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Short Communication

Long-lasting airway inflammation associated with equid herpesvirus-2 in experimentally challenged horses

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ABSTRACT

The aim of this trial was to investigate the putative involvement of equid herpesvirus 2 (EHV-2) in airway inflammation of adult horses. Six horses received corticosteroid treatment, before either mock infection ($n = 2$) or EHV-2 strain LK4 inoculation ($n = 4$). These four horses were also submitted to immunosuppression 84 days post inoculation. EHV-2 was detected by quantitative PCR in respiratory samples up to respectively 21 days and 14 days. Nested PCR, cloning and sequencing allowed the detection of five different 'field' strains throughout the trial. Neutrophils proportions were transiently increased in respiratory fluids; neutrophilia being significantly associated with concomitant EHV-2 detection. The laboratory findings reproduced in this trial were compatible with sub-clinical lower airway inflammation and suggest that EHV-2 infection should be suspected when investigating poorly-performing horses.

Equid herpesvirus 2 (EHV-2) is ubiquitous in the equine population, and its pathogenic role currently remains unclear. Clinical signs associated with EHV-2 lack specificity, ranging from mild respiratory signs in some animals to severe outbreaks in large groups of young horses (Fortier et al., 2010). The previous consensus statement on equine inflammatory airway disease (IAD) pointed out that there was insufficient knowledge concerning the relationship between viruses, IAD and tracheal inflammation (Couetil et al., 2007). In a recent study on 708 respiratory fluids (Fortier et al., 2009), EHV-2 was significantly more prevalent in tracheal wash (TW) of poor-performers, compared to clinically healthy horses. Furthermore, viral detection by PCR was significantly associated with neutrophilia of the corresponding fluid. Previously, only two experimental studies have been performed with EHV-2 on foals (Blakeslee et al., 1975; Borchers et al., 1998), but these studies did not investigate viral replication in association with cytological profile of respiratory fluids.

The aim of the present study was to investigate the putative involvement of EHV-2 in airway inflammation of adult horses. The trial was designed to investigate (1) the clinical signs exhibited following corticosteroid treatment and further nasal/tracheal inoculation, (2) EHV-2 detection by quantitative PCR in respiratory samples, and (3) the association between EHV-2 detection/quantification and modifications of cytological profiles in respiratory fluids. The study was approved by the Ethical Committee of Charles River Laboratories.

Six horses were randomly assigned to either control group (CG; horses 1 and 2) or infected group (IG; horses 3–6). Two days before inoculation (Day −2), IG horses were separated from the others and bedded in an isolated stall until the end of the study. During phase 1, both IG and CG horses received dexamethasone from Day −2 to Day 0 (0.2 mg/kg IV, once daily; Borchers et al., 1998). Nasal and tracheal inoculation was further performed on Day 0, using EHV-2 strain LK4 for IG horses and virus-free cell culture medium for CG horses (mock infection). Since EHV-2 DNA was detected in the airways of both groups following dexamethasone administration, phase 2 aimed to confirm that corticosteroids only (and not the inoculation procedure) may reactivate latent EHV-2. Dexamethasone was therefore administered to IG horses from Day +84 to Day +86 (1.0 mg/kg IV, once daily; Barrandeguy et al., 2008), and CG horses were submitted to mock infection on Day +84. Each horse regularly underwent clinical examination, blood sampling, conjunctival swabbing, nasal swabbing, TW and bronchoalveolar lavage (BAL) (Fig. 1; Appendix A). All laboratory

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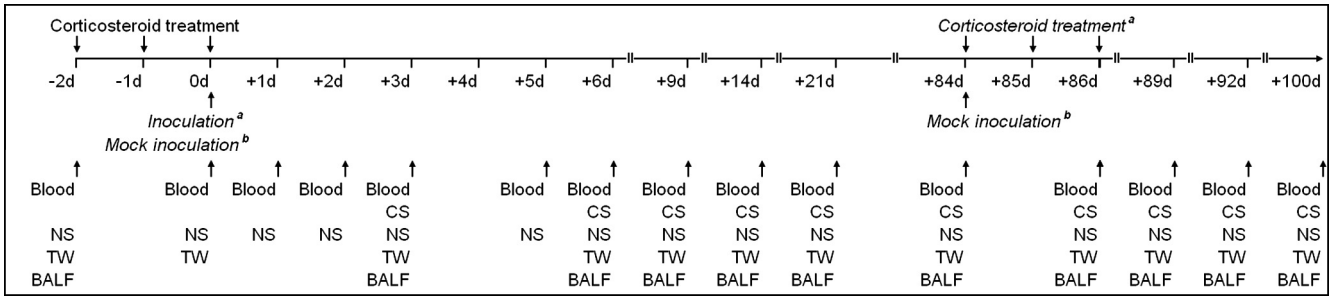

Fig. 1. Schematic representation of the experimental study design. Phase 1 is from Day −2 (2 days before inoculation) up to Day 21 (+21d); phase 2 is from Day 84 (+84d) up to Day 100 (+100d). CS conjunctival swab; NS, nasal swabs; TW, tracheal wash; BALF, bronchoalveolar lavage fluid. [a]infected group (IG) horses only [b]control group (CG) horses only.

