Pathogenesis of Intranasal Inoculation of Rift Valley Fever Virus In The Ferret

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Background

Rift Valley fever virus (RVFV) is an emerging arbovirus endemic to Africa and the Arabian peninsula which infects many domestic, wild and laboratory animals, as well as humans[1]. In people, infection is often through contact with body fluids or organs of infected animals, and although it usually manifests as a self-limiting influenza like illness, a proportion of cases progress to encephalitis and ophthalmitis, as well as haemorrhagic fever and hepatitis, with high morbidity and varying case mortality[2]. The ferret (*Mustela putorius furo*) has recently been proposed as a new animal model for studying human RVFV infections[3]. In this study the **neuropathogenesis, viral tropism and progression of RVFV infection** is investigated in ferrets through intranasal inoculation with RVFV.

Materials & Methods

18 ferrets were inoculated intranasally; 15 were given a single high dose (10^7pfu/ml) of RVFV strain ZH501, and 3 controls were mock inoculated with tissue culture medium, then clinically monitored over the course of the study. Serial blood samples and swabs were taken for virological and serological analyses. Following inoculation, 3 ferrets were euthanised at 3 days post infection (dpi), and the remainder upon reaching clinical end points. Tissues were collected and evaluated for histopathology and for RVFV-specific immunohistochemistry (IHC). After antigen retrieval at 100°C at pH 9, sections were incubated with 1:12000 rabbit polyclonal anti-RVFV nucleoprotein antiserum (ACDP CSIRO, Australia) or control serum, and the signal amplified using the DAKO EnVision+.

Results

Clinical Findings

The following clinical signs were observed at the first time point (3 dpi), and the remaining at clinical end-points. Euthanasia was necessary early in the time course.

Clinical Signs	3 dpi N=3	6dpi N=1	7dpi N=9	8dpi N=2	Controls N=3
Pyrexia*	1	0	5	2	0
Dyspnoea	0	1	2	0	0
Neurological signs	0	1	9	2	0

*Pyrexia defined as ≥ 40.1°C.

Gross Findings

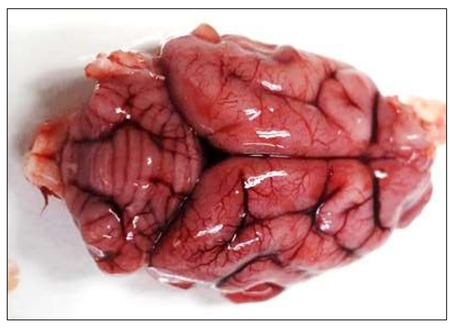
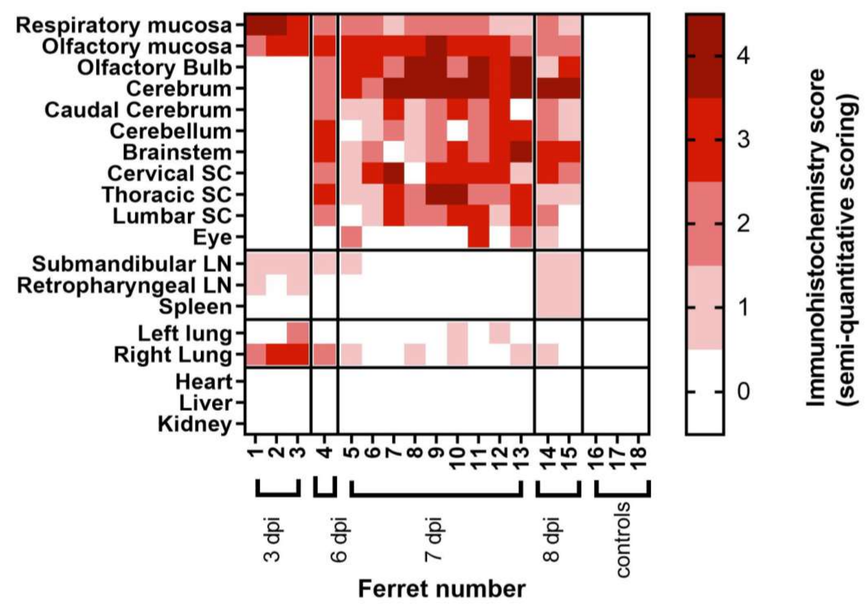


Figure 1: Gross image of the ferret brain at 6 dpi. Mild to moderate reddened meninges in **all** infected ferrets.

