First molecular detection of Equine Herpesvirus type 3 (EHV-3) in Chile.

Ignacio Troncoso[1], Rolando Calvanese[2], Fernando Saravia[3], Sebastián Muñoz-Leal[3], Nhur-Aischa Zegpi[3], and Rene Ortega[3]

[1]Universidad de Las Americas
[2]Ministerio de Agricultura
[3]Universidad de Concepcion - Campus Chillan

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Abstract

Equine coital rash (ECE) is a highly contagious benign disease that induces lesions on the external genitals, and it is caused by the equine herpesvirus type 3 (EHV-3). The disease is globally distributed and affects only equids. However, its presence in Chile has not been documented from a genetic point of view. Here, we performed PCR screenings for EHV-3 in genital lesions of external genitals in four horses belonging to a riding station at Bulnes, Ñuble Region, Chile. We sequenced a fragment of the glycoprotein g (*gG*) gene of this *alphaherpesvirus*, from three horses which signology compatible with ECE. The sequences were identical between them and 99.7% similar to a haplotype of EHV-3 detected in Brazil. A phylogenetic analysis pointed that our consensus sequence forms a clade with homologue isolates obtained from Japan, Russia and Brazil. Our results show the presence of EHV-3 for the first time in horses with ECE in Chile.

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Authors: Ignacio Troncoso[1,2*], Rolando Calvanese[3], Fernando Saravia[4], Sebastián Muñoz-Leal[4]

Nhur-Aischa Zegpi[5], René Ortega[5*].

*Co-corresponding authors

Affiliations:

[1] School of Veterinary Medicine, Faculty of Veterinary Medicine and Agronomy, Universidad de las Américas, Concepción, Chile. (I.T)

[2]Faculty of Agricultury Sciences, Universidad del Alba, Chillán, Chile. (I.T)

[3]Manager of the Equine Tracer Center. Chilean Army Agreement –INDAP. Municipality of Bulnes and San Carlos, Ñuble, Chile. (RC)

[4]Departament of Animal Sciences, Faculty of Veterinary Science, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile. (F.S, S.ML)

[5]Pathology Departament, Faculty of Veterinary Science, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile. (R.O, N.Z)

Summary

Equine coital rash (ECE) is a highly contagious benign disease that induces lesions on the external genitals, and it is caused by the equine herpesvirus type 3 (EHV-3). The disease is globally distributed and affects only equids. However, its presence in Chile has not been documented from a genetic point of view. Here, we performed PCR screenings for EHV-3 in genital lesions of external genitals in four horses belonging to a riding station at Bulnes, Ñuble Region, Chile. We sequenced a fragment of the glycoprotein g (*gG*) gene of this*alphaherpesvirus* , from three horses which signology compatible with ECE. The sequences were identical between them and 99.7% similar to a haplotype of EHV-3 detected in Brazil. A phylogenetic analysis pointed that our consensus sequence forms a clade with homologue isolates obtained from Japan, Russia and Brazil. Our results show the presence of EHV-3 for the first time in horses with ECE in Chile.

Keywords: equine, Chile, venereal, coital exanthema, herpesvirus, glycoprotein

Main Text

Introduction

Equine herpesvirus type 3 (EHV-3) belongs to the Order Herpesvirales, Family *Herpesviridae* and genus *Varicellovirus* (Davison, 2010). This virus is the causative agent of the equine coital rash (ECE), a venereal disease of worldwide distribution which is characterized by producing lesions in external genitals in foals and mares (Barrandeguy and Thiry, 2012). The main mechanism of transmission is the direct contact of genital mucous membranes during intercourse, or indirectly by fomites (Allen and Umphenour, 2004). Although EHV-3 causes a localized infection, some authors have speculated that systemic alterations such as infertility and abortion do occur as well; yet a systemic pathophysiology is still questionable (Van der Meulen et al., 2006; Léon et al., 2008; Barrandeguy, 2010).

The impact of the disease is associated with forced and temporary interruption of mating activities of the affected stallions and mares, which significantly decreases the number of entries at the end of the season, provokes a delay in delivery dates and a decrease in pregnancy rates (Allen and Umphenour, 2004, Barrandeguy and Thiry, 2012). In the case of infected stallions, a rigid gait, loss of libido and refuse to mate with mares is common (Barrandeguy and Thiry, 2012; Vissani et al., 2018). Diagnosis to distinguish between the different types of equine herpesvirus can be made through PCR (EHV1, EHV2, EHV3, EHV4 and EHV5) (Wagner et al. 1992; Borchers and Slater, 1993; Kirisawa et al., 1993; Wang et al., 2007).

Nowadays, the presence of the disease in Latin America has been detected by molecular tools only in Argentina and Brazil, in clinically healthy mares and from a foal, respectively (Barrandeguy, 2010). To date in Chile, the existence of this disease has not been reported (Berrios, 2005). Therefore, the objective of this study was to molecularly evaluate the presence of this alpha-herpesvirus in equines with the presence of genital lesions compatible with ECE in this country.

