Detection of *Equid gammaherpesvirus 2* and 5 DNA in the upper respiratory tract of asymptomatic horses from Southern Brazil

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Abstract

Equid gammaherpesvirus 2 (EHV-2) and 5 (EHV-5) are members of the *Herpesviridae* family and have been reported in horse populations worldwide. This study aimed to evaluate the presence of herpesvirus DNA in the upper respiratory tract of horses. Twenty-six nasal swabs were collected from asymptomatic adult horses of two different horse farms (A, $n = 18$; B, $n = 8$), both located in Southern Brazil. The EHV-1, EHV-2, EHV-4, and EHV-5 DNA analyses were performed using nested PCR assays targeting the glycoprotein B gene. Four (15.3%) and 12 (46.1%) of the 26 nasal swab samples were positive for the EHV-2 and EHV-5, respectively. Four (15.3%) horses were detected with both viruses simultaneously. DNA of EHV-2 and EHV-5 in both single and mixed infections was identified in horses from both herds. All swab samples were negative for EHV-1 and EHV-4. This study reports the first detection of EHV-2 and EHV-5 in the upper respiratory tracts of horses in Brazil. The high detection rate of EHV-2 and EHV-5 in asymptomatic adult horses demonstrates that these gammaherpesviruses are circulating in Brazil.

Keywords Equine · Herpesviruses · EHV-2 · EHV-5 · Molecular detection · Brazil

Introduction

Equid gammaherpesvirus 2 (EHV-2) and 5 (EHV-5) are members of the family *Herpesviridae*, subfamily

Gammaherpesvirinae, and genus *Percavirus* and have been reported in horse populations worldwide [1, 2]. Coinfections by these EHV-2 and EHV-5 are common in foals, which are first infected by EHV-2 and then later by EHV-5 [3]. Infection by EHV-2 occurs in young foals with periodic reactivation of the latent virus during the life of the animal. Adult horses are considered reservoirs of the virus and may contribute to the dissemination of the infection to other equines [1]. Although the EHV-2 DNA has been detected in other cells/tissues including leucocytes, macrophages, the central and peripheral nervous system, and lymphoid tissues [4], B lymphocytes are considered to be the major site of latency [5]. In addition, lung cells may be potential sites of latency for EHV-5 [6].

Horses infected with EHV-2 and/or EHV-5 develop sub-clinical or clinical disease depending on the level of immunity against the infecting virus strain. However, persistent infections and viral shedding may occur with a range of EHV strains, even in infected horses with high titers of antibodies [4]. There are a wide variety of modulatory genes of EHV-2 and EHV-5 that act on immune-response of the host [2]. The ability of these viruses to infect lymphocytes may reduce the immunity of the host with consequent increases in susceptibility to opportunistic viral or bacterial infections [7].

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Respiratory disease outbreaks associated with EHV-2 are characterized by coughing, nasal discharge, conjunctivitis, pneumonia, and lymphadenopathy [8, 9]. In addition, EHV-5 exhibits tropism for the respiratory tract. EHV-5 is associated with the occurrence of equine multinodular pulmonary fibrosis [6, 10], and coinfections with EHV-2 and EHV-5 are considered co-factors that exacerbate the clinical signs [7]. Other herpesviruses associated with respiratory disease in horses are alphaherpesviruses 1 and 4 (EHV-1 and EHV-4), although EHV-1 can also cause abortion and neurological disease in horses [11].

In Brazil, EHV-1-related infections were reported in the states of Sao Paulo, Minas Gerais, and Rio Grande do Sul and were associated with abortions or clinical neurological manifestations in horses [12]. In addition, there is a report of EHV-5 associated with equine multinodular pulmonary fibrosis in a mare from Rio Grande do Sul [13]. In Brazil, few studies have investigated infections induced by herpesviruses in horses. Because horses can be healthy carriers of herpesvirus, this study aimed to evaluate the occurrence of herpesvirus DNA in the upper respiratory tract of asymptomatic adult horses.

Materials and methods

Sample collection

Nasal swabs were collected from asymptomatic adult horses from two different horse farms in Southern Brazil. Samples ($n = 18$) from farm A were obtained from horses of the Appaloosa breed from Paraná state and samples ($n = 8$) from farm B were obtained from horses of the Crioula breed from Rio Grande do Sul state, totaling 26 nasal swab samples. All samples were stored in phosphate-buffered saline (PBS) buffer, pH 7.2 at -80°C until further analysis.

Nucleic acid extraction and molecular detection of EHV

Nasal swabs were homogenized in 0.01-M PBS buffer and clarified by centrifugation at $3000\times g$ for 10 min. Nucleic acid was extracted from aliquots of 500 μL of the suspensions which were pre-treated with proteinase K (Invitrogen™ Life Technologies, Carlsbad, CA, USA), using the silica/guanidine isothiocyanate nucleic acid extraction method [14]. The extracted nucleic acid was eluted in 50 μL of UltraPure™ diethylpyrocarbonate (DEPC)-treated water (Invitrogen™ Life Technologies, Carlsbad, CA, USA) and stored at -80°C . Sterile ultrapure water was used as a negative control in all nucleic acid extractions and amplification procedures.

