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CASE REPORT

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

Ignacio Troncoso[1,2] | Rolando Calvanese[3] | Fernando Saravia[4] | Sebastián Muñoz-Leal[4] | Nhur-Aischa Zegpi[5] | René Ortega[5]

[1]School of Veterinary Medicine, Faculty of Veterinary Medicine and Agronomy, Universidad de las Americas, Concepción, Chile

[2]Faculty of Agricultury Sciences, Universidad del Alba, Chillán, Chile

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

[4]Department of Animal Sciences, Faculty of Veterinary Science, Universidad de Concepción, Chillán, Chile

[5]Pathology Department, Faculty of Veterinary Science, Universidad de Concepción, Chillán, Chile

Correspondence
René Ortega, Pathology Department, Faculty of Veterinary Science, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile.
Email: reortega@udec.cl

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Abstract

Equine coital rash (ECE) is a highly contagious benign infection that induces lesions on external genitals, and it is caused by the equine herpesvirus type 3 (EHV-3). Although the disease is globally distributed, its presence in Chile has not been documented from a genetic point of view. Here, we performed polymerase chain reaction screenings for EHV-3 in 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 from three horses with clinical signs of ECE. The sequences were identical between them and 99.7% similar to a haplotype of EHV-3 detected in Brazil, and phylogenetically related with homologue 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

1 | INTRODUCTION

Equine herpesvirus type 3 (EHV-3) belongs to the Order Herpesvirales, family *Herpesviridae* and genus *Varicellovirus* (Davison, 2010). This virus is the aetiological agent of the equine coital rash (ECE), a venereal disease of worldwide distribution that produces lesions on the external genitals of foals and mares (Barrandeguy & Thiry, 2012). The main mechanism of transmission is through direct contact of genital mucous membranes during intercourse, or indirectly by fomites (Allen & Umphenour, 2004). Although EHV-3 causes a localised lesion in genitals, some authors speculate that infertility and abortion may also occur. However, the systemic pathophysiology is still questionable (Van der Meulen et al., 2006; Léon et al., 2008; Barrandeguy et al., 2010). EHV-3 directly impacts mating activities in the affected stallions and mares and leads to a significant decrease in the number of entries at the end of the season, with a subsequent delay in delivery dates and less pregnancy rates (Allen & Umphenour, 2004, Barrandeguy & Thiry, 2012). Rigid gait, loss of libido and a refusal to mate are common

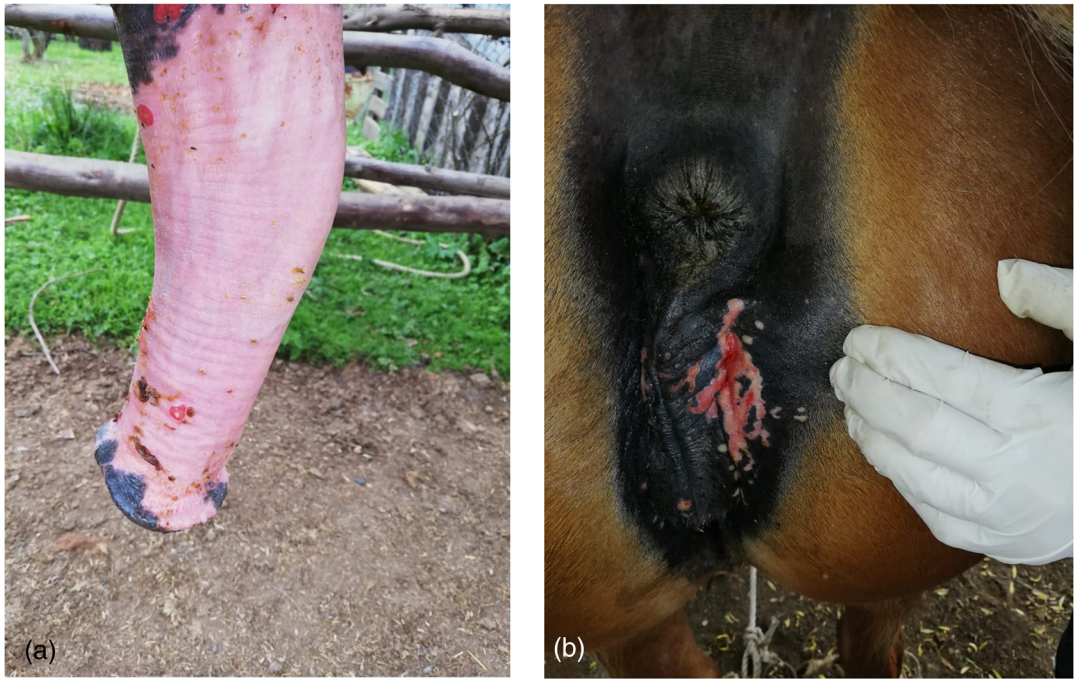

FIGURE 1 ECE. (a) Pustular lesions on the glans of a stallion's penis. Multiple multifocal ulcers to coalescers of 0.2–1 cm of diameter with raised edges. (b) Pustular and coalescing ulcerative lesions in the perivulvar zone of an affected female.

signs in infected stallions (Barrandeguy & Thiry, 2012; Vissani et al., 2018).

