causing fatalities in various avian species. After the initial European outbreaks in Austria in 2001 it appears that the virus has extended its range in Central Europe and has established a transmission cycle between local bird and mosquito species. Further episodes of increased avian mortality in the forthcoming years, with impact on wild and captive bird populations, predominantly Passeriformes and Strigiformes, can be anticipated. Furthermore, the possibility of broader dispersal of USUV in Europe during the next mosquito seasons must be considered and an increased mortality in Passeriformes and Strigiformes must be expected until protective “flock immunity” is established. Collections of valuable and endangered Passeriformes and Strigiformes, especially young of the year, should therefore be housed indoors or treated against ectoparasites at acceptable intervals between July and September each year.

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1. Introduction

Usutu virus (USUV) is a mosquito-borne flavivirus, which was first isolated from *Culex naevei* in South Africa in 1959 (Williams et al., 1964). In the first documented emergence of USUV outside Africa, it caused fatalities in birds, especially in wild blackbirds (*Turdus merula*) and captive great grey owls (*Strix nebulosa*) in and around Vienna, Austria, between 2001 and 2005 (Chvala et al., 2004; Weissenböck et al., 2002, 2003). Subsequently the virus spread to Hungary (Bakonyi et al., 2007) and Italy (Manarolla et al., 2010), and caused outbreaks in free and captive wild bird populations. While closely related to important human pathogens, such as Japanese encephalitis virus (JEV) and West Nile virus (WNV), human disease unequivocally caused by USUV has not been reported previously, although USUV was isolated from a patient with fever and rash in Africa (Digoutte and Adam, 2007), and viral RNA was detected in a patient with rash in Austria (Weissenböck et al., 2007). Recent reports, however, described a neuroinvasive USUV infection in a patient with diffuse large B cell lymphoma (Pecorari et al., 2009), and another neuroinvasive case in a patient who received an orthotopic liver transplant (Cavrini et al., 2009). Both cases occurred in Italy, in late summer 2009.

Pathogenicity of USUV in avian species varies. While domestic chickens (*Gallus domesticus*) (Chvala et al., 2005) and domestic geese (*Anser anser f. domestica*) (Chvala et al., 2006) seem to be resistant to USUV infection, especially blackbirds and owls were severely affected in the Austrian outbreak (Weissenböck et al., 2002, 2003). Pathologic alterations consisted of macroscopic enlarged liver and spleen, and histologically of necrotizing hepatitis, myocardial degeneration, and neuronal necrosis (Weissenböck et al., 2003).

From the end of July through mid September 2006, considerable acute mortality in wild and captive Passeriformes and Strigiformes was observed around Zurich zoo, Switzerland. Investigations determined USUV as the causative agent. Since then the virus caused significant mortality in captive Strigiformes during late summer every year. In view of the spread of USUV through Central Europe, the aims of the present study were (i) to describe clinical and pathological findings in affected avian species on the basis of the Swiss cases, and (ii) to compare pathological alterations, genome and disease epidemiology in Switzerland and Austria.

2. Materials and methods

2.1. Clinical history

From the end of July through mid-September 2006 a considerable mortality in wild and captive Passeriformes and Strigiformes was observed around Zurich zoo, Switzerland. All animals were found within a range of 2 km². Blood was collected from six captive Strigiformes from the right jugular vein for haematology and blood chemistry one to two days before death. The samples were analyzed within six hours at a clinical laboratory (Clinical Laboratory,

Vetsuisse Faculty, University of Zurich). Cloacal swabs were collected from dead and sick birds for avian influenza testing. Swabs were placed in Viral Culturette™ tubes (Becton Dickinson Microbiology Systems, Sparks, USA) and were analyzed within 24 h of collection according to described procedures at the Reference Laboratory, Institute for Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich (Dalessi et al., 2007). Blood was assayed for Newcastle disease virus antibodies using a real time RT-PCR (Camenisch et al., 2008) and for West Nile virus using a TaqMan reverse transcriptase-PCR assay (Lanciotti et al., 2000).

Due to the results of the pathological and virological analysis and an epidemiological evaluation, all Strigiformes at Zurich zoo born or acquired after October 2006 were housed indoors from July to September every year. Again from mid August to the end of September 2007, an increased number of dying wild Passeriformes were observed in the vicinity of Zurich zoo. Found carcasses in moderate to good postmortem condition were sent for necropsy and further virological analysis. In addition, one blackbird, which was found dead 15 km outside the zoo grounds, was included in the study because the animal showed clinical signs (incoordination) indicating USUV infection before death.

While in 2008 no suspicious USUV-associated avian case was detected at Zurich Zoo, in 2009 again, between July and September, captive Strigiformes and wild Passeriformes died either acutely or were seen with neurological disturbances at Zurich Zoo. After recognition of the increased mortality in Strigiformes, the remaining animals were treated for ectoparasites and transferred to indoor housing until end of September.

Data on climatic factors were obtained from the weather station Zürich Fluntern of the Swiss Weather Service and included: monthly mean air temperature in centigrade and monthly sum of rainfall from January 2000 until December 2009. Climatic factors during the last ten years were compared using a one-way analysis of variance (ANOVA). It was assumed that data from each sample were normally distributed and that each sample had the same variance, the latter were tested by Kolmogorov–Smirnov test and Hartley F_{\max} -test, respectively.

2.2. Pathology

Necropsies were performed either at the Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, or the Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, between 6 h and 48 h after the animal's death. Tissue samples were fixed in 4% buffered formalin for histopathology or immunohistochemistry (IHC) and, in addition, frozen tissue samples (brain and parenchymal organs) were stored at -80°C for further investigations. After embedding in paraffin wax, 4 μm sections were stained with hematoxylin and eosin. Besides all affected wild birds and captive Strigiformes, all birds were involved in the histopathological study that died from the collection of Zurich zoo between July and September of each year.

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