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Evidence of natural interspecific recombinant viruses between bovine alphaherpesviruses 1 and 5

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ABSTRACT

Closely related bovine alphaherpesviruses 1 (BoHV-1) and 5 (BoHV-5) co-circulate in certain countries, rendering cattle co-infection possible. This is a prerequisite for BoHV recombination. Here, we report the first identification of homologous recombination between field isolates of BoHV-1 and BoHV-5, two alphaherpesviruses belonging to two distinct species with an average genomic similarity of 82.3%. Three isolates of BoHV-5, previously classified as subtype "BoHV-5b", were phylogenetically studied and analyzed via eight PCR sequencing assays dispersed at regular intervals throughout the genome to discriminate between BoHV-1 and BoHV-5. In the phylogenetic analysis, differences of clustering were found in the UL27 gene which encodes the glycoprotein B (gB). We detected two recombination breakpoints in the open reading frame of the UL27 gene. We compared the amino acid sequences of the gB of BoHV-1.1 and 1.2, BoHV-5a and recombinant formerly named BoHV-5b (chimeric gB) and subsequently performed molecular modeling. All structures were alike and, simultaneously, similar to the chimeric gB. Neutralizing antibodies against BoHV-1, BoHV-5 and recombinant viruses were analyzed via serum virus neutralization test using polyclonal sera and a monoclonal antibody against gB to demonstrate an absence of viral escape for both assays.

Our results show that homologous recombination between two related species of ruminant alphaherpesviruses can occur in natural field conditions. We found three recombinant field isolates, previously classified as BoHV-5b subtypes, between BoHV-1 and BoHV-5.

1. Introduction

Within the *Herpesviridae* family, *Alphaherpesvirinae* is an extensive subfamily containing numerous viruses of great relevance for both human and animal health and associated economic production. As regards the latter, bovine herpesvirus 1 (BoHV-1) is the etiological agent of infectious bovine rhinotracheitis, infectious pustular vulvo-vaginitis, abortion and balanoposthitis, and it also is an important component of the bovine respiratory disease complex, causes severe economic losses and imposes restrictions to international livestock trade. Endemic in cattle populations worldwide (Raaperi et al., 2014), BoHV-1 co-circulates with the closely related bovine herpesvirus 5 (BoHV-5) rarely in Europe, Australia and the USA but with a strong geographical distribution in South America (Del Medico Zajac et al., 2010).

Nucleotide substitution rates are low in alphaherpesviruses, estimated to lie at around 3×10^{-8} per site per year (Sakaoka et al., 1994). The overall rate of herpesvirus protein sequence evolution has been estimated at 3×10^{-9} per amino acid per year (McGeoch et al., 2000). This is an order of magnitude greater than the evolutionary rate of mammalian nuclear genes (Shackelton and Holmes, 2004), suggesting that alphaherpesviruses may evolve faster than their hosts. However, the mutation rate is still low as compared to that of other

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viruses (e.g. RNA viruses), indicating that additional mechanisms must act together with mutations to mediate herpesvirus evolution (Schynts et al., 2003). Of these mechanisms, homologous recombination is the most frequent in herpesviruses, although illegitimate recombination has also been observed (Rijsewijk et al., 1999; Umene, 1999). Whether viral recombination in general is driven by mechanistic constraints associated with genome structures or is selectively favoured as a means to increase genetic diversity end eliminate deleterious mutations, it is undisputed that recombination can be considered as an essential driving force to increase the occurrence of rare but advantageous mutations within a viral species (Thiry et al., 2006). Highlighting the importance of recombination during herpesvirus history, sequence analysis has shown strong evidence of past natural homologous recombination events in several species, including human (Loncoman et al., 2017; Norberg et al., 2007, 2006, 2004; Peters et al., 2006) and animal (Hughes and Rivailler, 2007; Lee et al., 2011, 2015; Pagamjav et al., 2005) alphaherpesviruses.

In vitro and *in vivo*, herpesvirus recombination has been studied between distinguishable strains of herpes simplex virus 1 or 2 (HSV-1 and HSV-2) (Brown et al., 1992; Nishiyama et al., 1991), varicella-zoster virus (VZV) (Dohner et al., 1988), pseudorabies virus (PRV) (Dangler et al., 1993; Glazenburg et al., 1994; Henderson et al., 1990), feline herpesvirus 1 (FeHV1) (Fujita et al., 1998) and BoHV-1/5 (Del Medico Zajac et al., 2010; Meurens et al., 2004; Schynts et al., 2003; Schynts et al., 2001). Interspecies recombinants between BoHV-1 and BoHV-5, at a reduced degree of average similarity (82.3%), were obtained under *in vitro* co-infection by Meurens et al. (2004). More recently, these recombinants were characterized regarding both their genome structure and their virulence in natural hosts (Del Medico Zajac et al., 2011, 2009) and showed attenuated replication characteristics and established latency in the natural host.

