A new wild type mouse model paves the way for the study of Usutu virus pathogenicity in mammals

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Introduction

Usutu virus (USUV) is an emerging mosquito-borne Flavivirus closely related to West Nile virus (WNV) [1]. Originating from Africa, USUV emerged in Italy in 1996 [2], and now co-circulates in many European countries with WNV, causing seasonal mass mortalities in various bird species as well as occasional neurological diseases in both healthy and immunocompromised mammals, including humans [3, 4, 5]. The factors underlying the development of these events are still misunderstood and, thus, the increasing number of cases diagnosed in EU in the past few years generates growing interest in the establishment of a consistent, relevant mammalian model of USUV infection. Since adult immunocompetent mice proved to be quite resistant to USUV infection, with individuals showing a sporadic neuroinvasive disease [6], the most commonly used mammalian models so far are either immuncompromised or newborn mice [7]. However, we have recently developed a wild-type (WT) model based on the age-dependent susceptibility of mice to USUV infection, using older but genetically identical pups as controls. To identify the main host-pathogen interactions responsible for the observed difference in outcome of infection, we compared the viral dissemination and the immune response in the two groups of mice over time.

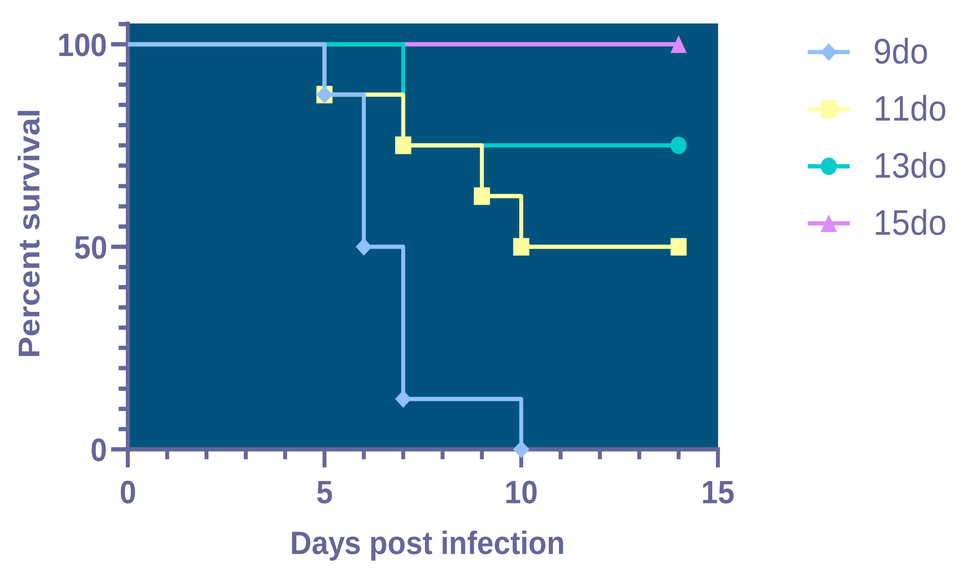
Materials and methods

We compared the susceptibility of 4 groups (9, 11, 13 or 15 days old) of 8 WT 129/Sv female pups. Each pup was inoculated in both footpads with a total dose of 10^6 TCID50 of Usutu virus (strain USU-BE-Seraing/2017, lineage Europe 3, *GenBank*: MK230892). Based on the results, the 9 day-old and 15 day-old pups were chosen as “susceptible” and “resistant” groups, respectively, and a kinetic comparison of the viral dissemination and the host’s response was conducted, using RT-qPCR and flow cytometry. The immunostainings were performed using a polyclonal anti-USUV antibody.

