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SHORT COMMUNICATIONS

Detection and first molecular characterization of bovine papular stomatitis virus in dairy calves in Argentina

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Abstract

Bovine papular stomatitis virus (BPSV) is a parapoxvirus associated with papular and erosive lesions on the muzzle, lips, and oral mucosa of cattle. BPSV infection occurs worldwide; however, it has still not been unequivocally diagnosed. The present report describes an outbreak of BPSV infection affecting dairy calves in northwestern Argentina and provides the first molecular characterization of this virus in the country. The disease was detected in a dairy farm, affecting 33 calves between 2 and 20 days of age. The signs included reddish papules, ulcers, and scabby proliferative lesions on muzzle, lips, and oral mucosa. The affected calves resisted to being fed due to severe local pain. Two necropsies were performed; papulas and ulcers were observed in ruminal and omasal mucosa. Histologically, the affected areas of the skin showed acanthosis, spongiosis, and parakeratotic hyperkeratosis with adjacent focally extensive ulcers and multifocal inflammatory infiltrate in the epidermis. Eosinophilic inclusion bodies were detected in the cytoplasm of epithelial cells. DNA extracted from scab samples was analyzed by PCR using pan-parapoxvirus primers for the B2L gene. The sequence analysis revealed 99%, 85%, and 84% similarity with BPSV, Pseudocowpox virus, and Orf virus, respectively. A phylogenetic tree constructed using the B2L sequence showed that the virus clustered with BPSV isolates. Although clinical cases compatible with BSPV infection have been frequently described in Argentina, the present report is the first to identify the agent associated with cattle disease in the country.

Keywords Bovine papular stomatitis virus · Cattle · Cutaneous disease · Parapoxvirus

Bovine papular stomatitis (BPS) is a common viral disease of calves characterized by the formation of papules or nodules that progress to vesicles and then crusts or scabs on the lips, gingiva, and tongue (Mark et al. 1991). The disease is caused by bovine papular stomatitis virus (BPSV) (Mayr and Büttner 1990). BPSV is a Parapoxvirus (PPV) belonging to the family Poxviridae, subfamily Chordopoxvirinae (Büttner and Rziha 2002). PPVs include four species: BPSV, Pseudocowpoxvirus (PCPV), parapoxvirus of red deer in New Zealand (PVNZ), and Orf virus (ORFV), which is the prototype of PPV. PPVs are epitheliotropic viruses that cause nonsystemic, proliferative skin disease in domestic and wild ruminants (Mark et al. 1991; Delhon et al. 2004). BPSV infection in young cattle is sometimes manifested with distinctive "horseshoe-shaped" popular lesions on the hard palate and oral mucosa, which can occur with or without concurrent inflammation of the gingiva (Dal Pozzo et al. 2011). BPS is widespread around the world and can occasionally be transmitted to humans (de Sant'Ana et al. 2012). In humans, BPSV infection is associated with nodules and pustules on the hands and sometimes on the face (Bowman et al. 1981). In South America, cases clinically compatible with BPSV infection have been frequently reported by veterinarians, and laboratory-confirmed cases have been reported in Uruguay and Brazil (Lemos and Riet-Correa 2007; de Sant'Ana et al. 2012). In Argentina, a BPS case was described by Rodriguez et al. (1988), but the agent

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has still not been unequivocally diagnosed. In this study, we report an outbreak of BPSV infection in cattle and provide the first molecular characterization of this virus for country.

In September 2018, 28 of 33 dairy calves housed on a farm in Salta province presented mildly erosive papules, coalescent scabby erosions, and ulcers mainly on the muzzle and oral cavity (Fig. 1). The affected farm held cattle for dairy production. Mean temperature at the time of the outbreak was approximately 22 °C, and the weather was dry and clean. Disease morbidity was 85% (28/33), and the affected calves were between 2 and 20 days old. Mortality was high (67%; 23/33). They resisted to being milked due to severe local pain. In addition, some animals had diarrhea and dehydration. Two recently dead calves were available for post-mortem examination. Necropsy revealed proliferative lesions and coalescent scabby erosions in oral mucosa. Additionally, in one calf, the ruminal and omasal mucosa were severe affected. Other animals recovered without treatment 15–18 days after the first appearance of clinical signs. On the farm, there were 250 adult cows in production; all of them were inspected for lesions compatible with BPS during milking. No cows presented lesions, suggesting the absence of clinical manifestation.

