

USUTU VIRUS INFECTION AND PLASMODIUM CO-INFECTION IN BLACKBIRDS (*TURDUS MERULA*) IN THE NETHERLANDS FROM 2016 TO 2018

G. Giglia^{*}, C.B.E.M. Reusken[†], F. Schaafsma[‡], M.T. Mandara^{*}, J. Rijks[§], R. Sikkema^{||}, A. Gröne[§], M. Koopmans^{||}, M.H. Verheije[‡] and J.M.A. van den Brand[‡]
^{*}Department of Veterinary Medicine, University of Perugia, Perugia, Italy, [†]National Institute for Public Health and the Environment, de Bilt, [‡]Department of Pathobiology, Veterinary Faculty, [§]Dutch Wildlife Health Centre, Utrecht University, Utrecht and ^{||}Department of Viroscience, Erasmus Medical Center, Rotterdam, The Netherlands

Introduction: Usutu virus (USUV), a mosquito-borne flavivirus, has been identified as the cause of die-offs in wild bird species. In particular, high mortality has been observed in blackbirds (*Turdus merula*) in Europe. To monitor the circulation of USUV in blackbirds in The Netherlands and describe associated lesions, blackbirds that were found dead were investigated in a passive surveillance programme from 2016 to 2018. Since *Plasmodium* sp. co-infection was previously reported in USUV-infected birds, the co-existence of these agents was evaluated.

Materials and Methods: From 2016 until 2018, 162 dead blackbirds were sent to the Dutch Wildlife Health Centre for necropsy examination. Various tissues were sampled for virology, histopathology and immunohistochemistry (IHC).

Results: By qPCR, 72% of the examined blackbirds were positive for USUV (117/162). The most prominent gross lesions were spleno- and hepatomegaly. Histologically, lymphoplasmacytic inflammation and necrosis were seen in the liver, spleen and heart; in the brain there were foci of gliosis. IHC confirmed the presence of USUV associated with the described lesions. In a substantial number of USUV-positive birds there was microscopical evidence of exo-erythrocytic stages of *Plasmodium* sp. in lung, liver, spleen, brain and heart.

Conclusions: This research demonstrates the presence of USUV and associated lesions in blackbirds in the Netherlands from 2016 to 2018. Additionally, co-infection with avian malaria, *Plasmodium* sp., was seen in a substantial proportion of the birds. Since the correlation between USUV and *Plasmodium* sp. in blackbirds is not known, further studies are needed.

TOWARDS IMMUNOCOMPETENT IN-VIVO MODELS OF USUTU VIRUS INFECTION

E. Benzarti, F. Rivas and M. Garigliani

Pathology, Faculty of Veterinary Medicine, University of Liège, Belgium

Introduction: Usutu virus is a mosquito-borne flavivirus, closely related to West Nile virus and originating from Africa, which emerged in Europe in the late 1990s. Birds are considered the main reservoir, while infection, often subclinical, is reported in several mammalian species. Usutu infection may result in severe, although transient, neurological impairment in human patients. In bats, the infection may be lethal. In birds, mass mortalities have been observed, mostly in the Eurasian blackbird (*Turdus merula*; Passeriformes) and nocturnal raptors (Strigiformes), while many bird species, including domestic chickens, are resistant. Little is known about the mechanisms underlying this selective pathogenicity. The only in-vivo infection model validated so far for Usutu virus is laboratory mice with knocked-out type I interferon receptor. To better understand Usutu virus pathogenicity, it is critical to develop and validate immunocompetent avian infection models.

Materials and Methods: Passeriforme birds were infected by different routes and infectious doses of Usutu virus. Animals were monitored twice daily for clinical signs, mortality and body weight. Similarly, embryonated chicken eggs were infected at different doses/strains. Samples were taken at different times post infection for histopathological, immunohistochemical and molecular analyses.

Results: The clinical signs, viral distribution in the different organs of the birds, as detected by PCR and immunohistochemistry, and excretion mimicked that observed with natural cases of infection.