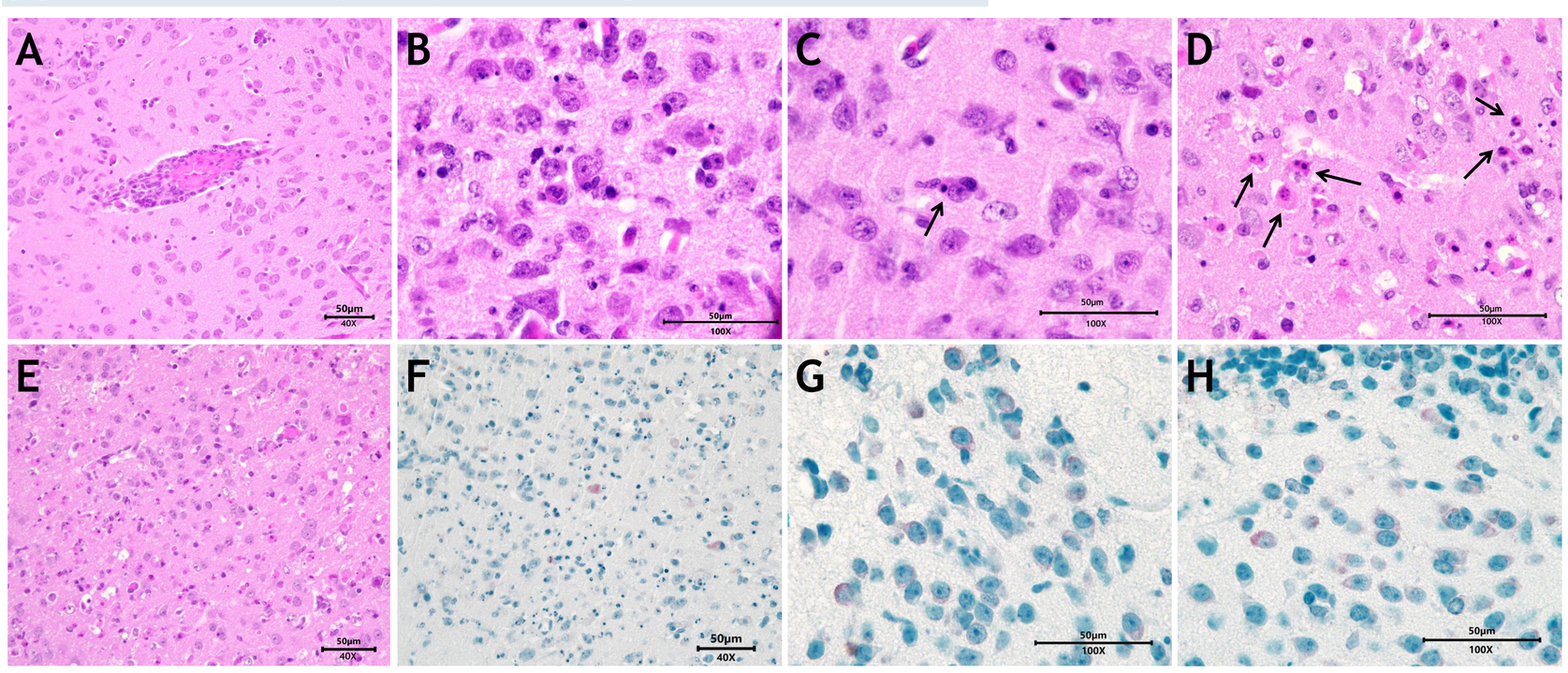
Results

While 100% of the pups infected at 9 days old died between 5 and 10dpi, mortality dropped to 50%, 25%, and 0% for the 11, 13 and 15 days old groups, respectively. While no clinical or histological consequence of the viral inoculation was found in resistant mice, the susceptible group suffered a neurotropic infection and displayed severe changes in the brain (extensive neuronal apoptosis and necrosis, satellitosis, neuronophagia and lymphoplasmacytic perivascular cuffs) and evident viral antigen expression in neurons. Based on preliminary flow cytometry data, infected pups seem to exhibit an early monocytosis when compared to the controls.