Different types of equine herpesvirus, namely EHV1, EHV2, EHV3, EHV4 and EHV5, circulate worldwide and the diagnosis can be done through PCR (Wagner et al. 1992; Borchers and Slater, 1993; Kirisawa et al., 1993; Wang et al., 2007). While in Latin America, the presence of the virus has been molecularly detected in clinically healthy mares from Argentina and in a foal from Brazil (Barrandeguy et al., 2010), the pathogen has not been genetically characterised in Chile (Berrios, 2005). Therefore, the objective of this study was to evaluate the presence of this alpha-herpesvirus in ECE-like lesions from Chilean equines using molecular tools

2 | MATERIALS AND METHODS

In October 2019, four equines (one Belgian Ardennes Breeder and three Mixed Shooting) were inspected at the Sector Agua Buena Chica, Bulnes commune, region of Ñuble, central Chile (Latitude: −36.7333 Longitude: −72.3). One male and three females presented genital lesions compatible with EHV-3 infection. The females showed the lesions after intercourse. The samples were taken with a sterile swab from papular or crustal lesions on vulvar or preputial areas (Figures 1a and b), and stored at −4°C 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 (Qiagen, Cat. 69506) following the manufacturer's specifications, and the samples 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 gene (*gG*) (Dynon et al., 2001), which is homologous in most alpha-herpesviruses sequenced to date (Hartley et al., 1999). DNA of Pneumabort-k®+1b vaccine was used as positive control for EHV-1 and ultra-pure water as negative control in each reaction.

Positive samples were sequenced in both directions, the sequences edited with BioEdit (Hall, 1999) and compared with the GenBank database using BLASTn (www.ncbi.nlm.nih.gov/BLAST). An alignment with homologue sequences was constructed with the Clustal W algorithm (Thompson et al., 1994), and phylogenetic relationships evaluated with the Bayesian method using MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001) with the General Time Reversible model and 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 (Figure 2).

3 | RESULTS AND DISCUSSION

Although we sampled lesions in four animals, only females were positive after PCR screening. Through BLASTn analyses, we confirmed the first EHV-3 positive case for Chile, since three identical sequences of *gG* 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 (Figure 2). Our sequence, clearly differs from types 1, 4, 8 and 9 of equine