Clinical samples of the noses and lips of affected calves were collected. For histopathology studies, samples of fresh tissue were fixed in 10% buffered formalin, processed for routine histopathological examination, and stained with hematoxylin and eosin. Histologically, multifocal areas of moderate acanthosis, spongiosis, and parakeratotic hyperkeratosis with adjacent focally extensive ulcers were identified in the epidermis (Fig. 1c). In the cytoplasm of epithelial cells of areas with degenerative changes numerous 2–6-μm circular, eosinophilic inclusion bodies were detected (insert in Fig. 1c). In more

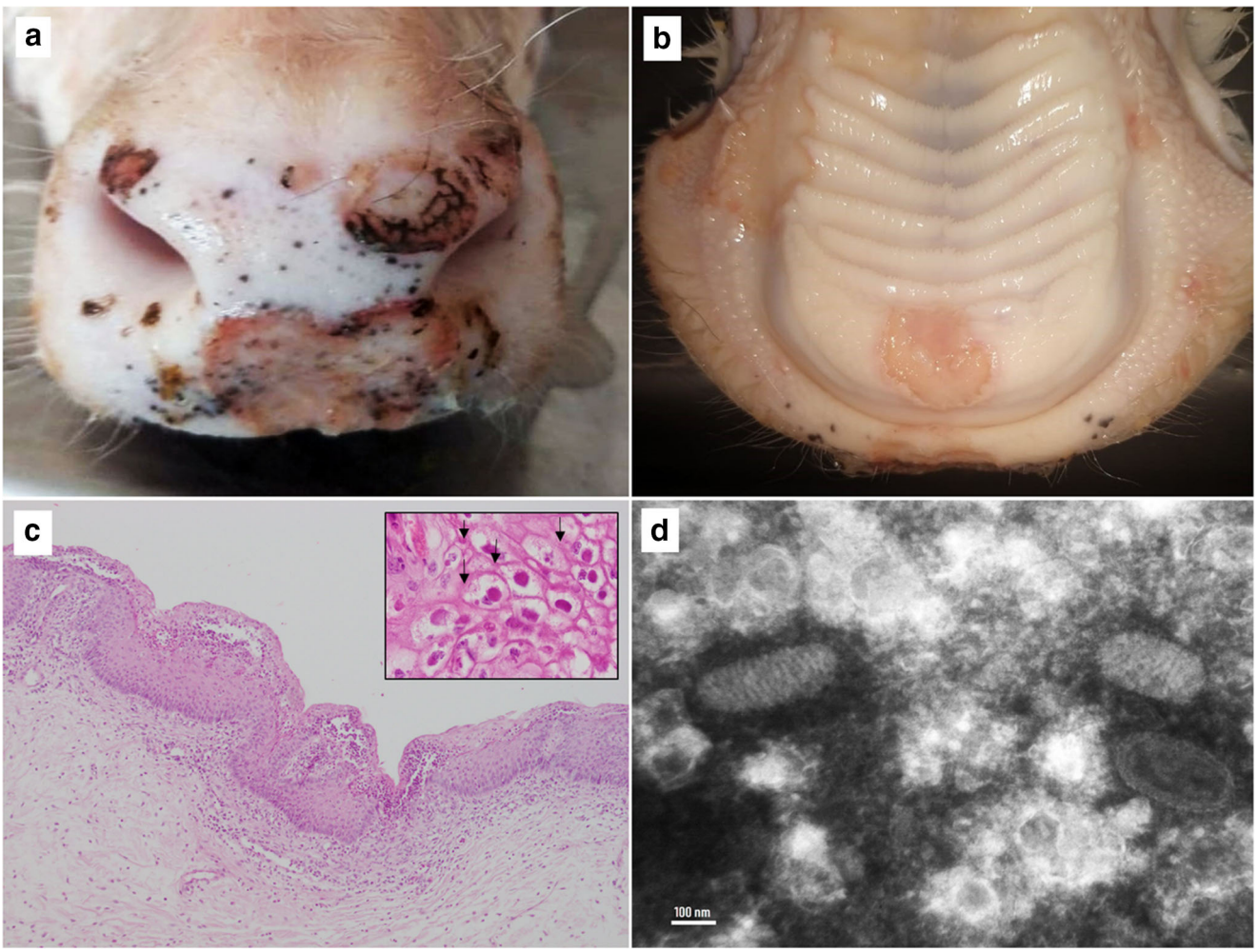

Fig. 1 Calves infected with BPSV-Salta. **a** Multiple ulcerative and erosive lesions in the nose and lips. **b** Typical lesions of disease on the palate. **c** Microphotography of lips. Pustular and necrotizing dermatitis with severe diffuse proliferative acanthosis and moderate lymphoplasmacytic infiltration. The insert shown ballooning degeneration of superficial keratinocytes and typical viral inclusion bodies (arrows) (H&E 20×). **d** Electron micrograph of a BPSV particle showing the typical helix-shaped morphology (bar = 100 nm)

