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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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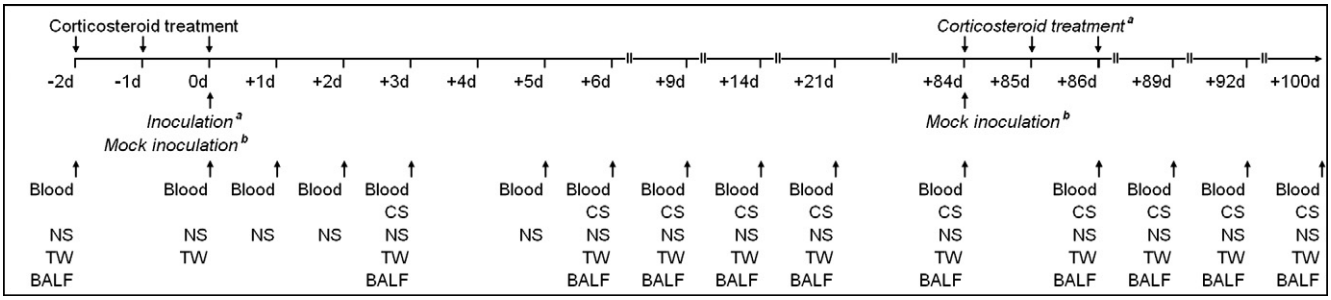

Fig. 1. Schematic representation of the experimental study design. Phase 1 is from Day −2 (2 days before inoculation) up to Day 21 (+21d); phase 2 is from Day 84 (+84d) up to Day 100 (+100d). CS conjunctival swab; NS, nasal swabs; TW, tracheal wash; BALF, bronchoalveolar lavage fluid. [a]infected group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