Kaplan-Meier survival curves of USUV-infected mice by age of infection

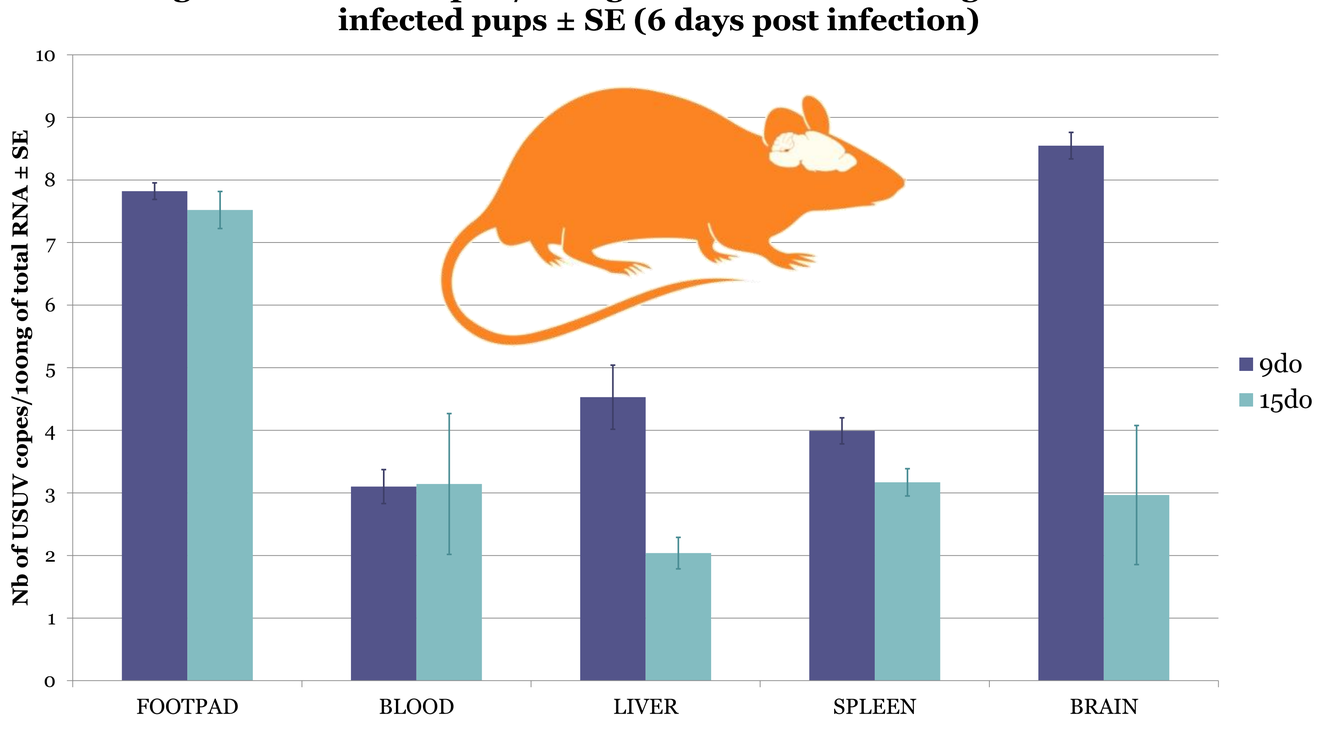


Log-Rank (Mantel-Cox), LogRank (trend) and Gehan-Breslow-Wilcoxon tests (performed on GraphPad Prism 9.3.1) show significant difference between curves ($p < 0.0001$)



(A) One to six cell layers-wide lymphoplasmacytic perivascular cuff in the brain cortex of a pup infected at 9 days old (do), 8 days post infection (dpi). Hematoxylin-eosin (HE) staining, magnification (Ma) x400. (B) Multifocal areas of massive satellitosis involving up to 25-30% of the cut surface of the brain cortex in a pup infected at 9 do, 6dpi. HE, Ma x1000. (C) Satellitosis and evident neuronophagia (arrow) in the brain cortex of a pup infected at 9 do, 6dpi. HE, Ma x1000. (D) Extensive neuronal apoptosis (arrows) in the brain cortex of a pup infected at 9 do, 6dpi. HE, Ma x1000. (E) Multifocal areas of massive neuronal death involving up to 25-30% of the cut surface of the brain cortex in a pup from the 9do group, 6dpi. HE, Ma x400. (F) Massive neuronal death correlated with USUV immunostaining in the brain cortex of a pup infected at 9 do, 6dpi. Hematoxylin counterstaining, Ma x400. (G,H) USUV-immunostained neurons in the brain cortex of a pup from the 9do group, 8dpi. Hematoxylin, Ma x1000.

Log10 USUV RNA copies/100ng of total RNA in the organs of USUV-infected pups ± SE (6 days post infection)



Mann-Whitney tests revealed significantly higher viral loads in the brain of susceptible (9do) pups compared to their liver, spleen, blood ($p<0.01$) and footpad ($p<0.05$).

Discussion and conclusion

These results pave the way for the study of the pathogenicity of Usutu virus in mammals, as they show a marked age-dependent susceptibility of mice to Usutu virus, and the evident neurotropism of the infection. Interestingly, survivors did not show any clinical signs and presented a marked decrease in viral titers and dissemination, suggesting that the outcome of the infection is determined at a very early stage after inoculation.

References

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