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Short communication

Partial sequence analysis of B2L gene of Brazilian orf viruses from sheep and goats

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ABSTRACT

We herein describe the partial nucleotide sequencing and phylogenetic analysis of the B2L gene of seventeen Brazilian orf viruses (ORFV). Seventeen viruses were recovered from outbreaks of contagious ecthyma in sheep and goats in four states in Southern and Northeast country, and three from commercial vaccines. Most analyzed viruses were associated with outbreaks of classical contagious ecthyma, with lip, nostrils and labial commissure involvement, yet udder/teat, feet, vulvar and disseminated lesions were also reported in some cases. Nucleotide sequence analysis revealed a high degree of B2L similarity among sheep sequences (>99%) regardless the geographic origin, and a remarkable high identity for the two goat isolates (>99.8%), with similarity dropping to below 99% when comparing viruses from the two species. A phylogenetic tree grouped most sheep and goat viruses on different branches. In addition, sequence alignment allowed the identification of up to six scattered nucleotide changes that were predominant and more consistent in goat isolates, including a number of sequences from other continents. Thus, in spite of the high nucleotide similarity, different degrees of similarity and discrete nucleotide changes in the B2L gene may help in grouping ORFV viruses according to host species.

Contagious ecthyma (or orf) is a debilitating disease of sheep and goats caused by orf virus (ORFV), the prototype member of the genus *Parapoxvirus* (PPV), family *Poxviridae* (Haig and Mercer, 1998). The disease is characterized by inflammatory, proliferative and scabby lesions in the lips, nostrils and muzzle and is particularly frequent and severe in lambs. Lesions may be occasionally observed on the teats of nursing animals and rarely on the internal organs such as tongue and gums (Hosamani et al., 2006). Depending on the location of the lesions, animals may be unwilling to nurse, eat, or walk. Primary lesions usually resolve spontaneously within 3–4 weeks (McKeever et al., 1988). Morbidity is usually high whereas mortality is low and, when occurs, is generally due to secondary infections or extreme debilitating condition (Robinson and Balassu, 1981). Contagious ecthyma is an economically important disease in most countries with commercial sheep and goat flocks (Hosamani et al., 2009). Likewise, the disease is widespread in Brazil and a number of outbreaks have been reported in sheep and goats and, occasionally, with human involvement (Abrahão et al., 2009; Catroxo et al., 2002; Langoni et al., 1995; Nóbrega et al., 2008; Salles et al., 1992; Torres, 1939). A few vaccines are commercially available in the country, yet vaccination is not an usual practice in most herds. In contrast, vaccination is more an exception than a rule and is usually adopted only in herds that had previously experienced cases of the disease.

The ORFV genome consists of a linear double stranded DNA, of 138 kbp in length and encodes 132 putative gene products (Mercer et al., 2006). The central genomic core

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region encodes proteins involved in the virus structure and assembly, while the terminal regions contain genes whose products are implicated in host range and virulence (Delhon et al., 2004). The ORFV B2L gene (1137 bp) encodes a major and highly immunogenic envelope protein of about 42 kDa, which is a homologue of vaccinia virus major envelope antigen p37K (Sullivan et al., 1994). The B2L gene is highly conserved among ORFV isolates and has been used for detection, molecular characterization and phylogenetic analysis of ORFV; and several B2L nucleotide (*nt*) and amino acid sequences are available in the GenBank (Abrahão et al., 2009; Hosamani et al., 2006; Lojkic et al., 2010).

In spite of the widespread distribution of ORFV infection in Brazil (Abrahão et al., 2009; Catroxo et al., 2002; de Oliveira et al., 2012; Langoni et al., 1995; Mazur et al., 2000; Mazur and Machado, 1989; Nóbrega et al., 2008; Salles et al., 1992) very few isolates have been characterized at a molecular level (Abrahão et al., 2009; de Oliveira et al., 2012; Mazur et al., 2000). Similarly, most of the genetic studies conducted in other countries focused on a single or a few isolates (Billinis et al., 2012; Chan et al., 2007; Lojkic et al., 2010; Venkatesan et al., 2011; Zhao et al., 2009). Thus, the present study is justified by the significant sheep and goat production in some Brazilian regions; the widespread distribution of contagious ecthyma in sheep and goat producing regions and the lack of genetic data on circulating ORF viruses (Table 1).