Amplification of EHV-2 and EHV-5 DNA was performed using nested polymerase chain reaction (PCR) targeting the

partial glycoprotein B gene (gB), according to Wang et al. [15]. The presence of EHV-1 and EHV-4 DNA was determined by nested PCR assays targeting the partial gB gene using primers recommended by the World Organization for Animal Health (OIE) [16, 17].

Sequencing analysis

To confirm the specificity of the amplicons obtained in this study, four nested PCR-amplified products representative of each EHV species obtained from each farm were selected for sequencing analyses. The amplicons were purified by the PureLink® Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen® Life Technologies, Carlsbad, CA, USA) and then were quantified using a Qubit® Fluorometer (Invitrogen® Life Technologies, Eugene, OR, USA) and sequenced in both directions with the same forward and reverse primers used in the PCR assay. Sequencing was performed in an ABI3500 Genetic Analyzer sequencer with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®, Foster City, CA, USA). Sequence quality analyses and consensus sequences were obtained using PHRED and CAP3 software (<http://asparagin.cenargen.embrapa.br/phph/>). Similarity searches were performed with nucleotide (nt) sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results and discussion

Four (15.3%) and 12 (46.1%) of the 26 upper respiratory tract swabs of asymptomatic adult horses analyzed in this study were positive for EHV-2 and EHV-5, respectively. Four (15.3%) of the horses were positive for both viruses (EHV-2 and EHV-5 DNA) simultaneously. EHV-2 and EHV-5 were identified in horses from both farms in single or in mixed infections. Table 1 shows the results of the nested PCR assays

Table 1 Distribution of the nested PCR results to identify DNA of herpesvirus-1, herpesvirus-2, herpesvirus-4, and herpesvirus-5 in the upper respiratory tract of asymptomatic adult horses according to the evaluated herds in Brazil

Virus	Samples ($n = 26$)		Total
	Farm A ($n = 18$) ¹	Farm B ($n = 8$) ²	
EHV-1	–	–	–
EHV-2	4	–	4
EHV-4	–	–	–
EHV-5	9	3	12
EHV-2 and EHV-5	1	3	4
Total	14	6	20

¹ Paraná State. ² Rio Grande do Sul State

for identification of EHV-1, EHV-2, EHV-4, and EHV-5 DNA in nasal swabs, according to the horse farms in this study.

Sequencing analyses confirmed the specificity of the EHV-2 and EHV-5 amplicons of both farms. The EHV-2 and EHV-5 nt sequences herein identified were named BR/EHV2-PR1/2013 (GenBank accession number MG252619) and BR/EHV2-RS1/2014 (MG252620), and BR/EHV5-PR1/2013 (MG252621) and BR/EHV5-RS1/2014 (MG252622), respectively.

This is the first report of EHV-2 and EHV-5 DNA in the upper respiratory tracts of asymptomatic adult horses from Brazil. Gammaherpesviruses have been detected in horse populations in several countries worldwide [1, 15, 18, 19]. In South America, EHV-2 was successfully isolated from cells cultured from symptomatic horses in Argentina [20, 21]. The elevated detection rates of EHV-5 (46.1%) compared with that of EHV-2 (15.3%) in the upper respiratory tract of adult asymptomatic horses in our study are similar to those from a study in Australia, in which EHV-5 DNA was more prevalent (56.7%) than EHV-2 (2.8%) in nasal swabs of weaned asymptomatic foals [15]. Alternatively, a higher prevalence of EHV-2 compared with that of EHV-5 was identified in foals from farms with annual respiratory problems [3, 22]. These findings and results from previous studies [3, 15, 22, 23] indicate that EHV-2 is more frequently correlated with equine respiratory disease when compared with EHV-5, which is more frequently detected in asymptomatic animals. The detection of both EHV-2 and EHV-5 from nasal swabs of the same horses has also been reported in animals from Switzerland and the USA [23, 24]. Although the pathogenicity of gammaherpesviruses in horses is not fully elucidated, infections by these viruses are important predisposing factors for respiratory disease complexes in horses [25]. Studies have demonstrated that infection by other pathogenic agents, such as viruses and bacteria, may contribute to the development and/or aggravation of respiratory disease complexes in horses [25, 26].

Additionally, all nasal swab samples analyzed in this study did not contain EHV-1 and EHV-4 DNA; similar results were described in which it was suggested that EHV-1 and EHV-4 are not commonly detected in healthy horses [15]. Moreover, EHV-4 has never been reported in horses from Brazil.

This study reports for the first time the detection of EHV-2 and EHV-5 DNA in the upper respiratory tracts of horses from Brazil. The high detection rate of these viruses in asymptomatic adult horses demonstrates that gammaherpesviruses EHV-2 and EHV-5 are circulating in Brazil. Additionally, considering the higher frequencies of both viruses detected in a limited geographical region, we presume that EHV-2 and EHV-5 may be endemic in other regions of the country. However, the effects of these results are unknown; thus, additional epidemiological studies are necessary to understand the distribution of these viral agents in horse populations from different geographical regions of Brazil and their related diseases.

Moreover, additional studies are needed to elucidate the importance of these viruses in the development of respiratory disease in horses.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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