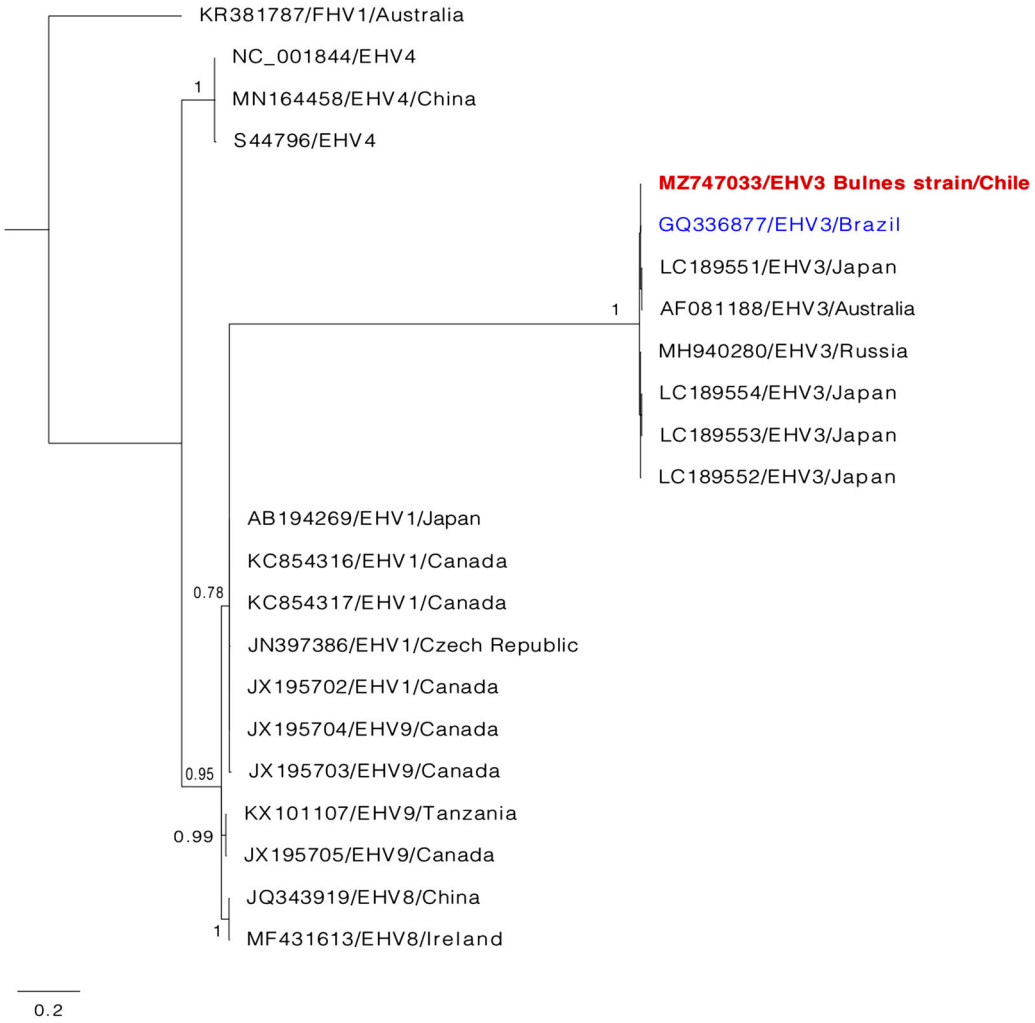

FIGURE 2 Bayesian phylogenetic tree inferred for a partial fragment of the EHV-3 *gG* gene, using 22 reference sequences obtained from GenBank. Bulnes strain (highlighted in red) is a unique isolate genetically similar to reference strains. GQ336877 (in blue) is a Brazilian strain of EHV-3. Posterior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

herpesviruses, and was deposited in GenBank with accession number MZ747033.

Although fetus abortion because of EHV infection was reported in Chile decades ago (Ruiz et al., 1998), this is the first study that genetically characterises an EHV-3 strain in the country isolated from equines with genital lesions. EHV-3 has been detected in countries such as Japan, in horses with symptoms of ECE (Kirasawa et al., 2017). Yet this strain can also cause subclinical infection, as reported from asymptomatic infected mares in Argentina (Barrandeguy, 2010).

While first detection of EHV-3 occurred in the USA, Canada and Australia in 1968 (Kirasawa et al., 2017), the strain is highly contagious (Sijmons et al., 2014), so it is currently of worldwide distribution, and its pathogenesis, genetics and antigenic features differ from other EHV types. The presence of EHV was suspected in Chile since 2013, because of lesions on breeding equines' genitalia. However, efforts to molecularly confirm the pathogen was not possible perform due to the remission lesions when the samples were obtained. Although complaint on the possible occurrence of this virus was made to the Chilean agricultural and livestock service, further samples were not collected since EHV is not of mandatory report in the country. There are no previous studies for this disease and this pathogen in our country.

EHV abruptly hinders reproductive activity and might negatively impact farms with infected animals (Barrandeguy, 2010). For this reason, preventive actions such as vaginal washing should be carried out routinely to reduce the risk of transmission (Toishi et al., 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 should be undertaken. In conclusion, in this study, ECE-like lesions are described for the first time in Chile. In addition, for the first time, an EHV-3-like genomic sequence is detected in Chile. The recognition of the pathology will allow for the identification of distributional areas and therefore predict reproduction risks in the Chilean equine population.

AUTHOR CONTRIBUTIONS

Conceptualisation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing: Ignacio Troncoso. *Conceptualisation; investigation; methodology*: Rolando Calvanese. *Conceptualisation;*

formal analysis: Fernando Saravia. *Investigation; methodology*: Sebastián Muñoz-Leal. *Methodology; writing – original draft*: Nhur-Aischa Zegpi.

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CONFLICT OF INTEREST

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.

The study was reviewed and approved by Comité de Bioetica de la Facultad de Ciencias Veterinarias, Universidad de Concepción. The horse samples were obtained with the informed consent of the owners with the commitment not to identify the animals individually.

ORCID

René Ortega https://orcid.org/0000-0003-4416-6400

PEER REVIEW

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