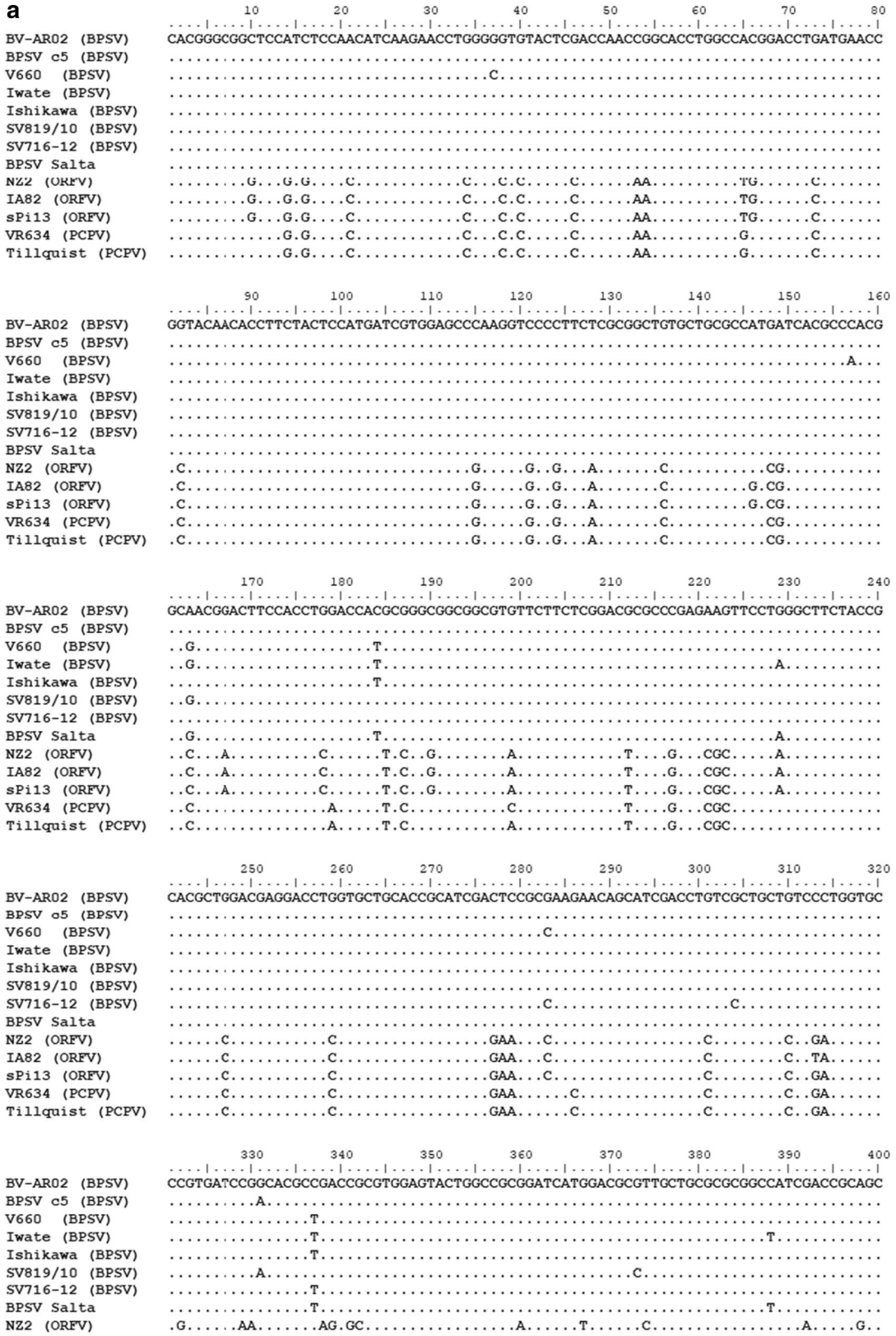

Fig. 2 Molecular characterization of BPSV-Salta based on internal region of B2L gene. **a** Alignment of nucleotide sequences from internal region of B2L gene among BPSV-Salta and several parapoxvirus: BPSV, ORFV, and PCPV. **b** Phylogenetic analysis of Argentine BPSV outbreak based on B2L gene. The phylogenetic relationships were constructed by the neighbor-joining algorithm using MEGA 6.0 