While natural homologous recombination has been described in different species of alphaherpesviruses, it occurs most commonly as intraspecies recombination. Interspecies recombination however appears to be rare with a single reported detection between equine herpesviruses 1 and 4 in field samples (Pagamjav et al., 2005). It has not yet been reported between human alphaherpesviruses or other animal alphaherpesviruses.

The examination of natural intra- and interspecies recombination of bovine herpesviruses is especially relevant with respect to the widespread use of attenuated BoHV-1 vaccines (Muylkens et al., 2006; van Drunen Littel-van den Hurk, 2007) and risks to animal health posed by the potential of recombination between bovine herpesviruses and live attenuated vaccine strains. While live attenuated vaccines against BoHV-1 are not used in Argentina, the co-circulation of BoHV-1 and BoHV-5 presents a unique opportunity for co-infection and subsequent recombination between strains.

In this work, we a report the first molecular detection of interspecies recombinant field isolates between two alphaherpesviruses BoHV-1 and BoHV-5, previously isolated in Argentina and known as BoHV-5 subtype b (Maidana et al., 2013).

2. Materials and methods

2.1. Virus population samples and cell culture

Viruses used in this study were propagated in Madin Darby bovine kidney (MDBK) cells and viral stocks were produced after infection of MDBK cells at a multiplicity of infection (MOI) of 0.01, as previously described (Romera et al., 2000) (Table 1). MDBK monolayers were grown in 12-well plates, and infected with 1:10 serial dilutions of the isolates A663, 166/84 (Maidana et al., 2011) and 674/10 (Maidana et al., 2013). After incubation for 2 h at 37 °C, viral inocula were removed by washing. To detect the progeny generated specifically by each viral particle, 2 ml carboxymethyl cellulose (CMC)/FBS (7,5 g/L CMC, 480 ml/L MEM-D 2X, 20 ml/L SFB) semisolid overlay were added to each well. Plates were incubated for 72 h at 37 °C. Finally, several (n = 30) isolated lysis plaques were selected and replicated individually to isolate and amplify a single viral clone.

Table 1
Bovine herpesvirus isolates and reference strain used in this study.

Viruses	Strain	Subtype	Reference
BoHV1	Cooper	1.1	d'Offay et al. (1993)
	K22	1.2b	
	688/10[a]	1.2b	
BoHV5	SV507/99	a	Delhon et al. (2003)
	06/13[a]	a	
	A663	b	Carrillo BJ et al. (1983)
	166/84	b	Maidana et al. (2011)
	674/10	b	

[a] Field isolates with low passage number used in *in vitro* assays.

2.2. PCR, DNA sequence and phylogenetic studies

Eight different sets of primers, spread along the genome were used in this study. They served to amplify regions located in the unique long (UL) region (UL41, UL40, UL29, UL28, UL27 and UL22) (Del Medico Zajac et al., 2009; Maidana et al., 2011) and the short (US) region (US6 and US8) as previously described by (Thiry et al., 2007) (Table 1). Nucleotide sequences of the isolates were edited and analyzed with BioEdit version 7.0.5.3 (Hall et al., 1999). Alignments were performed with Clustal W. MEGA 5.0 software (Tamura et al., 2011). A phylogenetic inference was performed using the MEGA version 5 software package based on UL27 and US6, which are conventionally used to study the phylogenetic relationships amongst ruminant alphaherpesviruses. Tree topology was constructed via maximum composite likelihood method with the Tamura and Nei model (1000 replicate bootstrap values).

2.3. Recombination analysis

The complete UL27 gene of the three BoHV5b isolates, responsible for the differential clustering in the phylogenetic analysis, was amplified by PCR and sequenced. Subsequently, genetic recombination was analyzed using a sliding-window genetic diversity plot (Simplot software version 3.5.1 available at http://sray.med.som.jhmi.edu/SCRoftware) (Lole et al., 1999) and the Recombinant Detection Program (RDP) (Martin et al., 2015), version 3, available at http://darwin.uvigo.es/rdp/rdp.html.

2.4. Amino acid sequence comparison

Amino acid (aa) sequences for the entire gB were compared between the BoHVs. Alignments and sequence identity matrices were performed with Clustal W. MEGA 5.0 software (Tamura et al., 2011). To evaluate potential antigenicity changes in gB due to limited aa sequence variations in BoHV1.2b, BoHV5a and BoHV5b isolates, we performed epitope predictions of these sequences using BepiPred (http://www.iedb.org/). This program predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and surface propensity scale method. The residues with scores above the threshold (1.5) are predicted to be part of an epitope. The number of epitopes predicted in recombinant gB was compared to that of the parental viruses.

2.5. Molecular modeling

Structural models of gB from BoHV-1.2b, BoHV-5 and field isolates were constructed with the program SWISS-MODEL (http://swissmodel.expasy.org/; (Guex and Peitsch, 1997; Kiefer et al., 2009; Schwede et al., 2003), using the HSV-1 gB crystal structure 3NW8 as template