Development of a peptide ELISA for discrimination between serological responses to equine herpesvirus type 1 and 4 (EHV-1 and EHV-4)

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Infections with equine herpesviruses type 1 (EHV-1) and 4 (EHV-4) can result in important economic losses for the horse industry worldwide. Both viruses are common causes of acute respiratory disease, while EHV-1 frequently induces abortion but also perinatal foal mortality and devastating neurological disease. EHV-1 and -4 are closely related alphaherpesviruses. Detection of EHV antibodies in serum is a common tool to determine whether a horse has been exposed to EHV. Despite the differences in their biological characteristics and genomic sequences, both viruses show strong antigenic cross-reactivity in any immunological assay that uses polyclonal serum as the source of antibodies. An effective serological tool capable of discriminating between antibodies responses to EHV-1 or EHV-4 in horses is of importance in disease control. In the present study, we describe the development and application of a type-specific EHV-1/EHV-4 ELISA based on peptide antigens. Seven and four peptides for EHV-1 and EHV-4, respectively, were initially studied with respect to their discriminatory potential in an ELISA setup using sera from experimentally infected foals. In our optimized protocol, plates were coated overnight at 4°C with 100 µl/well streptavidin at 1 µg/ml in 50 mM carbonate/bicarbonate buffer (pH 9.6), washed 3 times with washing buffer (PBS containing 0.1% Tween 20) and coated with 100 µl/well of respective biotinylated peptide at a concentration of 2 µg/ml in carbonate/bicarbonate buffer. After incubation for 2 h at 37°C and extensive washing, wells were blocked with 100 µl/well of blocking buffer (1% goat serum in washing buffer) for 1 h at 37°C. After washing, 100 µl of horse serum diluted 1/400 or 1/1200 in washing buffer was added in triplicate and incubated for 1 h at 37°C. After 3 further washes, plates were incubated with 100 µl/well of HRP goat anti-horse immunoglobulin diluted 1/20,000 in blocking buffer for 1 h at 37°C. Following a final wash, the color reaction was developed for 10 min at room temperature by adding 100 µl/well of a chromogen/substrate mixture of TMB (240 µg/ml 3,3′,5,5′-tetramethylbenzidine) in Gallatti buffer (42 µg/ml citric acid, pH 3.95/0.01% H₂O₂). The reaction was stopped with 100 µl/well of 1 M sulfuric acid and the optical density measured at 450 nm. Results were expressed as absorbance OD values and sera tested in triplicate against the EHV-1 and EHV-4 antigens. The most promising pair of peptides, EHV-1 glycoprotein E and EHV-4 glycoprotein G peptides, was further evaluated using acute and convalescent sera from experimentally and naturally infected horses (Figure 1) as well as a panel of field sera. The results show that our peptide ELISA clearly identifies horses that have been infected with EHV-1 or EHV-4 using acute and convalescent sera and, when applied to a large number of field samples, revealed to be a robust assay for determining the EHV-1 and EHV-4 antibody status. With further validation, the developed EHV-1/EHV-4 peptide ELISA could serve as an effective and cheaper alternative to other current tools for EHV-1 and -4 serodiagnosis.

Surveillance of equine influenza viruses through the RESPE network in France from November 2005 to October 2010

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Outbreaks of equine influenza (EI) in endemic population cause economic loss despite a greater surveillance and vaccination. According to the Federation Equestre Internationale (FEI), all horses attending to their competitions have to be vaccinated in the previous 6 months. Vaccination...