Conclusions: The models developed in this study will permit the detailed study of the innate immune response to the infection, the modes of excretion and transmission, and the virulence determinants of Usutu virus in birds.

HISTOLOGICAL LESIONS AND VIRUS DETECTION IN RATS, BANK VOLES AND MICE INFECTED EXPERIMENTALLY WITH VARIEGATED SQUIRREL BORNAVIRUS 1

D. Nobach^{*}, K. Schlottau[†], D. Hoffmann[‡], M. Beer[‡], C. Herden^{*} and C. Herden^{*†}

^{*}Institute of Veterinary Pathology, [†]Center of Mind, Brain and Behaviour, Justus-Liebig-University Giessen, Giessen and [‡]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald, Germany

Introduction: Variegated squirrel bornavirus 1 (VSBV-1) is a zoonotic RNA virus of the family Bornaviridae and was identified as a cause of human lethal encephalitis. Since 2015, it has been shown to be present in various exotic squirrel populations in zoos and private collections.

Materials and Methods: For the development of small animal models representing either the reservoir (squirrel) or the accidental host (man), neonatal and adult rats ($n = 94$), bank voles ($n = 55$) and mice ($n = 96$) were infected with VSBV-1 by different infection routes (intracerebral, intranasal or subcutaneous). After 3 or 5 months, animals were examined for gross and histological lesions. Virus distribution was analysed by immunohistochemistry detecting the viral phosphoprotein.

Results: Gross lesions were not found in any of the animals. Some rats ($n = 3$) and bank voles ($n = 2$) displayed lymphohistocytic encephalitis with perivascular cuffs and reactive microglia. In the CNS, viral antigen was present in neurons and astrocytes in the hippocampus, cerebral cortex, thalamus and brainstem in intracerebrally infected rats ($n = 3$) and bank voles ($n = 3$). Additionally, in one subcutaneously infected bank vole, neurons and astrocytes of spinal ganglia and spinal cord harboured viral antigen. No virus antigen was found in peripheral organs.

Conclusions: These results might indicate different courses of infection depending on the species, the age and the route of infection. The presence of viral antigen in the CNS reflects a strict neurotropism like in the accidental host, but the duration of the infection could influence the results and must be considered in future studies.

Education and Research

VIRTUAL PATHOLOGY ROUNDS – A BRIDGE BETWEEN PATHOLOGISTS, CASES, STUDENTS, TEACHING AND TECHNOLOGY

P. Bolfa^{*}, C. Fuentealba[†] and O. Illanes[†]

^{*}Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, St. Kitts & Nevis and [†]College of Veterinary Medicine, Long Island University, Brookville, USA

Introduction: Worldwide, most veterinary schools host weekly or bi-weekly gross pathology rounds with cases submitted to their pathology service. The aim of this project was, with the use of a modern digital learning tool, to maximize the value of this activity by making it available to a larger audience of technologically aware students, offering them the opportunity to learn at their own pace and from anywhere.

Materials and Methods: Cases submitted to the pathology services at RUSVM are being routinely archived (gross images, pathology reports, histology slides, clinical pathology data, ancillary testing etc.). Cases with high learning potential are assigned to fourth semester student volunteers (four to six students per semester) enrolled in the systemic pathology course, who compile these cases into a presentation containing the most significant findings, high-quality gross/histology/cytology images, pathology interpretation and a discussion that includes a literature search. Subsequently the document is reviewed by a pathologist and submitted to the academic technology team for conversion into the Virtual Pathology Rounds (VPR) eBook format.

Results: As all RUSVM students receive an iPad on acceptance into the Doctor of Veterinary Medicine programme, the eBook is uploaded in the iPad and updated every semester. Students are currently using the VPR as part of their systemic pathology course.

Conclusions: Surveys indicate that the VPR is a great interactive learning resource valued by the students. Future plans include integration of the eBook into the Clinical Pathology course and its use as a formative assessment tool.