software. All positions with less than 50% site coverage were removed. Numbers at nodes represent percentage of 1000 bootstrap replicates. Black circle pointed to BPSV-Salta

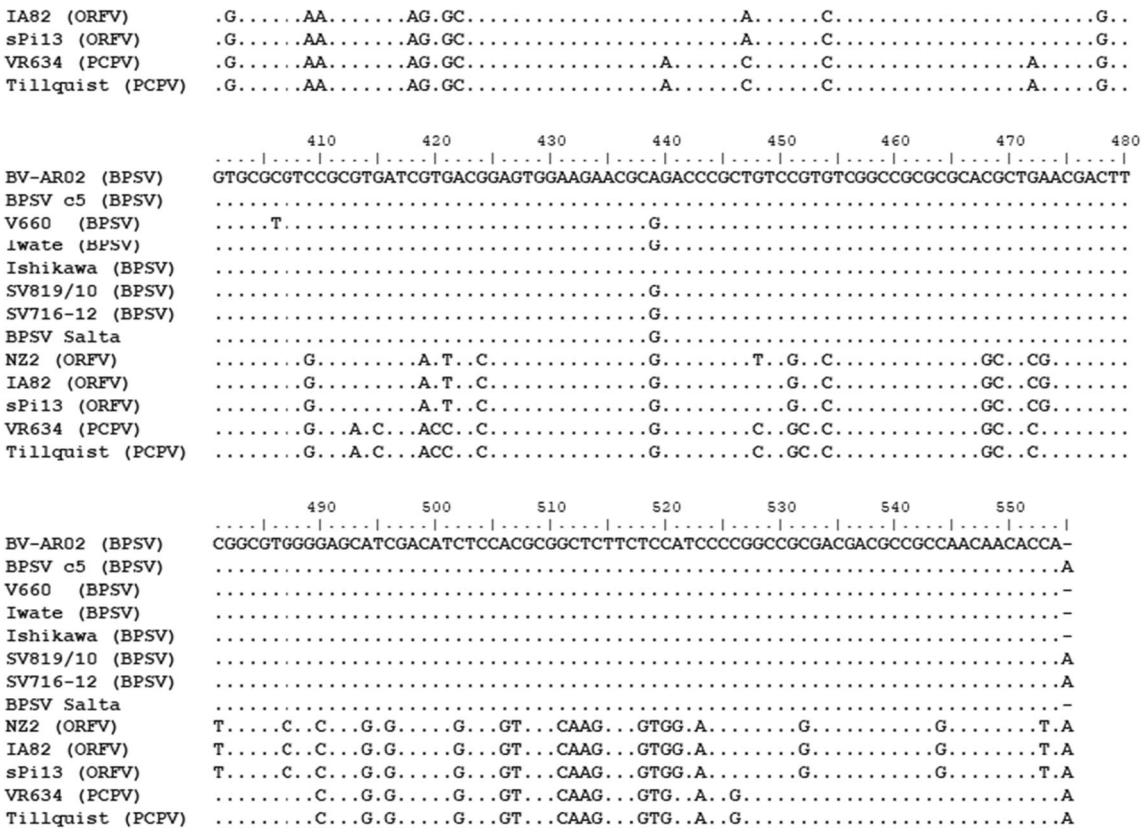

Fig. 2 continued.

affected areas, tissues of the superficial dermis presented moderate inflammatory infiltrate of lymphocytes, neutrophils, and macrophages. The adjacent dermis showed the same type of perivascular infiltrate.