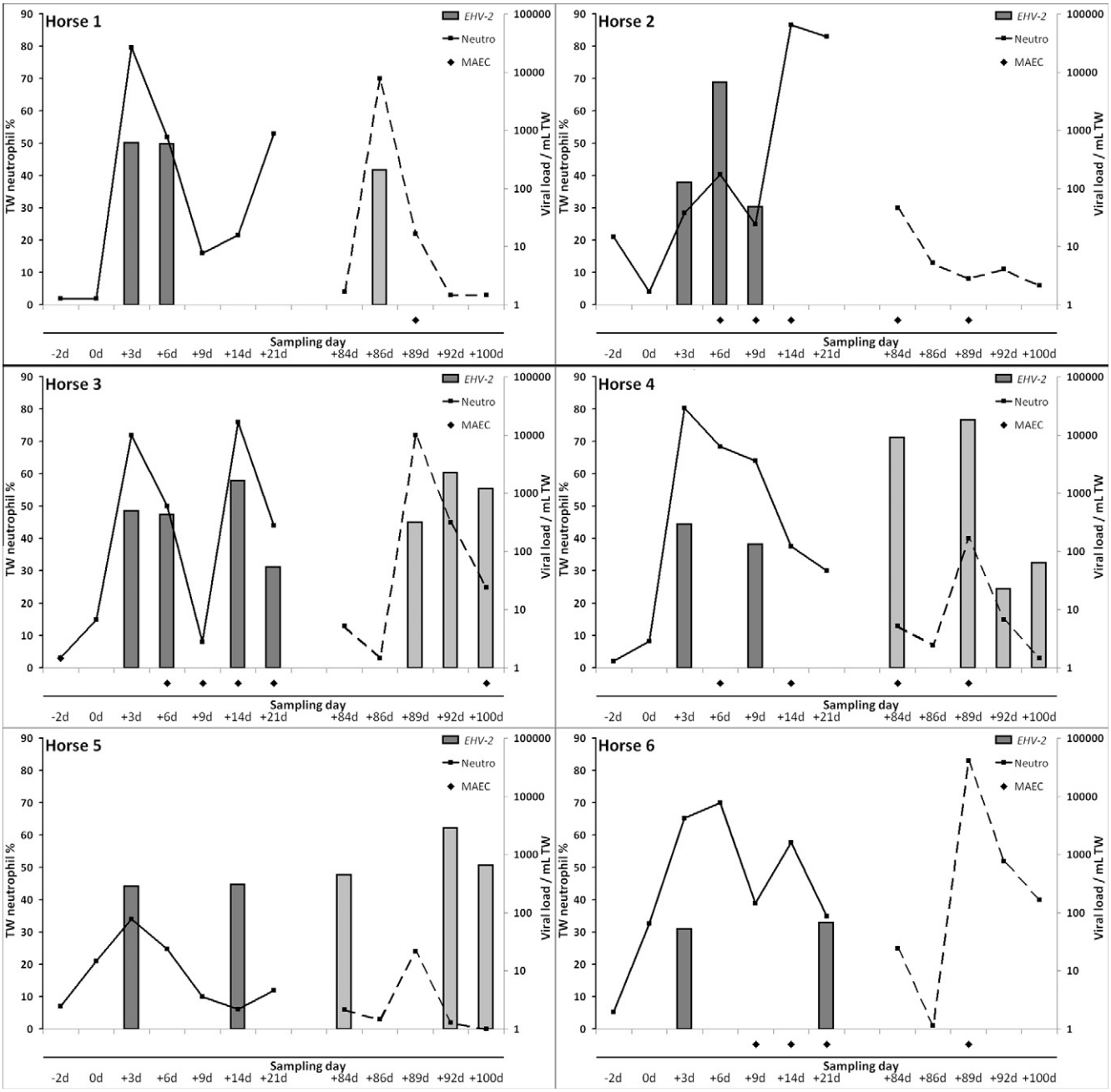

Fig. 2. Neutrophil counts and EHV-2 viral load in tracheal wash of the control group (horses 1 and 2) and the infected group (horses 3–6). TW, tracheal wash; Neutro, neutrophil; MAEC, morphological abnormalities of epithelial cells; EHV-2, viral load in TW (logarithmic scale). Day 0 (0 d) and Day 86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

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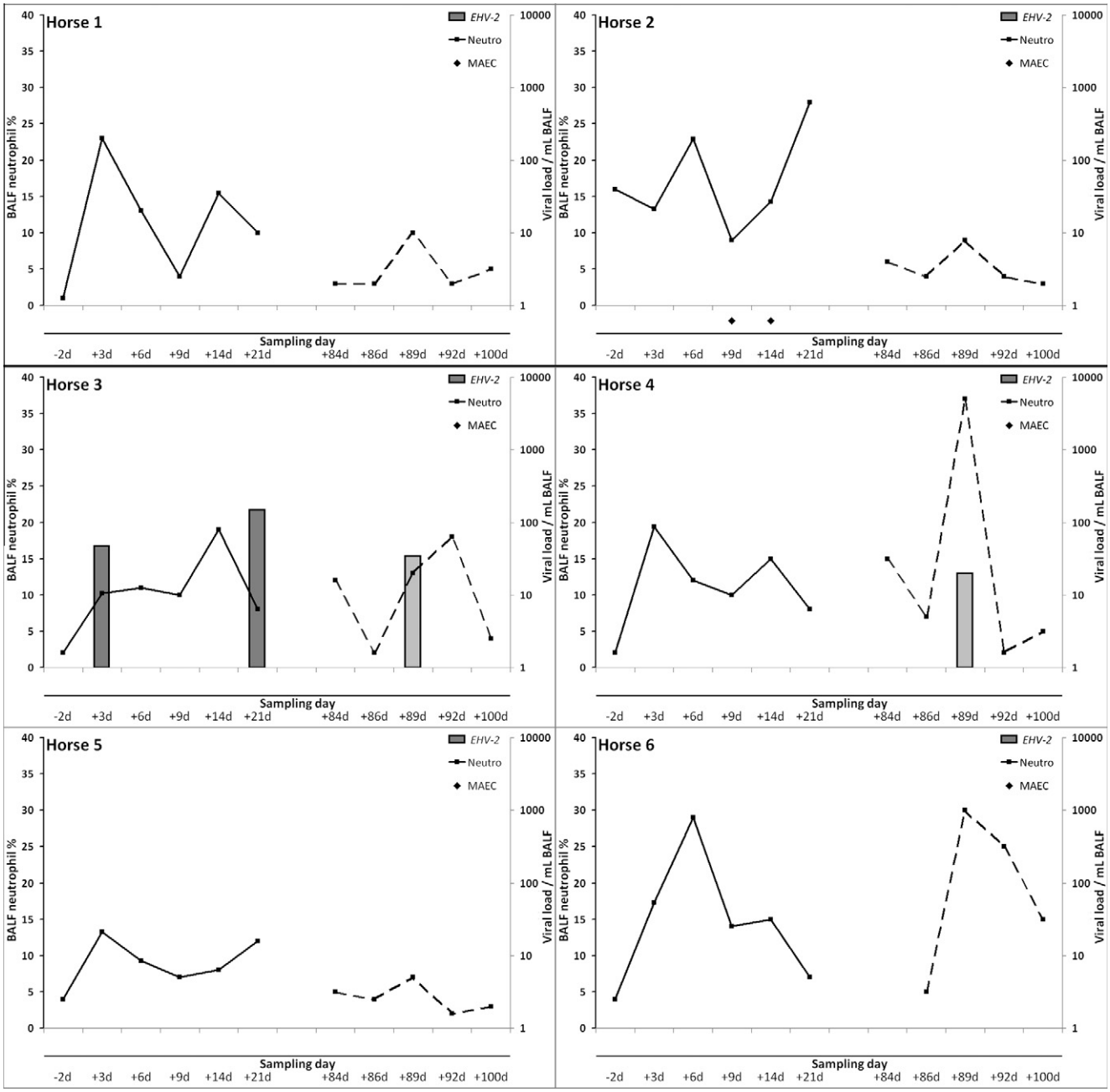

