Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 3, pp: (24-31), Month: May - June 2019, Available at: www.noveltyjournals.com

Detection and phylogenetic analysis of the Orf virus from sheep in Uruguay

E.R. Castro[1,2], R. Pérez S[1], R. Negro[1], L. Bassetti[1], S. Rodríguez[1]

(1) Departamento Virología - División de Laboratorios Veterinarios "M.C.Rubino" (DILAVE-DGSG-MGAP), Ruta 8 Km. 17.500, Montevideo, Uruguay.

(2) Posgrado Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.

*Corresponding author: ercastro72@gmail.com; racastro@mgap.gub.uy

Abstract: **The Orf virus (ORFV) is the etiological agent of contagious ecthyma (CE), a pustular dermatitis of sheep and goats. Outbreaks of ORFV have been observed in all geographical regions of the world, including Uruguay.**

In this study, we describe the partial nucleotide sequencing and phylogenetic analysis of the B2L gene from two samples recovered from outbreaks of CE which occurred during 2018 in Uruguay. Phylogenetic analysis and the derived amino acid sequences from the B2L gene suggest that the Uruguayan virus displays similarities with American ORFV isolates as well as current vaccine strains used in the region.

Keywords: **Phylogenetic analysis, Orf virus, Sheep, Uruguay.**

1. INTRODUCTION

Bovine papular stomatitis virus (BPSV), ORF virus (ORFV) and Pseudocowpox virus (PCPV) are epithelotrophic and zoonotic DNA viruses included into the Parapoxvirus genus as a part of the Poxviridae family, which affect ruminants worldwide. ORFV is maintained in sheep and goats, whereas BPSV and PCPV are maintained in cattle. The viruses are often transmissible to humans. ORFV is the etiological agent of contagious ecthyma (CE), a severe exanthematous dermatitis that affects small domestic and wild ruminants. CE is a zoonosis, associated with occupational activities with sheep and goats (Damon, 2007). The disease is usually characterized by highly infectious pustules on the lips, tongue, and around the mouth as well as, although less commonly, on the feet, genitals, teats, navel, intestines, and respiratory tract. Transmission occurs by direct contact or via environmental contamination (Haig and Mercer, 1998). ORFV has a worldwide distribution, and is an economically important disease in most countries with commercial sheep and goat flocks (Hosamani et al., 2009). South American ORFV outbreaks have been diagnosed by veterinarians who recognized the clinical symptoms (Abrahão et al., 2009, Mazur et al., 2000), De Oliveira et al., 2012). Sheep production in Uruguay shares many similarities with its counterparts in neighboring Argentina and Brazil, which are among the largest sheep producers in the region. Every year the reference laboratory responsible for the diagnosis of FMD in Uruguay (Dilave-MGAP) receives epithelial samples that show clinically a vesicular disease similar to FMD but test negative for FMDV, and the diagnostic is EC. Contagious ecthyma is endemic in many flocks and has been partially controlled by vaccination. Commercially available vaccines have been derived from field viruses and were obtained by the traditional method of skin scarification of lambs. A few vaccines are used interchangeably in Uruguayan, Brazilian and Argentinean flocks. ORFV can be distinguished from other PPV species e.g., pseudocowpoxvirus (PCPV) or bovine popular stomatitis virus (BPSV), by genomic analyses (Inoshima et al., 2000). The ORFV genome consists of a linear double stranded DNA, of 138 kb in length which encodes 132 putative gene products (Mercer et al., 2006). The terminal regions contain genes, particularly the B2L gene (ORF 011), that 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 (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 in different outbreaks (Abrahão et al., 2009, Hosamani et al., 2006). Although it has been previously described, molecular characterization of ORFV outbreaks occurred in Uruguay during 2004-2011 (Olivero et al, 2018), at the present, there is no information available about recently circulating strains in this country. In the present report we have characterized two virus isolates, from two recent Uruguayan outbreaks of CE, on the basis of partial envelope gene (B2L) sequence. Comparative sequence analysis of this gene and its phylogenetic relationship with the published sequences of various Parapoxvirus isolates, and three commercial vaccine strains used in Southern countries (Vaccine A – Ectisan Ceva®, B – Ectisan Santa Elena®, and C– Rosenbush®) were determined.

