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Equid alphaherpesviruses 8 and 9: strain isolation, ORF30 sequencing and antiviral assay

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time mares and stallions are segregated from reproduction in the face of an ECE outbreak.

Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: INTA and INTA-HARAS Agreement.

92 | Circulation and characterisation of Equid herpesvirus-3 strains in France between 2010 and 2021

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Background: Equine coital exanthema (ECE) is an acute viral infection of horses caused by equid herpesvirus-3 (EHV-3). The presence of the virus and its economic impact is well described in different countries such as Argentina or Japan. However, even though it is included in the HBLB code of practice (UK) and in the list of diseases monitored by the RESPE (FR), only a few studies have been performed in Europe.

Objectives: To determine the circulation of EHV-3 in France and measure the efficacy of new antiviral compounds against EHV-3.

Study design: Retrospective field study and *in vitro* experiments.

Methods: 71 field samples from suspected ECE cases, and collected between 2010 and 2021, were analysed by qPCR (DNA polymerase gene) and phylogenetic analysis (gG gene) was performed on 10 positive samples. Antiviral compounds (aciclovir, ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, cidofovir, aphidicolin, vidarabine) previously described with antiviral properties against EHV-1, were evaluated using Real-Time Cell Analysis (RTCA) on equine dermal cells with the reference strain EHV-3 VR3522, cytopathic effect monitoring (microscopy) and viral load quantification (qPCR).

Results: 16/71 (22%) suspected ECE cases were confirmed positive by PCR with 0 to 3 cases per year. French strains were clustered into two of the three observed phylogenic groups, but there was no correlation between the phylogeny and the time of collection or location. Aphidicolin was the most effective compound with an EC_{50} value of 2.43±0.02 µM (RTCA) and 1.63±0.48 µM (qPCR). Pritelivir and cidofovir were less potent molecules against EHV-3 (EC_{50} >50 µM).

Main limitation: Antiviral compounds were tested against the reference strain only.

Conclusions: This study demonstrates for the first time the presence of EHV-3 over a twelve-year period in France. *In vitro* studies show the antiviral efficacy of aphidicolin.

Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data and archived samples.

Informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.

Competing interests: None declared.

Sources of funding: LABÉO, IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon (SAVE, OVERLORD N12-2017), Normandy County Council (17E01598/17EP04324), Région Normandie (CPER R25 P3) and CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

Other Equine Herpesviruses

Oral Presentations

93 | Equid alphaherpesviruses 8 and 9: strain isolation, ORF30 sequencing and antiviral assay

G. Sutton[1,2]; C. Normand[1,2]; C. Thieulent[1,2]; C. Fortier[1,2,3]; E. Hue[1,2,3]; A. Leclerc[4]; B. Mulot[4]; N. Goddard[4]; L. Legrand[1,2]; R. Paillot[1,2,5,#] and S. Pronost[1,2,3,#]

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Background: While rare, Equid alphaherpesvirus (EHV) infections could be devastating for exotic endangered species which are part of conservation programmes. When such an infection occurs in an animal park setting, antiviral treatments may be available as a therapeutic option. Such an approach was recently attempted in a French zoo to save two Grevy's zebras infected with EHV-9 and displaying severe respiratory and neurological disease. Valaciclovir was administered, with mixed results obtained. Several studies have determined *in vitro* antivirals' efficacy against EHV-1 in recent years. While EHV-9 but also EHV-8 show strong genetic homology with EHV-1, these EHVs remain largely uncharacterised.

Objectives: To highlight the genetic proximity of field EHV-1, EHV-8 and EHV-9 strains and compare their *in vitro* sensitivity to antiviral compounds previously described as efficacious against EHV-1.

Study design: *in vitro* analysis of micro-organisms.

Methods: Field EHV-8 and EHV-9 strains were isolated (from donkey and Grevy's zebra, respectively) and cultured *in vitro*. ORF30 sequences and antiviral sensitivity were compared with field and reference EHV-1 strains. Complete ORF30 genes were sequenced and compared with 33 EHV sequences referenced in GenBank (18

EHV-1, 6 EHV-9, 7 EHV-8, 1 EHV-3 and 1 EHV-4). Seven antiviral molecules (ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, aciclovir and aphidicolin) were tested using Real-time Cell Analysis (xCELLigence®).

Results: ORF30 phylogenetic analysis confirmed the genetic proximity of EHV-1, -8 and -9. While aphidicolin, ganciclovir and valganciclovir showed clear *in vitro* antiviral activity against the EHV-1 and EHV-8, aciclovir was found to be the least effective compound tested.

Main limitations: Due to poor reproducibility, the EHV-9 antiviral assay will require some adjustments (cell lines).

Conclusions: This is the first report of *in vitro* tests of an antiviral compound panel against EHV-8/-9. While field use of valaciclovir is anecdotally reported against EHV-1 and other EHVs, other compounds may provide greater effectiveness.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number 2017-008, CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational program 2014-2020.

94 | Equine multinodular pulmonary fibrosis in a Thoroughbred with pituitary pars intermedia dysfunction

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Background: Despite a strong association between equine multinodular pulmonary fibrosis (EMPF) and equine herpesvirus-5 (EHV-5), the vast majority of horses infected with this gammaherpesvirus remain free of clinical disease. Pituitary pars intermedia dysfunction (PPID) is well described in older horses resulting in greater susceptibility to infectious agents. Both disease processes are considered the result of acquired immune dysfunction.

Objectives: To investigate a cause of pulmonary disease in a 21-year-old Thoroughbred stallion with clinical signs and laboratory diagnosis consistent with PPID.

Study design: Case report.

Methods: Details of clinical investigation, clinical pathology (serum ACTH determination), gross post-mortem and histopathological examination were reviewed.

Results: Pathological findings in the lung included a severe pneumonia that was interstitial, nodular and fibrosing, multifocally extensive to coalescing with type II pneumocyte hyperplasia and intra-histiocytic intranuclear viral inclusion bodies; consistent with equine multinodular pulmonary fibrosis (EMPF). Enlargement of the pituitary gland was confirmed histologically as an adenoma of the *pars intermedia*. Cross section of the hooves revealed downward rotation of the third pedal bone more pronounced in the forelimbs, consistent with chronic laminitis.

Main limitations: No cytokine analysis to demonstrate putative Th2 pro-fibrotic immune response.

Conclusions: This is the first report of a horse suffering from EMPF with confirmed PPID. The association of EHV-5 infection with the profibrotic condition EMPF raises questions regarding co-factors that may produce this condition in the vast minority of infected horses. Given the immune suppression associated with PPID, scrutiny of host factors leading to immune dysregulation may be worth further investigation. Further understanding of the complex aetiology of EMPF is warranted given the debilitating, often fatal, fibrosis associated with EHV-5 infection.

Ethical animal research: Research ethics committee oversight not required: case report.

Informed consent: The case was donated by the owner for post mortem investigation for diagnostic and teaching purposes.

Competing interests: None declared.

Source of funding: No external funding.

Equine Arteritis Virus

Oral Presentations

95 | Development of a high-throughput screening assay to identify equine virus arteritis replication inhibitors

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Background: During the last 60 years, the equine arteritis virus (EAV) has induced respiratory and reproductive problems in equids