Materials and Methods

In October of 2019, at the Sector Agua Buena Chica, Bulnes commune, region of Ñuble, central Chile (Latitude: -36.7333 Longitude: -72.3), four samples (one from a Belgian Ardennes Breeder and three from Mixed Shooting) were obtained from three females and one male that presented clinical signology in the genital area compatible with EHV-3 infection. The samples were taken with a sterile swab from papular or crustal lesions of the vulvar or prepucial area (Fig. 1a and 1b), and then transported to -4ºC and stored in the Virology Laboratory of the Faculty of Veterinary Sciences of the University de Concepción, Chillán Campus.

DNA extraction was carried out using the Dneasy Blood & Tissue kit (Quiagen, Cat. 69506) following the manufacturer's specifications, and the samples were stored at -20 °C until use. To detect EHV-3 we implemented a conventional PCR with primers targeting a conserved fragment (520 bp) of the glycoprotein G (*gG*) gene (Dynon et al., 2001), which is homologous in most alpha-herpesviruses sequenced to date (Hartley et al., 1999).

Positive samples were sequenced in both directions, and then compared with the GenBank database using

BLASTn (*www.ncbi.nlm.nih.gov/BLAST*). The sequences were edited using BioEdit (Hall, 1999), with manual edition when needed, and aligned with the Clustal W algorithm (Thompson et al., 1994). Phylogenetic relationships were evaluated with the Bayesian method with MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) using the General Time Reversible model, with 1,000,000 generations. Each tree was sampled every 100 generations, beginning with random seeds and ran four times. The first 25% of the trees was considered burn-in, and the remaining trees used to calculate Bayesian posterior probabilities. A sequence of Feline herpesvirus 1 (KR381787) was employed as out group (Fig. 2).

Results and discussion

Through a molecular analysis and using BLASTn we confirmed the first EHV-3 record in Chile, since three identical the sequences of *gG*gene were obtained from three mares. The consensus sequence was 99.7% (428/429 bp) identical to a homologue haplotype from Brazil (GQ336877), and clustered into a monophyletic group with EHV-3 sequences obtained from Japan, Russia and Brazil (Fig. 2). Our sequence, clearly differs from types 1, 4, 8 and 9 of equine herpesviruses, and was deposited in GenBank with accession number MZ747033.

Although the presence of aborted fetuses as a result of EHV-1 was reported in Chile decades ago (Ruiz, 1998), this is the first study that molecularly characterizes a EHV-3 strain in the country isolated from equines with genital lesions. Likewise, EHV-3 has been described in countries such as Japan, in horses with symptoms of ECE (Kirasawa, 2017). On the other hand, in Argentina, positive females were diagnosed with a subclinical infection, without clinical symptoms (Barrandeguy, 2010).

The first detections of EHV-3 occurred in the USA, Canada and Australia in 1968 (Kirasawa, 2017). Currently, EHV-3 is distributed worldwide, and its pathogenesis, genetics and antigenic features vary with respect to the other types of EHV. The virus is also highly contagious (Sijmons, 2014), so it is likely its prevalence in Chile is underestimated. Because the virus abruptly hinders reproductive activity, it might negatively impact farms with infected animals (Barrandeguy, 2010). For this reason, preventive actions such as vaginal washing, that reduces the risk of transmission, should be carried out routinely (Toishi, 2017). As our finding suggests that Chilean equine populations would be at risk of infection, studies looking to understand the prevalence of this pathogen along the country must be undertaken.

In conclusion, the presence of EHV-3 is confirmed for the first time in Chile. The recognition of the pathology as well as its association with venereal transmission will afford to deduce areas of distribution and, therefore, evaluate the potential for reproduction risks in the Chilean equine population.

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Conflict of interest statement

The authors declare no conflict of interest

Data Availability Statement

The data that support the findings of this study are openly available in GenBank at Accession numbers MZ747033.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

Was reviewed and approved by Comité de Bioetica de la Facultad de Ciencias Veterinarias, Universidad de Concepción. Horses samples were written informed consent for participation was not obtained from the owners because the animals were not identified individually.

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Figures Legends

Fig. 1. ECE. (1a). Pustular lesions on the glans of a stallion's penis. Multiple multifocal ulcers to coalescers of 0.2 to 1 cm of diameter with raised edges. (1b) Pustular and coalescing ulcerative lesions in the perivulvar zone of an affected female.

Fig. 2. Bayesian phylogenetic tree inferred for a partial fragment of the EHV-3 *gG* gene, using 22 reference sequences obtained from GenBank. Bulnes strain (highlighted in red) is a unique isolate genetically similar to reference strains. GQ336877 (in blue) is a Brazilian strain of EHV-3. Posterior probabilities are to the left of each clade respectively.