2. MATERIALS AND METHODS

Origin of samples

In January of 2018, an outbreak of CE occurred in an unvaccinated crossbreed sheep (Ovis aries) flock from Curtina, a town in the department of Tacuarembó, Uruguay. Eight sheep and one lamb presented wart-like lesions on the lips and around the mouth. The presumptive diagnosis was CE. Dried scabs from the lamb were collected, and the samples were stored at refrigerated temperatures until arrival to the laboratory and stored at -70° C until processing. In November of 2018 another outbreak occurred in a partially vaccinated crossbreed sheep flock from Sauce de Tranqueras, also in the department of Tacuarembó, Uruguay. Thirty sheep presented exanthematic lesions on the lips and around the mouth. Scab samples from two affected sheep were collected and incorporated into our analysis.

Extraction of genomic DNA

Total DNA was extracted from the collected scabs using the QIAamp DNA Mini kit (QIAGEN) according to the manufacturer´s instructions. Briefly, 20 mg of the sample scabs were resuspended in 0,18 mL of PBS in a microcentrifuge tube. Then, lysis buffer and proteinase K were added into the microcentrifuge tube. After incubation at 56º C in a water bath, until complete tissues lysis, DNA was extracted, according to the manufacturer's instructions, eluted with 0.2 mL of elution buffer and finally stored at -20º C.

qPCR assays for the detection of species generic Parapoxviruses (PPV)

For species generic parapoxvirus genome sequence detection in the samples, we ran the assay established by Hiu et al.,2013. It is a TaqMan-based parapoxvirus specific qPCR test which targets the conserved parapoxvirus RNA polymerase subunit RPO147 gene sequence (ORF56 in ORFV IA82), the homolog of the J6R gene of orthopoxvirus vaccinia virus. This assay detects three species of parapoxvirus. The detection probe contains a 5' reporter molecule FAM and a 3'quencher molecule QSY7 (Applied Biosystems, USA). The primers and probe sequences of the Parapoxvirus generic assay (PAPV J6R) are: Forward primer CGCGGTCTGGTCCTTG Reverse primer CAGCATCAACC TCTCCTACATCA Probe CCACGAAGCTGCGCAGCAT. The reactions were carried out in 25 uL volumes for analysis on the Stratagene™ MX3005p qPCR. Each reaction mixture contained 1X PCR Master Mix (Tiangen Biotech ET101 Beijing), 0.4 umol/L each primer, 200 nmol/L TaqMan probe, and 8 uL of template DNA. Thermal cycling for the Stratagene™ was: one cycle 95°C for 2 min; 45 cycles of 95°C for 5 s, and 62.5°C for 20 s. The measurement of PCR amplification is based on the recording of fluorescent emission after the annealing/elongation step.

PCR reactions and sequencing of ORFV genome regions

The partial sequences of the B2L gene (ORF011) from the extracted DNA were amplified by the PCR kit (Tiangen Biotech ET101 Beijing) using a t 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 (Inoshima et al 2000). DNA was amplified at 95ºC for 9 min, and 30 cycles of denaturation (94º C,1 min), annealing (55º C, 1 min) and extension (72º C, 1 min), and final extension by 72º C for 7 min. An aliquot (5 ul) of the PCR product was analyzed by 1% agarose gel electrophoresis to visualize the amplicons. The PCR products (50 ul) were purified by using commercial DNA Clean & Concentrator^{TM} -5 kit (Zymo Research Corp, USA), according to the manufacturer's instructions. The PCR products were purified and sequenced by LGC Genomics, Germany (http://lgcgenomics.com). The B2L nucleotide sequences were edited with the BIOEDIT software 7.2.5 Sequence Alignment Editor (Hall, 1999). Multiple nucleotide and amino acid sequence alignments were constructed in CLUSTAL_X v.1.4 (1Thompson et al., 1994) and subsequently edited in MEGA X (Kumar et al., 2018).

Phylogenetic analysis using MEGA X software was performed with the B2L gene sequences determined, and selected sequences from the NCBI GenBank database are shown in Table I. A phylogenetic tree was constructed using the maximum-likelihood method with 1000 bootstrap replicates. The phylogenetic tree of isolates and reference strains of PPV was conducted in Table 1.

The sequences were annotated in the GenBank (accession numbers in Table 1).