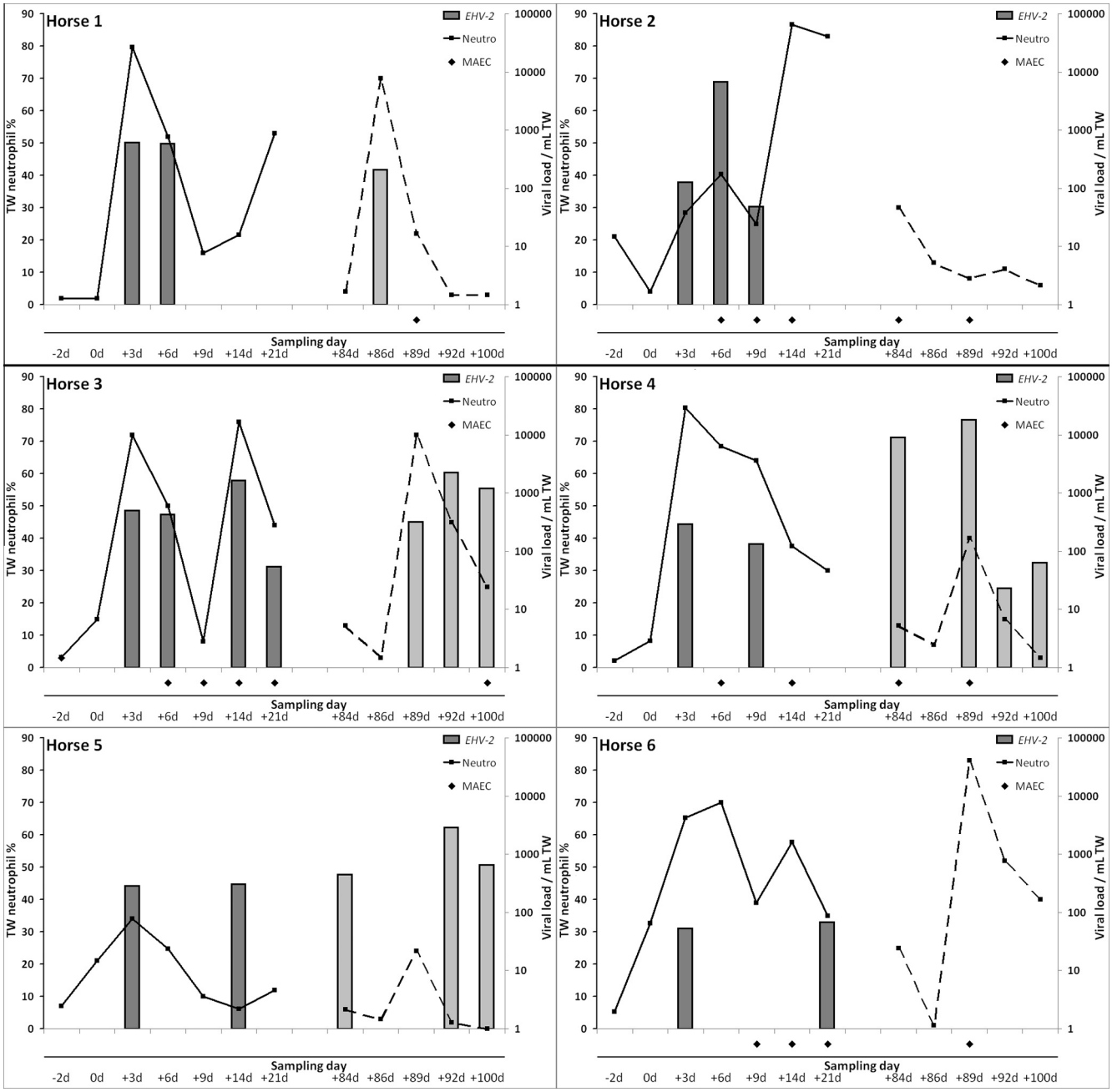

Fig. 2. Neutrophil counts and EHV-2 viral load in tracheal wash of the control group (horses 1 and 2) and the infected group (horses 3–6). TW, tracheal wash; Neutro, neutrophil; MAEC, morphological abnormalities of epithelial cells; EHV-2, viral load in TW (logarithmic scale). Day 0 (0 d) and Day 86 (+86 d) correspond to the day of inoculation and/or the last day of corticoid treatment.