



## Subclinical hepatitis E virus infection in the laboratory ferrets in the UK

Fabian Z. X. Lean<sup>1^\*</sup>, Anne-Laure Leblond<sup>2</sup>, Alexander M. P. Byrne<sup>1</sup>, Benjamin Mollett<sup>1</sup>, Joe James<sup>1</sup>, Samantha Watson<sup>1</sup>, Shellene Hurley<sup>1</sup>, Sharon M. Brookes<sup>1</sup>, Achim Weber<sup>2</sup> and Alejandro Núñez<sup>1</sup>

<sup>1</sup> Animal and Plant Health Agency (APHA), Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK

<sup>2</sup> Department of Pathology and Molecular Pathology, University Zurich and University Hospital Zurich, Zurich, Switzerland.

<sup>^</sup>Current address: Department of Pathobiology & Population Sciences, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, AL9 7TA, UK

\*Correspondence: Fabian Z. X. Lean, [flean22@rvc.ac.uk](mailto:flean22@rvc.ac.uk)

### Abstract

Ferrets are widely utilised as experimental models for studying viral infections. However, the presence of pre-existing diseases in ferrets can complicate the interpretation of experimental findings. In this study, we detected a subclinical infection of ferret hepatitis E virus (FRHEV) in a subgroup of female laboratory ferrets enrolled in a SARS-CoV-2 study. Histopathological examination of liver samples collected at various experimental endpoints, ranging from day 3 to day 24 post-infection, revealed the presence of lymphoplasmacytic cuffing in periportal spaces. However, testing for SARS-CoV-2 RNA and antigens yielded negative results. Viral metagenomic analysis conducted on liver specimens detected FRHEV-associated sequences, which were subsequently confirmed using reverse-transcriptase polymerase chain reaction. *In situ* hybridization by RNAScope® confirmed the presence of HEV-specific RNA in hepatocytes, while immunohistochemistry demonstrated the presence of the HEV open reading frame 2 (ORF2) protein in hepatocytes and biliary canaliculi. In conclusion, our report demonstrates the association of FRHEV infection, as detected through microscopy and molecular virology, and the presence of lymphoplasmacytic cuffing of hepatic periportal areas in ferrets naturally infected with FRHEV. Although the epidemiology of this infection remains unclear, it is recommended to perform virological testing in cases exhibiting these histological changes to determine the involvement of FRHEV and prevent erroneous attribution of observed liver disease in *in vivo* studies.