3. RESULTS

The clinical diagnosis of CE was assessed by qPCR (ORF56 gene) in the lamb samples from the Curtina 2018 outbreak and in two sheep samples from the Sauce de Tranqueras 2018 outbreak. All tested samples, except one, showed a positive result in this analysis (data not shown), which confirmed the presence of ORFV in both outbreaks. The partial-length B2L genes were amplified from the positive samples. Primers PP1/PP4 complementary to flanking regions of the B2L gene yielded a PCR product with the expected length of 595 bp. All qPCR positive samples were confirmed as ORFV by direct sequencing of the retrieved sequences. The B2L gene from all Uruguayan samples demonstrated high identity with other ORFV sequences. The nucleotide sequences of the virus identified in this study (submitted as ORF B2L Uy 0118 and ORF B2L Uy 0618 to the NCBI database, accession number: MK482520 and MK482521 respectively), revealed a high degree of B2L similarity among Uruguayan sequences and also with the reference US strain IA82 (Genbank accession:AY386263). The maximum likelihood tree accomplished with partial sequences of the B2L gene (nt 408- 886) is shown in Fig. 1.

4. DISCUSSION

The B2L gene (ORF011) is an important PPV molecular target, and several PPV B2L nucleotide and amino acid sequences are available in GenBank. Moreover, detection of the B2L gene is the most sensitive method for virus detection because it harbors epidemiologically relevant sequence information (Hosamani et al., 2006). The presence of ORFV in samples tested positive by qPCR assay was confirmed by similarity with B2L gene sequences retrieved from direct sequencing using NCBI BLAST. A high degree of nt identity was observed among Uruguayan sheep isolates and between these and the vaccine strains analyzed. The vaccine strains showed a remarkably high similarity to the other ovine isolates, including the reference IA82. This is not surprising since the vaccine ORFV strains used in Brazil, Uruguay and Argentina are usually derived from field strains circulating in these regions. The maximum likelihood tree was accomplished with partial sequences of 478 bp, in the full-length ORFV B2L nucleotide sequence (Fig. 1). As previously described (Olivero et al., 2018), it showed that the Uruguayan sequences were broadly split into two main groups. One, comprising only the UY05/04 sample grouped with sequences predominantly from Asia and one Brazilian strain isolated in 1992 (Accession number JN088052.1). The more closely grouped sequences share distinctive residues at variable positions. Moreover, 185I, 256Q and 267N residues are only present in UY05/04 and the Brazilian strain isolated in 1992 (Accession number JN088052.1), when compared with the rest of the Uruguayan samples from 2018 (Fig. 2). Nevertheless, B2L sequence analysis showed that the 2004 sample (UY05/04) from a herd previously immunized comes from the only animal infected in a herd of 1500 individuals, which probably escaped vaccination and was infected with an exogenous virus, introduced to the northern frontier of the country (Olivero et al., 2018). All remaining Uruguayan sequences, including those from the 2018 outbreaks, formed a cluster of American viruses detected in Brazil, USA, Mexico, Argentina and Uruguay (Fig.1), along with the South American vaccine strains, also observed by other authors (17, 18). As with Olivero's findings, our 2018 Uruguayan outbreak sequences are grouped in different sub clusters. However, the analysis showed low bootstrap values (53). This effect could be caused by the high level of identity observed within these samples which results in a low phylogenetic signal. More complete viral genome sequences or, at least, multiple gene sequencing studies could help to differentiate strains that are identical in the commonly studied gene (as with the three South American vaccine strains with B2L) but different in other regions of the genome. For example, the analysis of the virulence gene ORF109, one of the most variable genes of the PPV genus according to the comparison of reference genomes, confirmed that two Argentinian isolates still stayed together in the same phylogenetic group with a high bootstrap value (Peralta et al., 2015). Otherwise, the nt sequence analysis confirmed the high degree of conservation of the B2L gene among ORFV from different countries and continents (Damon, 2007, Hui et al., 2013, Peralta et al., 2015, Schmidt et al., 2013, Zaho et al., 2010, Velazquez-Salinas et al., 2018). The molecular characterization achieved in this study provides information that improves our knowledge of the scale of the ORFV problem in Uruguay.

ACKNOWLEDGMENTS

We wish to thank Edviges Maristela Pituco for her valuable comments, and Ruth Santestevan for preparing the manuscript. This work would not have been possible without the cooperation of Cristina Gonzalez and Ricardo Rossano for sampling and clinical description of the outbreaks. We thank to IAEA / APH and LGC Genomics for their cooperation in the sequencing service of the samples.