Fig. 3. Neutrophil counts and EHV-2 viral load in bronchoalveolar lavage fluid of the control group (horses 1 and 2) and the infected group (horses 3–6). BALF, bronchoalveolar lavage fluid; Neutro, neutrophil; MAEC, morphological abnormalities of epithelial cells; EHV-2, viral load in BALF (logarithmic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

analyses, including PCR assays and cytology of respiratory fluids (Appendix A) were conducted in a blinded manner.

Overall, experimentally challenged horses revealed mild to moderate clinical signs during both phases of the trial (Appendix A). EHV-1, EHV-4 and EHV-5 were not detected at any time in any sample. On Day −2, EHV-2 was detected in the blood of all horses, in the nasal swabs of IG5 but not found in respiratory fluids of any horse. Supplementary Tables 1 and 2 (Appendix A) summarise viral loads in blood and nasal swabs at other time points. Figs. 2 and 3 illustrate EHV-2 viral loads, neutrophil counts and morphological abnormalities of epithelial cells (MAEC; Appendix A) in respiratory fluids throughout both phases. EHV-2 detection was significantly associated with concomitant TW neutrophilia ($P = 0.008$) and MAEC ($P = 0.028$), whereas viral loads were not significantly correlated with neutrophil counts ($P = 0.53$).

EHV-2 was detected in, respectively, 23% of TW without cytological modifications, 50% of TW with either neutrophilia or MAEC, and 73% of TW with both criteria; the trend being statistically significant ($P = 0.001$). Moderate BAL fluid neutrophilia was also present, whilst no significant association was found with EHV-2 detection. Moreover, five different 'field' EHV-2 strains were identified in nasal swabs from three horses on Day 0 (two strains for CG1 and IG3, respectively and one for IG5). On Day +2, 'inoculated' EHV-2 strain was detected in nasal swabs of IG5, along with two different 'field' strains (Appendix A; Fig. 1).

This is, to our knowledge, the first study reporting systematic evaluation of viral DNA loads and cytological profiles during an experimental EHV-2 challenge. Since EHV-2 was detected in blood but not in any respiratory fluid before immunosuppression, corticosteroid treatment successfully stimulated viral shedding as

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appraised by quantitative PCR, as well as associated airway inflammation. Viral loads and concomitant neutrophil counts in respiratory fluids were however not significantly correlated. These findings are in accordance with a study by Brault et al. (2011) in which clinical signs of respiratory disease were not significantly associated with either viral loads or any specific EHV-2 genotype.

Since clinical signs related to EHV-2 infections were previously reported in foals only, we aimed to determine whether EHV-2 could be experimentally associated with subclinical airway troubles in adult horses. No clinical manifestation was reported, whilst hyperaemia of the tracheal mucosa and tracheal hyper-reactivity were noticed. Moreover, both neutrophilia and late detection of viral DNA up to Day +21 and Day +14 post-infection, respectively during phase 1 and 2, are compatible with subclinical forms of IAD as currently defined (Couetil et al., 2007).

Quantitative PCR also revealed that each horse was previously infected with EHV-2, while the 'inoculated' EHV-2 strain was detected only on a single occasion. Phase 1 of the experiment then resulted in an unsuccessful super-infection of IG horses, possibly due to a protective immune response or competition with reactivated 'field' EHV-2 strains. The results also confirmed previous data revealing that multiple EHV-2 strains may be concomitantly detected in respiratory samples of a single horse (Brault et al., 2011). EHV-2 is ubiquitous and found to early infect foals (Bell et al., 2006). The molecular tools being used in the current study were nonetheless highly targeted for tracking purposes of the 'inoculated' EHV-2 (Appendix A) so the precise number of different strains being shed was not definitively determined.

In conclusion, the present trial allowed experimental confirmation that EHV-2 DNA detection in TW was significantly associated with concomitant neutrophilia. The economic consequences of such long-lasting airway inflammation may be of paramount importance in racehorses (Holcombe et al., 2006). EHV-2 status should probably be evaluated when investigating poorly-performing horses.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tvjl.2012.12.027.

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