Scabs obtained from naturally occurring cases of ecthyma in sheep in Rio Grande do Sul ($n = 14$), Paraíba ($n = 1$); and from goats in Bahia ($n = 1$) and Pernambuco ($n = 1$) between 2008 and 2012 were analyzed. Three commercial vaccine strains used in Southern country (Vaccine A – Ectisan Ceva®, B – Ectisan Santa Elena®, and C – Rosenbush®) were also analyzed.

Total DNA was extracted from 200 mg of triturated scabs or from 500 μl of vaccine suspension, using DNAzol® reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. A set of primers which amplifies a 594 bp fragment of the B2L gene (position in OV-IA82 strain: PPP1 – at *nt* 9809 and PPP4 – at *nt* 10,402) was used (Inoshima et al., 2000). DNA was amplified by 95 °C for 9 min, and 30 cycles of denaturation (94 °C, 1 min), annealing (55 °C, 1 min) and extension (72 °C, 1 min), and final extension by 72 °C for 7 min. An aliquot (5 μl) of the PCR product was analyzed by 1% agarose gel electrophoresis to visualize the amplicons. The PCR products (95 μl) were purified by using commercial Illustra™ GFX™ PCR DNA and Gel Band purification kit (GE Healthcare, Pittsburgh, PA, USA), according to manufacturer's instructions. Samples were submitted to *nt* sequencing in duplicate in an automatic sequencer ABI-PRISM 3100 Genetic Analyzer armed with 50 cm capillaries and POP6 polymer (Applied Biosystems, Carlsbad, CA, USA).

The sequences were analyzed by the Staden Package (Staden, 1996), to obtain consensus sequence of each sample, which were subsequently submitted to BLAST software (Basic Local Alignment Search Tool) (Altschul et al., 1997) for comparison with sequences deposited in the GenBank, using NCBI database. The sequences alignment was performed using the ClustalW, which is within the BioEdit Sequence Alignment Editor software suite, version 7.0.5.3 (Hall, 1999). The *nt* identity was also obtained by BioEdit software. The phylogenetic tree of isolates and reference strains of PPV was conducted in

Table 1
Clinical and epidemiological information on Brazilian ORFV isolates analyzed in the present study (2008–2012).

Identification	Species	Number of animal affected/total	Local of lesions	Origin	Year	GenBank accession number
SV 578/08	Sheep	4/30	Teats, udders	RS	2008	JX485983
Bahia	Goat	n.i.	Lips, vulva	BA	2010	JX485987
São Martinho	Sheep	n.i.	Lips, nostrils	RS	2009	JX485980
Caprino SJE	Goat	n.i.	Lips, nostrils	PE	2009	JX485992
Patos	Sheep	n.i.	Lips, nostrils	PB	2009	JX485993
Canguçú	Sheep	250/1000	Lips, nostrils, teats, udders, feet	RS	2010	JX485989
SV 252/11	Sheep	n.i.	Teats	RS	2011	JX485997
SV 269/11	Sheep	n.i.	Lips, nostrils, udders, teats	RS	2011	JX485979
SV 520/11	Sheep	n.i.	Lips, nostrils, udders	RS	2011	JX485986
SV 561/11	Sheep	n.i.	Lips, nostrils, gums, udders	RS	2011	JX485990
SV 581/11	Sheep	n.i.	Lips	RS	2011	JX485985
SV28/12	Sheep	114/380	Lips	RS	2011	JX485996
SV 26/12	Sheep	11/400	Lips	RS	2012	JX485988
SV27/12	Sheep	20/50	Lips, feet	RS	2012	JX485995
SV29/12	Sheep	15/40	Lips	RS	2012	JX485991
SV 168/12	Sheep	4/60	Lips, udders	RS	2012	JX485982
SV178/12	Sheep	n.i.	Lips	RS	2012	JX485994
Vaccine A	Sheep	n.a.	n.a.	Ectisan Ceva®	n.a.	JX485981
Vaccine B	Sheep	n.a.	n.a.	Ectisan St. Elena®	n.a.	JX485984
Vaccine C	Sheep	n.a.	n.a.	Rosenbush®	n.a.	JX485978

RS, Rio Grande do Sul; BA, Bahia; PE, Pernambuco; PB, Paraíba; n.a., non aplicable; and n.i., non informed.