REFERENCES

[1] Abrahão JS, Campos RK, Trindade GS, Guedes MI, Lobato ZI, Mazur C, Ferreira PC, Bonjardim CA, Kroon EG (2009) Detection and phylogenetic analysis of Orf virus from sheep in Brazil: a case report. Virol. J. 6:47. doi.org/10.1186/1743-422X-6-47.

[2] Damon I. (2007) Poxviridae and their replication. In : Knipe DM, Howley PM, editors. Fields Virology. Raven Press . pp. 2079-2081.

[3] De Oliveira C, Assis F, Neto J, Oliveira C, Tavora C, Lopes C, Bomjardim H, Vinhote W, Silva A, Abrahão J, Kroon E (2012) Multifocal cutaneous Orf virus infection in goats in the Amazon Region, Brazil. Vector Borne Zoonotic Dis. 12(4):336–340. https//doi.org/10.1089/vbz.2011.0725.

[4] Delhon G, Tulman ER, Afonso CL, Lu Z, de la Concha-Bermejillo A, Lehmkuhl HD, Piccone ME, Kutish GF, Rock DL (2004) Genomes of the parapoxviruses ORF virus and bovine papular stomatitis virus. J. Virol. 78(1):168–177.

[5] Haig DM, Mercer AA. (1998) Ovine diseases. Orf. Vet. Res. 29:311-26.

[6] Hall TA. (1999) BioEdit: a user friendly biological sequence alignment and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser(Oxf): 41:95–98.

[7] Hosamani M, Bhanuprakash V, Scagliarini A, Singh RK. (2006) Comparative sequence analysis of major envelope protein gene (B2L) of Indian orf viruses isolated from sheep and goats. Vet Microbiol. 116(4):317-24.

[8] Hosamani M, ScagliariniA, Bhanuprakash V, McInnes CJ, Singh RK (2009) Orf: an update on current research and future perspectives. Expert Rev. Anti Infect. Ther. 7:879–893.

[9] Inoshima Y, Morooka A, Sentsui H. (2000) Detection and diagnosis of parapoxvirus by the polymerase chain reaction. J. Virol. Methods 84(2): 201-208.

[10] Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35(6):1547-1549. doi: 10.1093/molbev/msy096.

[11] Mazur C, Ferreira I, Rangel Filho R, Galler R (2000) Molecular characterization of Brazilian isolates of orf virus. Vet. Microbiol. 73(4):253–259.

[12] Mercer AA, Ueda N, Friederichs SM, Hofmann K, Fraser KM, Bateman T, Fleming SB (2006) Comparative analysis of genome sequences of three isolates of Orf virus reveals unexpected sequence variation. Virus Res. 116(1-2):146–158.

[13] Olivero N, Reolon JE, Arbiza J, Berois M (2018) Genetic diversity of Orf virus isolated from sheep in Uruguay. Arch.Virol. 163(5):1285-1291. https://doi.org/10.1007/s00705-018-3717.

[14] Peralta A, Robles C, Martınez A, Alvarez L, Valera A, Calamante C, Konig G. (2015) Identification and molecular characterization of Orf virus in Argentina. Virus Genes 50(3):381–388. //doi. org/10.1007/s1126 2-015-1189-6.

[15] Schmidt C, Cargnelutti J, Brum M, Traesel C, Weiblen R, Flores E (2013) Partial sequence analysis of B2L gene of Brazilian orf viruses from sheep and goats. Vet Microbiol. 162(1):245–253. doi.org/10.1016/j.vetmi c.2012.10.031.

[16] Sullivan JT, Mercer AA, Fleming SB, Robinson AJ, (1994) Identification and characterization of an orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K. Virology 202(2):968–973.

[17] Thompson JD, Higgins DG, Gibson TJ. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22(22):4673-80.

