



Short communication

Equid herpesvirus 5-associated dermatitis in a horse—Resembling herpes-associated erythema multiforme

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ABSTRACT

An equid herpesvirus 5 (EHV-5) infection was detected in lesioned skin from a nine-year-old Holsteiner stallion in the south of Germany. Macroscopically, the animal displayed a non-pruritic, multifocal, pustular dermatitis around both eyes, nostrils and the muzzle, which had been ongoing for one year. Histopathologically, skin lesions were characterized by orthokeratotic to parakeratotic hyperkeratosis, pustular dermatitis, epidermal hyperplasia, apoptotic keratinocytes, a lympho-plasmahistiocytic interface dermatitis with hydropic degeneration of keratinocytes, and perivascular to diffuse, lympho-histiocytic infiltrations. The stratum granulosum and the upper part of the stratum spinosum contained multiple amphophilic, intranuclear inclusion bodies. By *in situ* hybridization and immunohistochemistry herpesvirus DNA and protein, respectively, were detected within keratinocytes containing inclusion bodies. Sequencing of the PCR-product revealed the presence of EHV-5 DNA. This is the first description of a dermatitis associated with EHV-5 in a horse, indicating that EHV-5 should be considered as an etiology of lymphohistiocytic interface dermatitis with intranuclear inclusion bodies in horses and is similar to herpes-associated erythema multiforme in humans.

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1. Introduction

Herpesviruses are double-stranded, enveloped DNA-viruses with a size ranging from 100 to 180 nm. Only eleven of 120 identified herpesviruses have the potential to infect equids and they belong to the sub-families *alpha*- and *gammaherpesvirinae* (Fortier et al., 2010). In general, induction of latency is an important feature of herpesviruses. Latency results in life-long infection with potential reactivation. This leads to the necessity to vaccinate horses

in order to suppress virus shedding and clinically apparent disease. Equine herpesvirus (EHV) type 1 and 4 infections occur frequently and have a worldwide distribution. Equine rhinopneumonitis, abortion and myeloencephalopathy caused by EHV-1 and -4 are major diseases threatening equine health, thus having considerable economic relevance (Patel and Heldens, 2005). Although herpesviruses represent viruses with a high species-specificity, some EHV-1 strains can break the species barrier and cause disease in several other species like llamas, black bears, Thompson's gazelles, and guinea pigs (Rebhun et al., 1988; Wohlsein et al., 2010). EHV-3 is the etiologic agent of equine coital exanthema, a venereal disease in horses displaying abortigenic properties (Gleeson et al., 1976). All over the world the prevalence of EHV-2 and -5 in horses is high and age-independent. EHV-2 and -5 are detectable in healthy horses (Nordengrahn et al., 2002), and may be associated with

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dermal (EHV-2) and pulmonary (EHV-5) alterations (Sledge et al., 2006; Williams et al., 2007). EHV 7, 8 and 9 are often identified in donkeys (Fortier et al., 2010). EHV-7, which is also termed asinine herpesvirus 2 (AHV-2) was found in nasal swabs of a healthy mule in the USA and shows a prevalence of 8% in 127 healthy mules and donkeys (Bell et al., 2008). The Burchell's zebra in East Africa is suggested to be the natural host of EHV-9, and a transmission from equids to gazelles can cause disease (Borchers et al., 2005).

This is the first description on an EHV 5-associated dermatitis in a horse. By PCR and sequencing EHV-5 DNA was detected and shown by *in situ* hybridization to be localized within the affected skin sample.