Parapoxvirus infections are usually considered diseases that should be differentiated from important vesicular diseases in ruminants. Therefore, we extracted DNA (QIAamp DNA mini kit, QIAGEN) and RNA (Trizol Reagent, Invitrogen) from scab samples to determine the etiological agent of the lesion. The presence of bovine viral diarrhoea virus (BVDV) was discounted by RT-PCR based on 5′UTR BVDV genome sequence (Pellerin et al. 1994), foot-and-mouth disease virus (FMDV) was rejected by RT-PCR based on 3D gene sequence, and vesicular stomatitis virus (VSV) was discounted by RT-PCR based on an internal region of VSV glycoprotein G gene sequence. Possible *Orthopoxvirus* infection was discounted by pan-pox low-GC PCR assay (Li et al. 2010), whereas pan-pox high-GC PCR indicated a member of PPVs as responsible for the animal injury (data not shown).

Tissue samples were homogenized mechanically in phosphate-buffered saline (PBS) using a pellet pestle device. Homogenates were then centrifuged at 3000×*g* for 5 min, and the supernatant was loaded on 200 mesh copper grid and stained with 2% phosphotungstic acid for transmission electron microscopic examination. This study revealed typical PPV particles: oval (260 nm × 160 nm), enveloped, and with a helical structure (Fig. 1d).

Once PPV was confirmed as the etiological agent, a PCR using pan-parapoxvirus primers for the B2L gene (Inoshima et al. 2000) was performed. The amplified 590-bp product was directly sequenced in both directions using the amplification primers. Genomic information was derived from overlapping sequences covered by the forward and reverse primers. The sequence obtained was aligned with BPSV, ORFV, and PCPV parapoxvirus sequences available in the GenBank (Fig. 2a). In addition to the BPS case, ORFV sequences obtained from previous cases occurred in the same Argentine province were also included. The identity matrix revealed a nucleotide identity of 99%, 85%, and 84% with BPSV, PCPV, and ORFV, respectively. The sequence was called “Salta” and was submitted to the GenBank databases under accession number MK285566.

Given that our neighboring country Brazil has several reports on outbreaks and characterization of BPSV strains, we decided to determine if the BPSV-Salta was related to Brazilian strains. Then, a phylogenetic analysis was performed using the neighbor-joining method with 1000 bootstrap replicates, using MEGA software version 6 (Tamura et al. 2013), and the Tamura-3 parameters (Tamura and Nei 1993) were selected as the fitted evolution model. As expected, the NJ tree showed that BPSV-Salta clustered with other

