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# Technical transfer and validation of an EHV-1 and EHV-4 ELISA.

Establishment and validation of an EHV-1/4 specific serology ELISA in order to improve current EHV diagnostic methods and overcome limitation of the EHV complement fixation test (CFT).

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#### **Reasons to perform the study:**

- Equine Herpes Virus (EHV)-1 and EHV-4 are highly contagious respiratory viruses in horses.
- EHV-1 induces abortion, respiratory and neurological diseases. In recent years, several outbreaks of EHV-1 neurological disease were reported in England. EHV-4 is mostly associated with respiratory infection.
- Rapid detection of exposure is required to identify and manage outbreaks of these viruses. Current serological assays need to be modernised to increase outbreak management efficiency.



The complement fixation test (CFT) is the serological test of choice for diagnosis.

<u>Main Advantages</u>: CFT determines levels of relatively <u>short-lasting</u> antibody that may be interpreted with reasonable confidence, in absence of recent vaccination, as <u>evidence of EHV-1 exposure</u>.

**Significant disadvantages**: CFT requires <u>qualitative interpretation</u> by experienced technicians, EHV-1/4 <u>cross-reactivity</u>, difficulty to automate for high throughput capacity and <u>extended turnaround</u> <u>time</u>.

#### **Project objectives:**

This project aimed to establish and validate a serology ELISA recently described, in order to overcome the CFT disadvantages.



#### **Experimental approach:**

Peptide specific to EHV-1 and EHV-4 were evaluated to develop an EHV-1/4 discriminatory ELISA. Negative and clinical positive serum panels were used. In parallel, the EHV CFT was optimised.

### **Key results:**

- Of all the peptides tested, the EHV-1 antigen "KKPP" best measured sero-conversion subsequent to EHV-1 infection.
- However, the "KKPP" ELISA seemed to detect a late-stage IgG response specific to EHV-1 infection.
- The EHV CFT can be improved by automation of the reading process using a colorimetric revelation assay.





#### **Conclusions and implications:**

The EHV-1 antigen "KKPP" can detect EHV-1 specific sero-conversion, but is not suitable for developing a rapid diagnostic EHV ELISA able to replace and/or complement the current EHV-1/4 CFT.

However, the EHV CFT may be optimised by the application of a colorimetric assay, reducing the subjectivity of the reading process and improving standardisation.