


# Usutu virus in cerebrospinal fluid: A 2-year survey in a Tertiary Care Hospital, Geneva, Switzerland

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In 2009, the Usutu virus (USUV) was first reported as a cause of human neuroinvasive disorders. In Switzerland, USUV has been detected in wild birds with a seroprevalence of up to 6.59% in captive specimens sampled from zoo enclosures. This study investigates the clinical prevalence of USUV in human acute neuroinvasive disorders in Switzerland. Two hundred and fifty-eight cerebrospinal fluid samples collected between 2015 and 2017 for routine clinical care in a tertiary level hospital (Geneva) were tested for USUV by rRT-PCR. No samples were found positive, suggesting the absence, or the extremely low circulation of USUV in Western Switzerland.

## KEYWORDS

cerebrospinal fluid, survey, Switzerland, Tertiary Care Hospital, Usutu virus

## 1 | INTRODUCTION

Usutu virus (USUV) is a mosquito-borne ssRNA flavivirus belonging to the Japanese encephalitis virus group. In the USUV life-cycle, mosquitoes vector the virus to wild and captive birds, which are the main amplifying hosts, while humans are considered incidental hosts. Since its initial isolation from a South African mosquito (*Culex neavei*) in 1959, USUV has been reported in mosquitoes from several other African countries.<sup>1</sup> In 2001, it was identified in Austrian bird species in the context of significant die-off of Eurasian blackbirds.<sup>2</sup> USUV has since spread to surrounding European countries such as Switzerland, Italy, Germany, France, Hungary, the Czech Republic, Spain, Belgium, Poland, and the United Kingdom.<sup>1,3</sup> In the summer of 2016, multiple western European countries experienced what is considered to be the largest epizootic outbreak of USUV,<sup>4</sup> which coincided with an increased number of USUV human infections. Thus, while humans are only incidental hosts, the emergence of such transmission events justifies the implementation of an arbovirus surveillance program in susceptible regions.<sup>5</sup> The first European USUV-related human infections were reported in 2009 in two immunocompromised patients presenting with severe encephalitis

from the Emilia Romagna region in northern Italy.<sup>6,7</sup> Retrospective studies performed on cerebrospinal fluid (CSF) samples collected in 2008 from the same region revealed that neuroinvasive infections also occurred among immunocompetent patients<sup>8,9</sup>; a finding later confirmed in a Croatian cohort.<sup>10,11</sup> Interestingly, Grottole and colleagues observed a higher prevalence of USUV (8/306 positive CSF samples) than West Nile virus (0/306)<sup>9</sup>; an observation they confirmed by serology.

As for any pathogen, it could be anticipated that USUV infection would have a spectrum of phenotypic presentations, ranging from severe encephalitis to asymptomatic carriage and commensal infection. This potentially introduces new contamination risk for blood transfusion products. Indeed, among 4200 serum samples collected from healthy blood donors in 2012 from south-west Germany, one sample (0.02%) tested positive for USUV-IgM and IgG.<sup>12</sup> The donor was asymptomatic and the authors concluded that the infection was probably autochthonous. Similarly, another healthy blood donor in Germany in whom an acute USUV infection was discovered (viral RNA detected by RT-PCR in plasma products) presented no signs of illness in the 6 weeks prior donation and had no travel history in the 7 months prior to the donation.<sup>13</sup>

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In the summer of 2006, USUV was identified for the first time in several bird species in Zurich, Switzerland, which was related to a significant die-off in wild and captive birds (Passeriformes and Strigiformes).<sup>14</sup> Further studies in captive birds sampled between October 2006 and August 2007 from zoos in Zurich and Basel, revealed a seroprevalence of 5.3% and 6.59%, respectively.<sup>15</sup> Thus, although no human cases have been documented in Switzerland, the presence of pathogenic avian infection within Swiss territory and bordering countries, justifies the surveillance of this emerging pathogen, especially in cases of meningoencephalitis of unknown etiology. This study reports the findings of a 2-year surveillance effort (2015-2017) on the prevalence and potential clinical relevance of USUV in human acute neuroinvasive illness in western Switzerland.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics statement

This study was approved by the Research Ethics Committee of Geneva (project # 2016-01096).

### 2.2 | Cohort

The specimens used in this study were collected between May 1st, 2015 and July 31st, 2017 and included all CSF samples (paediatric and adult patients) sent to the laboratory of virology of the University Hospitals of Geneva (Geneva, Switzerland) for routine viral screening. Inclusion criteria were a total leukocyte count of  $>5$  cells/ $\mu$ L and those having sufficient leftover volume after all other clinical tests were performed. All samples were stored at  $-80^{\circ}\text{C}$  before analysis.

