Generation of EHV-1 pseudotype virus for cell tropism studies and virus-neutralising assays

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ranked titres (e.g. 4, 8 etc.), the EHV-1-VN RTNA is an automated, sensitive and objective measurement that reports continuous titres.
Ethical animal research: All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19).
Informed consent: Informed consent was given by the horses' owners.
Competing interests: None declared.
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80 | Detection of *Equid herpesvirus-1* in serum samples collected from infected horses

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Background: Abortion and myeloencephalopathy are caused by *Equid herpesvirus-1* (EHV-1) infection as a consequence of its transmission to susceptible organs by viraemia. Since EHV-1 circulates in the bloodstream in a cell-associated manner, serum samples collected from infected or febrile horses are rarely used for virus detection.
Objectives: To determine the usefulness of horse serum samples for the detection of EHV-1.
Study design: Assay assessment.
Methods: Archived sera and peripheral blood mononuclear cells (PBMCs) collected daily from three horses that had been experimentally inoculated with EHV-1 during the subsequent two-week observation period [1] were investigated. Acute-phase serum samples collected from 40 febrile (≥ 38.5°C) horses, including 11 serologically confirmed field EHV-1 cases were also examined. Real-time PCR was used to detect EHV-1 in the samples.
Results: EHV-1 was detected in experimental PBMC and serum samples for 6 to 7 days (from post-infection day [PID] 5 or 6 to 11 and sporadically in one sample at PID 1) and 5 to 7 days (from PID 5 or 7 to 11), respectively. Six of 11 acute-phase serum samples collected from field EHV-1 cases were positive for the virus, whereas the rest of the field sera were negative.
Main limitation: The presence of cell-free intact particles of EHV-1 in horse sera was unclear.
Conclusions: EHV-1 was detected almost simultaneously in PBMC and serum samples collected from experimentally infected horses. Additionally, more than half of the field acute-phase sera collected from EHV-1 infected horses tested positive for the virus, which suggests that the pyrexia observed in these horses was caused by viraemia. These results show that serum samples collected from EHV-1 infected or febrile horses can be used to detect the virus if PBMC samples are not available.

Reference

[1] Bannai, H., Nemoto, M., Tsujimura, K., Yamanaka, T., Kokado, H., Kondo, T. and Matsumura, T. (2018) Comparison of protective efficacies between intranasal and intramuscular vaccination of horses with a modified live equine herpesvirus type-1 vaccine. *Vet. Microbiol.* **222**, 18–24.

Ethical animal research: This study was approved by the Research Planning and Ethics Committee of the Equine Research Institute with accession number 2018-3263-07.
Informed consent: Owner consent was obtained for all field samples.
Competing interests: None declared.
Source of funding: Japan Racing Association.

81 | Generation of EHV-1 pseudotype virus for cell tropism studies and virus-neutralising assays

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Background: Equid herpesvirus-1 (EHV-1) can cause respiratory disease, abortion, neonatal death and myeloencephalopathy. Thus, EHV-1 represents a threat to the equine industry. EHV-1 exhibits 12 glycoproteins on its surface envelope, but those important for cell entry/host immune responses remains partially unclear. To investigate the contribution of these glycoproteins, pseudotype viruses (PVs) may provide a useful study tool.
Objectives: Generate high titre EHV-1 PV particles for cell tropism studies and develop tests for virus-neutralising (VN) antibody detection in naturally/experimentally infected horses.
Study design: Assay development.
Methods: 5 EHV-1 glycoprotein gene sequences were obtained from an aborted fetus strain isolated during a large EHV-1 outbreak in France in 2010. Sequences were synthesised and subcloned into expression vectors and employed in lentivirus PV generation. PVs were utilised in a Pseudotype Virus Neutralisation Test (PVNT), a sensitive technique to measure levels of specific VN antibodies. Serum samples (n=48) tested were taken longitudinally (Days 0 to 18 pi) from ponies experimentally infected with EHV-1, compared with uninfected controls (n=4). Plasmids expressing PV components' genes were co-transfected into HEK293T/17 using polyethylenimine (PEI). PV production and quantification were assessed by fluorescence and luminescence, respectively. For PVNT, two-fold serial dilution of equine sera were incubated with PV and target cells. As for

traditional VN tests, the antibody titre was expressed as the highest serum dilution causing 50% inhibition (IC50).

Results: Titres of EHV-1 PV were optimised and PVNT successfully performed and compared with a conventional EHV-1 VN assay (r=0.82).

Main limitations: Cross-reactivity studies with other EHVs need further investigation.

Conclusions: Functional EHV-1 PVs can be generated using a minimum of four glycoproteins gB, gD, gH and gL. The addition of gC neither enhances PV production nor is essential for cell entry. EHV-1 neutralising antibodies can be quantified in experimentally infected horse sera.

Ethical animal research: The use of sera was authorised by the Loire Valley ethical review board (CEEA VdL, committee number 19).

Informed consent: Not stated.

Competing interests: None declared.

Source(s) of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324) and the University of Kent.

82 | Oral administration of valganciclovir reduces clinical signs, virus shedding and cell-associated viraemia in ponies experimentally infected with equid herpesvirus-1

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Background: Equid alphaherpesvirus-1 (EHV-1) is a frequent respiratory pathogen of the horse, causing mild disease and occasionally myeloencephalopathy (EHM) or abortion. Current vaccines reduce the nasopharyngeal excretion and dissemination of the virus and therefore the extent of an epizooty, but their efficacy against secondary forms of diseases (abortion and EHM) is either limited or remains untested respectively. Several antiviral compounds are active against EHV-1 *in vitro* but no pharmaceuticals are licenced for *in vivo* treatment to date.

Objectives: To measure the *in vivo* efficacy of antiviral compounds, starting on the day of experimental infection of the target species with EHV-1 (C2254), as assessed by any reduction of clinical signs, virus shedding and viraemia.

Study design: Randomised semi-blinded experiment.

Methods: Four ponies were treated with valganciclovir (VGCV, the oral prodrug of ganciclovir [GCV]) at 6.5 mg/kg bodyweight, three times on day 1 and twice daily until day 14 inclusive. Four other ponies received a placebo. All ponies were experimentally infected with a field EHV-1 strain (5e07 $TCID_{50}$/pony). Clinical signs of disease, virus shedding and blood/cell associated viraemia were recorded and measured for 3 weeks.

Results: Serum GCV concentration was maintained above the EC_{50} (0.153 μg/mL) for at least 15 days. The overall cumulative clinical score was significantly reduced in VGCV treated ponies when compared with controls ($p<0.009$; pyrexia duration, nasal discharge and coughing). Infectious EHV-1 shedding measured on RK13 cells was significantly reduced in the VGCV treated group when compared with the control group between D+1 and D+12 ($p=0.006$). Blood and cell-associated viraemia were also both significantly reduced in the VGCV treated group ($p=0.02$ and 0.03, respectively). All ponies seroconverted after infection.

Main limitations: Due to animal management procedures, blinding was not possible for clinical evaluation.

Conclusions: Oral administration of valganciclovir for 14 days from the first day of experimental EHV-1 infection induced no noticeable side effects but significantly reduced clinical signs, virus shedding and cell-associated viremia.

Ethical animal research: All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19, authorisation number APAFiS#22708).

Informed consent: Not applicable.

Competing interests: None declared.

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83 | Identification of antiviral compounds against equid herpesvirus-1 using Real-Time Cell Analysis: screening of 2,891 molecules

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