2. Materials and methods

2.1. History

In January 2011, a nine-year-old grey Holsteiner stallion (show jumper) presented with lethargy resulting in poor performance of a few weeks duration. In addition, the horse suffered from non-pruritic, hyperkeratotic and pustular dermatitis around both eyes, nostrils and the muzzle, which have been observed and treated rather unsuccessfully for approximately one year. Further, multiple nodular, exophytic-growing masses were located in the pectoral area. Physical examination, gait evaluation, serum biochemistry and sonography of the thoracic and abdominal cavities did not reveal any abnormalities. Complete blood count showed a mild monocytosis (6% monocytes). The facial skin lesions had previously been investigated by skin scrapings, which were negative for ectoparasites and dermatophytes, and bacterial culture revealed moderate growth of *Staphylococcus aureus*. According to the owners, the skin lesions had transiently responded to topical administration of a gentamicin-betamethasone ointment. Due to lack of evidence of an underlying systemic disease, and the favorable response to corticosteroid-containing ointments, an immune-mediated dermatitis was suspected, possibly causing poor performance and lethargy. Differential diagnoses included systemic and discoid lupus erythematosus, erythema multiforme, pemphigus foliaceus, atopy and drug eruption. The nodular lesions of the chest had not been addressed previously and were suspected to be equine sarcoids, likely unrelated to the facial lesions. Skin samples of all lesions were taken for histopathological evaluation.

2.2. Histology, immunohistochemistry and electron microscopy

Six formalin-fixed skin biopsies from nose, eyes and chest (two samples from each localization) were submitted for histopathological examination. Each sample was cut into two pieces and embedded in paraffin-wax, sectioned at 2–5 µm and stained with hematoxylin and eosin. Immunohistochemistry for equine rhinopneumonitis virus/equine herpesvirus type-1 and electron microscopy on 5% glutaraldehyde-fixed skin samples were performed as described (Wohlsein et al., 2010).

2.3. *In situ* hybridization

In situ hybridization was performed using a digoxigenin-labeled 293 base pair probe (generated by PCR) of the EHV-5 genome which hybridizes within the highly conserved glycoprotein B (gB) open reading frame. Briefly, 3 µm sections were dewaxed before proteolysis by proteinase K (20 µg/ml; 30 min; 37 °C). After refixation in absolute ethanol, they were air-dried. Hybridization was carried out in a humid chamber at 45 °C overnight with approximately 100 ng probe. After repeated washing, color reaction was performed during 30 min, and tissues were sealed with aqueous mounting medium (Teifke et al., 2003).

2.4. DNA extraction and PCR

DNA extraction from the dewaxed paraffin-embedded sections samples was accomplished using the QIAamp DNA Mini Kit, "Isolation of genomic DNA from paraffin-embedded tissue" exactly as recommended by the manufacturer (Qiagen, Hilden, Germany). A first panHerpes PCR reaction with degenerated and inosine substituted primers specific for the herpesviral DNA polymerase gene was performed (Ehlers et al., 1999). The PCR product (approximately 220 bp) obtained from the nested PCR reaction was purified after agarose gel electrophoresis with the QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. In a further, EHV-5 specific PCR reaction, a PCR product with a size of 293 base pairs could be detected after amplification with EHV-5 gB specific primers (Holloway et al., 1999). No amplification products were detectable after conventional PCR reactions with primers specific for EHV-1, -2, and -4 (Kirisawa et al., 1993; Rizvi et al., 1997).

2.5. Sequence determination

Sequencing of the DNA polymerase encoding region obtained from the nested PCR reaction was performed using the Big Dye[®] Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Nucleotide sequences (double stranded sequenced) were read with an automatic sequencer (3130 Genetic Analyzer, Applied Biosystems) and analyzed using the Genetics Computer Group (GCG) software version 11.1 (Accelrys Inc., San Diego, USA).

3. Results and discussion

Macroscopically, the skin around muzzle, nostrils and eyes showed erythema, hyperkeratosis, pustules and alopecia (Fig. 1). Affected skin areas lacked pruritus and pain, and the physical examination revealed no further abnormal findings. Additionally, hematological and ultrasonographic investigations were unremarkable. Histopathologically, equine sarcoids were diagnosed in the skin sample of the chest. The skin of nose and eyes showed a moderate to severe, orthokeratotic to parakeratotic hyperkeratosis (Fig. 2A, asterisk), a moderate, slightly irregular hyperplasia of the epidermis, a minimal pigmentary incontinence and a lympho-plasmahistiocytic interface

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