Histopathology and Immunohistochemistry

The distribution of viral antigen in tissues using a semiquantitative scoring (0-4 where 0 is no staining, 4 is widespread) is shown below:



Results (Contd.)

Figure 2: At **3 dpi**, an acute mild to moderate, multifocal suppurative rhinitis was seen in respiratory (2a and 2b, x20) and olfactory (2c and 2d, x40) nasal turbinates. Virus was detected in presumptive sensory cells and associated nerve fascicles in the respiratory and olfactory mucosa and submucosa (brown immunolabelling). A mild to moderate acute multifocal angiocentric pneumonia was identified, with virus detected in interstitial macrophages, in the perivascular space (2e and 2f, x20), and occasionally in type 1 and 2 pneumocytes.

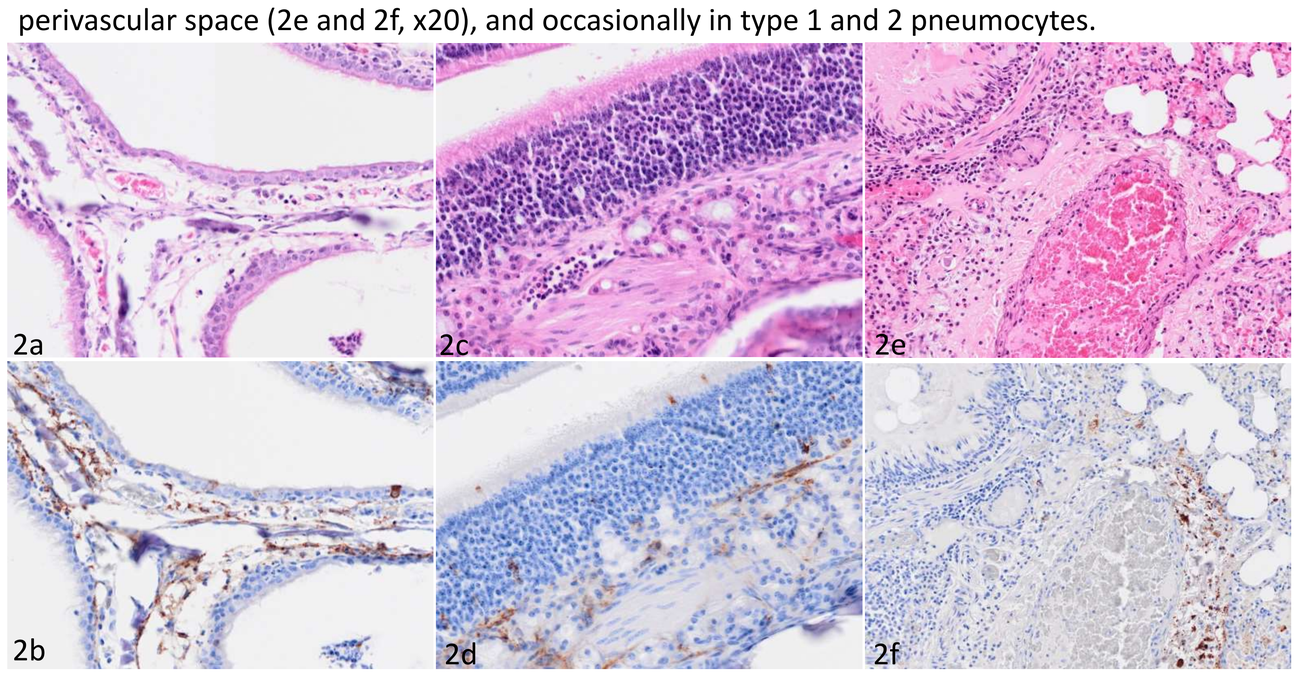
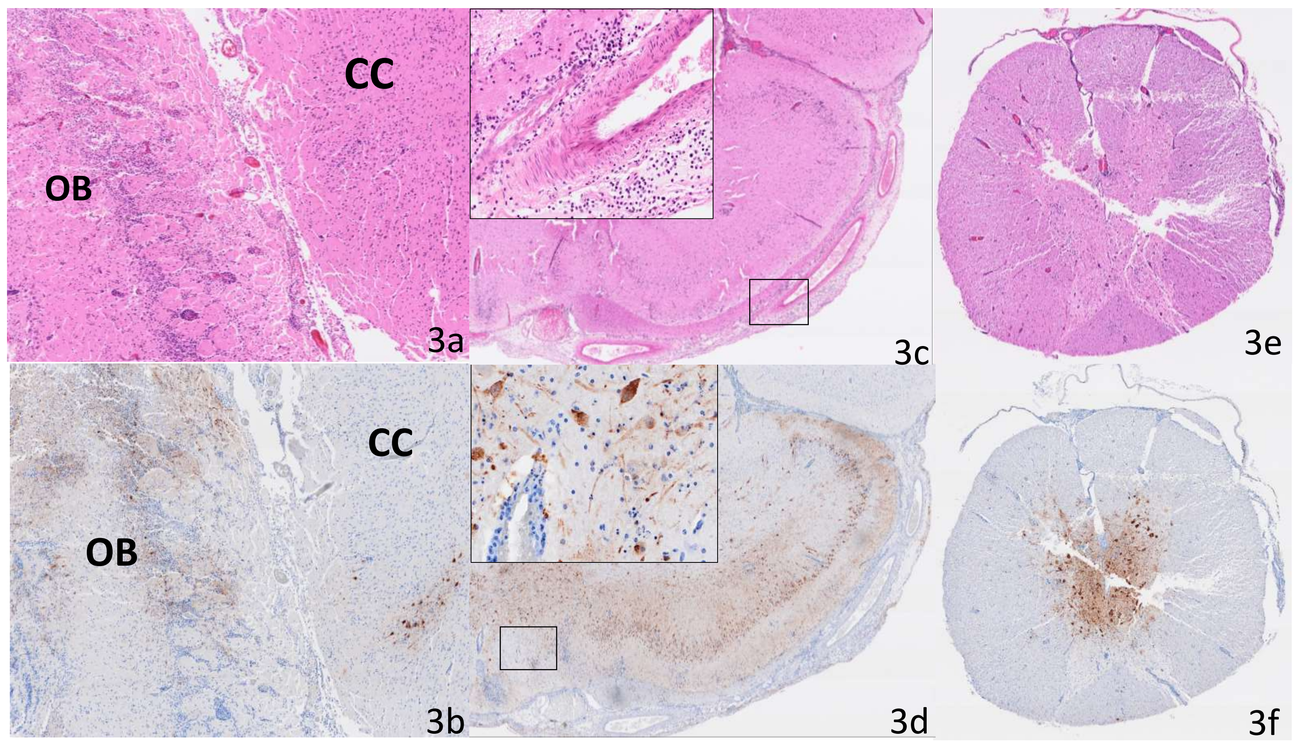