### 2.3 | Screening of Usutu virus

Nucleic acids were extracted using the NucliSENS easyMAG (bioMérieux, Geneva, Switzerland). Before extraction, a standardized

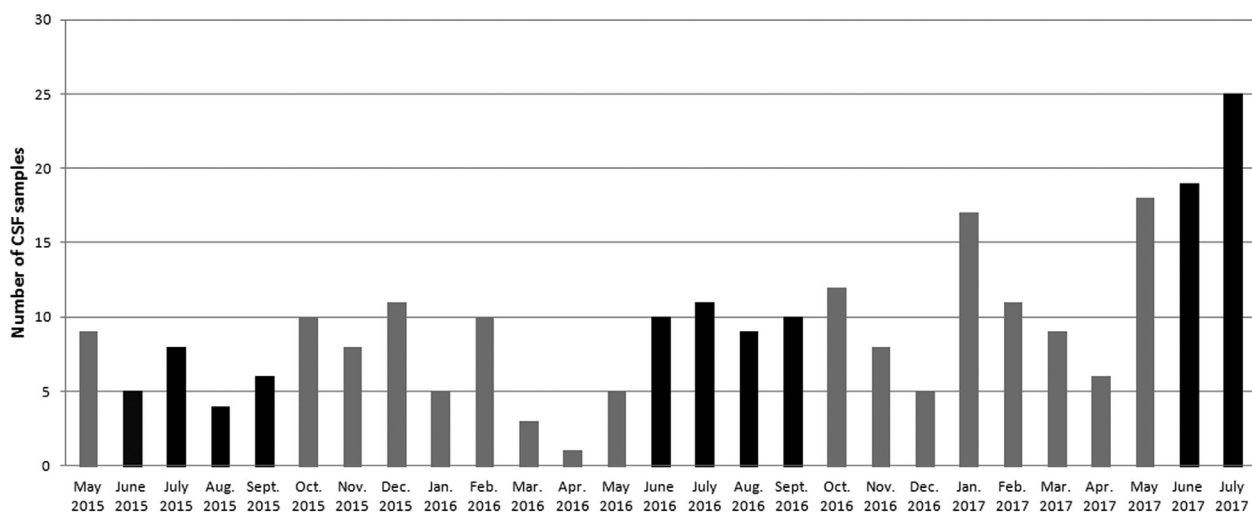
canine distemper virus was added to each sample and used as an internal control, as previously described.<sup>16</sup> The Usutu virus screening was performed using the rRT-PCR published by Nikolay B and colleagues,<sup>17</sup> under the same conditions. Data were analyzed with the StepOne software V.2 (Applied Biosystems, Rotkreuz, Switzerland). For each assay, duplicates of 10-fold dilution plasmid-derived transcribed RNA (corresponding to the GenBank reference sequence NC\_006551) were included as positive controls.

## 3 | RESULTS

During the 2-year surveillance period, a total of 258 CSF samples were collected from 240 individual patients (55 paediatric and 185 adult patients). The median age was 38-year-old (range, 0-93). Three samples showed a strong inhibitory signal and were therefore excluded. Among the 255 CSF samples (107 were collected during the warm seasons, Figure 1), none were found positive for USUV by rRT-PCR.

## 4 | DISCUSSION

The recent detection of USUV virus and specific antibodies in the captive and wild birds of Switzerland and bordering countries raises concerns about its potential as an emerging human pathogen in the region. Human cases in these new endemic counties must be investigated to better understand the provenance and prevalence of transmission events and thus better predict the clinical relevance and potential of future outbreaks. A recent phylogenetic analysis based on whole genome sequences obtained from mosquitoes and birds between 2010 and 2014 in the northern part of Italy suggests that although there is a continuous exchange of USUV strains between Africa and Europe, an autochthonous evolution exists in European USUV strains related to a common ancestor that could be traced back



**FIGURE 1** Monthly distribution of the 255 CSF samples screened during the 2-year survey. Black and grey columns correspond to samples collected during the warm (June-September) and cold seasons, respectively

to the early 1990s.<sup>18</sup> Indeed, the virus seems to find a favorable terrain in Europe thanks to the presence of numerous reservoir hosts including a large number of wild bird species but also potentially bats.<sup>19</sup>

The study surveilled patients over a wide temporal margin (spanning 2 years) and the results suggests the absence, or very low prevalence of circulating USUV in the western part of Switzerland, the canton of Geneva. As, a- or pauci-symptomatic presentations have been described in some human USUV infections (eg, rash, fever, jaundice), this conclusion based on the screening by real-time RT-PCR of acute neuroinvasive infections should be confirmed in larger serological studies for a more complete picture of clinically relevant USUV infection. The limitations of this study are: 1) the blood samples from patients tested in this study were not available to supplement our investigations with serological analysis; and 2) although all CSF with a total leukocyte count of >5 cells/ $\mu$ L and a sufficient leftover volume collected during the 2-year survey were tested further analyzes on a larger number of CSF and blood samples collected from different Swiss regions are needed. Of note, as no laboratory surveillance system exists at the national level (Federal Food Safety and Veterinary Office) for USUV, there is no information available on USUV circulation in birds in Switzerland during the study period.

Finally, this pilot study indicates that the CSF-USUV screening should be restricted to specific patients presenting with acute meningo-encephalitis of unknown etiology during the warm season and for whom a viral cause is suspected.

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## CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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