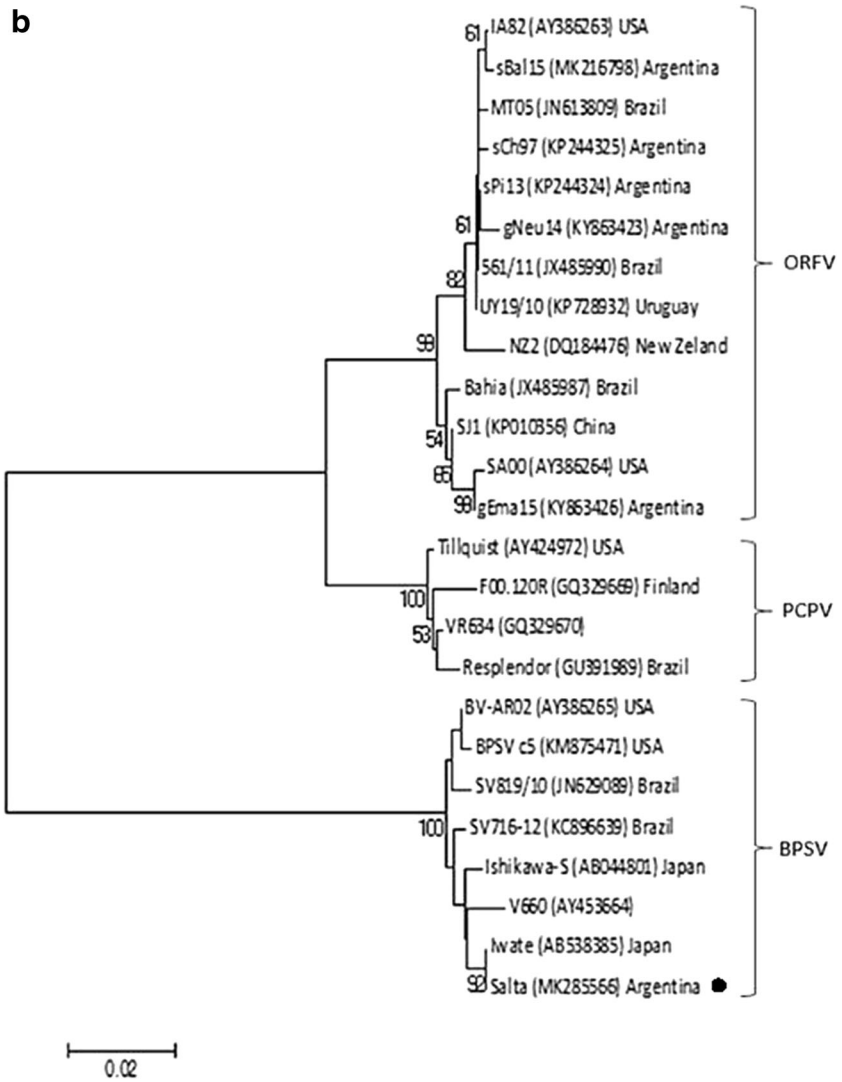

Fig. 2 continued.

BPSV isolates, with high bootstrap value, and separated from PCPV and ORFV strains (Fig. 2b). BPSV-Salta showed 100% identity with Iwate strain (from Japan) and 98.9% identity with Brazilian and North American BPSV strain (see Fig. 2b). Given that there is no import record of cattle from Japan to Argentina, we hypothesize that this observation is due to a low phylogenetic signal caused by the high degree of identity in this internal region of the B2L gene.

This sequence was able to distinguish viral species within the PPV genus. However, within each viral species node, many of the branches have nodes with low or intermediate bootstrap values. This phenomenon was also reported in ORFV and BPSV phylogenetic studies (Schmidt et al. 2013; Billinis et al. 2012; Li et al. 2013; Oem et al. 2013; Yaegashi et al. 2013; Peralta et al. 2018). This effect could be caused by the high level of identity observed within these gene sequences, which results in a low phylogenetic signal among isolates from different countries and continents.

The clinical and pathological findings are similar to those reported for other BPS outbreaks (de Sant'Ana et al. 2012; Dal Pozzo et al. 2011; Jeckel et al. 2011). Findings include papules, often mildly erosive mainly on the muzzle, lips, hard palate, and oral mucosa of calves and, occasionally, on the tongue, esophagus, and forestomach (Brown et al. 2007). In both necropsied calves, the esophagus was unaffected; however, ruminal and omasal mucosa presented necrotic and proliferative lesion. Parapoxvirus infection generally occurs through scarified or damaged skin, followed by virus replication in keratinocytes (Büttner and Rziha 2002). Histologically, this infection produced acanthosis, spongiosis, and

parakeratotic or orthokeratotic hyperkeratosis with adjacent focally extensive ulcers in the epidermis. In early lesions, eosinophilic inclusion bodies were noted in the cytoplasm of epithelial cells of areas with acanthosis or necrosis (Brown et al. 2007).

In this report, the control of the outbreak was carried out through biosecurity and hygiene measures. Infected calves were separated by a physical division on one side and healthy newborns on the other. Using gloves and other biosecurity attire was indicated for employees in charge of caring for the calves. BPS is considered a neglected zoonosis and several case reports in humans were previously reported (de Sant'Ana et al. 2012; Bowman et al. 1981).

Parapoxvirus infections are usually considered important diseases that should be differentiated from other vesicular diseases in ruminants, such as infection by foot-and-mouth virus. However, BPS does not have to be reported to the World Organization for Animal Health (OIE). On the other hand, the presence and wide distribution of parapoxvirus infection in cattle and humans have been frequently reported (Büttner and Rziha 2002; Dal Pozzo et al. 2011; Inoshima et al. 2009; Jeckel et al. 2011; de Sant'Ana et al. 2012; Oem et al. 2013; Lederman et al. 2014). In addition, severe atypical cases of parapoxvirus infection have been recently reported (Inoshima et al. 2009; Leonard et al. 2009; Jeckel et al. 2011). Therefore, the importance of parapoxvirus infection in cattle should be re-evaluated. Further studies are needed to determine the prevalence of infection and to identify risk factors for both animals and humans.

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Compliance with ethical standards

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

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