***Figure 3*:** From **6 dpi**, rapid extension of infection into the central nervous system was evident, characterised by a multifocal, peracute to acute lymphohistiocytic meningoencephalomyelitis of varying severity, with a mild neutrophilic response, spreading from the olfactory bulb (**OB**) (3a and 3b, x20, **7 dpi**) to the whole of the remaining central nervous system, including the cerebral cortex (**CC**), (3c and 3d, x20, **7 dpi**) and the spinal cord (3e and 3f, x2 **6 dpi**). Inset of 3c. HE x40 shows moderate perivascular and meningeal infiltration. Inset of 3d. RVFV IHC x40 illustrates the widespread immunolabelling seen in neurons, and moderate perivascular cuffing in the brain.



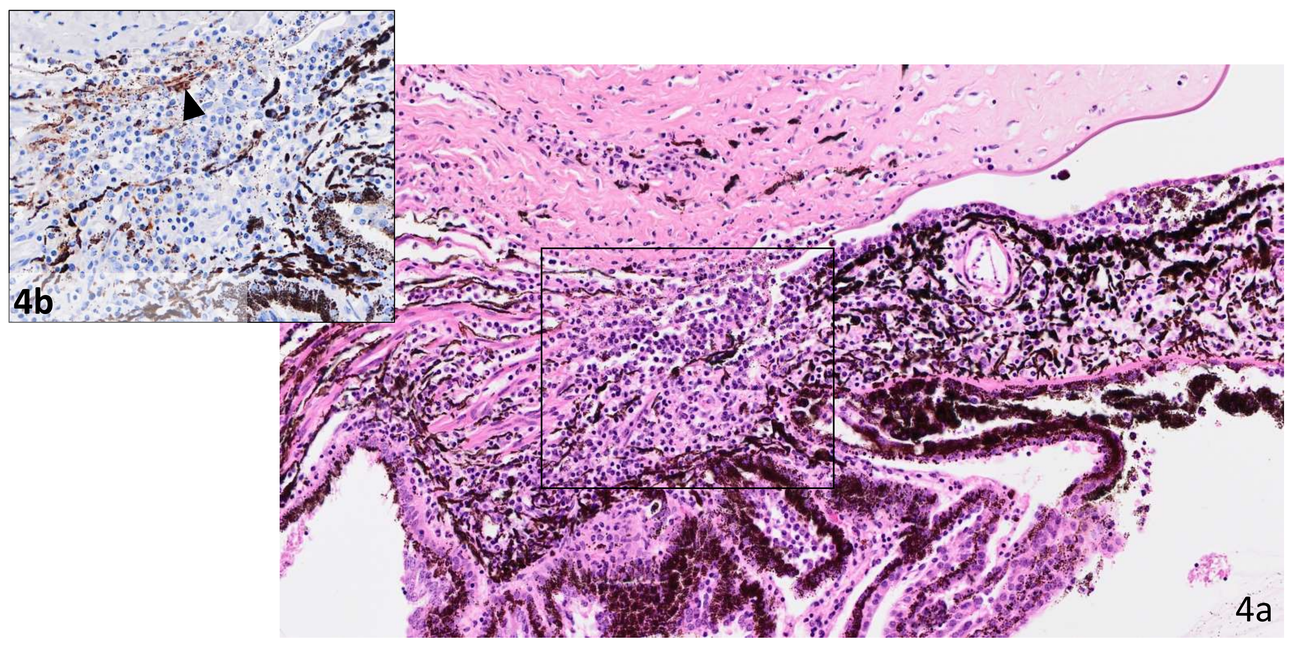


Figure 4: In the eye, (4a, x20) a moderate subacute diffuse lymphohistiocytic anterior uveitis, visible at the iridocorneal angle and extending into the iris, with viral antigen localised in presumptive nerve fascicles (see black arrowhead, inset 4b x20) was identified at **8 dpi**.

Discussion and Conclusions

- Following intranasal inoculation, this ferret model demonstrates the strong neuronal tropism of RVFV, where virus is taken up through both respiratory and olfactory mucosal neural pathways, and rapidly progresses anterograde into the central nervous system, leading to moderate to severe acute, multifocal meningoencephalomyelitis.
- Viral antigen was detected in the eye of four ferrets with clinical signs on days 7 and 8 dpi, one of which had anterior uveitis, and two with mild optic neuritis. Ocular complications occur in up to 10% of human cases, with 50% of patients permanently blind[2]. Only one rodent model has been established to study the pathogenesis of ocular disease, so the finding of ocular changes in these ferrets highlights the potential for further studies.
- Changes identified in the lungs suggest systemic spread of infection, highlighting the virus's ability to disseminate to its target organs through multiple routes.

References:

1. ***Dodd, K.A. et al. (2014). PLoS Negl Trop Dis 8(6): e2874***
2. ***Schwarz, M.M. et al (2022). J Virol 2022 Vol. 96 Issue 20 Pages e0111222***
3. ***Barbeau DJ et all, (2020). mSphere. 2020 Oct 28;5(5):e00798-20:***

Acknowledgements: *Many thanks to all staff of the Animal Sciences, Laboratory Services, Pathology and Virology departments of the Animal Plant and Health Agency (APHA) for their hard work and contribution. This study was funded by VetBioNet (grant number 731014) and DEFRA (England and Wales).*