[18] Velazquez-Salinas L, Ramirez-Medina E, Bracht AJ, Hole K, Brito BP, Gladue DP, Carrillo C. (2018) Phylodynamics of parapoxvirus genus in Mexico (2007-2011). Infect.Genet.Evol.65:12–14. doi.org/10.1016/ j.meegid.2018.07.005

[19] Zhao H, Wilkins K, Damon IK, Li Y (2013) Specific qPCR assays for the detection of orf virus, pseudocowpoxvirus and bovine papular stomatitis virus. J. Virol. Methods 194(1-2):229– 234. doi: 10.1016/ j.jviromet. 2013.08.027

[20] Zhao K, Song D, He W, Lu H, Zhang B, Li C, Chen K, Gao F (2010) Identification and phylogenetic analysis of an Orf virus isolated from an outbreak in sheep in the Jilin province of China. Vet. Microbiol. 142(3-4):408–415. doi.org/10.1016/j.vetmi c.2009.10.006

Table 1: Parapoxviruses (PPV) viruses included in phylogenetic analysis

Strain	Host	Country	Accession number
NZ2	Sheep	New Zealand	DQ184476 (complete genome)
Uy 05/04	Sheep	Uruguay	KP728919.1
Orf virus strain SD/DY	Sheep	China	JQ904794.1
reference strain OV-SA00	goat	USA	AY386264.1
Orf virus isolate UY24/11	Sheep	Uruguay	KP728936.1
Orf virus isolate UY18b/09	Sheep	Uruguay	KP728931.1
Orf virus isolate UY18a/09	Sheep	Uruguay	KP728930.1
Orf virus isolate UY17/09	Sheep	Uruguay	KP728929.1
Orf virus isolate UY15/09	Sheep	Uruguay	KP728927.1
Orf virus isolate UY13/08	Sheep	Uruguay	KP728925.1
Orf virus isolate UY12/07	Sheep	Uruguay	KP728924.1
Orf virus isolate UY11/07	Sheep	Uruguay	KP728923.1
Orf virus isolate UY21/10	Sheep	Uruguay	KP728934.1
Orf virus isolate UY09/79	Sheep	Uruguay	KP728922.1
Orf virus isolate UY08/79	Sheep	Uruguay	KP728921.1
Orf virus isolate UY20/10	Sheep	Uruguay	KP728933.1
Orf virus isolate UY16/09	Sheep	Uruguay	KP728928.1
Orf virus isolate UY14/07	Sheep	Uruguay	KP728926.1
Orf virus isolate UY02/07	Sheep	Uruguay	KP728917.1
ORFV	Sheep	Brazil	JX485986.1
ORFV Arg 13 sCh97	Sheep	Argentina	KP244325.1
ORFV Arg 13 sPi13	Sheep	Argentina	KP244324.1
Curtina Uy 0118	Sheep	Uruguay	MK 482520
Sauce de Tranqueras Uy 0618	Sheep	Uruguay	MK 482521
Uy 2311	Sheep	Uruguay	KP728935.1
Uy 1910	Sheep	Uruguay	KP728932.1

Uy 2110	Sheep	Uruguay	KP728934.1
Uy 0307	Sheep	Uruguay	KP728918.1
Uy0107	Sheep	Uruguay	KP728916.1
ORFV 12	Sheep	Brazil	JX485991.1
PCPV Eth13	Camel	Ethiopia	KU645548.1
ORFV 10	Goat	China	JQ904798.1
ORFV 10	Sheep	China	JQ904794.1
ORFV 10	Goat	China	JQ904792.1
ORFV	Sheep	China	JQ904789.1
ORFV 06	Sheep	China	GU903501.1
ORFV 92	Sheep	Brazil	JN088052.1
Vaccine A	Sheep	Brazil	JX485981.1
ORFV 04	Sheep	India	DQ263303.1
PCPV 04	Bovine	nd	AY424972.1
Vaccine C	Sheep	Argentina	JX485978.1
ORFV 09	Goat	Korea	GQ328006.1
ORFV 10	Sheep	Mexico	KJ137702.1
ORFV 10	Sheep	Mexico	KJ137690.1
ORFV 14	Goat	Canada	MF497431.1
Uy0107	Sheep	Uruguay	KP728916.1
ORFV Taiwan 07	Goat	Taiwan	EU327506.1
ORFV 04	Sheep	India	DQ263305.1
Vaccine B Uy	Sheep	Uruguay	JX485984.1
ORFV 04	Sheep	India	DQ263303.1
ORFV 10	Sheep	Mexico	KJ137690.1
Uy0107	Sheep	Uruguay	KP728916.1
ORFV 14	Sheep	Canada	MF497429.1
PCPV 08	Bovine	Mexico	KJ137718.1
BPS 10	Bovine	USA	KJ137717.1
ORFV 2007	Goat	USA	KJ137709.1
ORFV 08	Sheep	Mexico	KJ137705.1
ORFV 11	Goat	Mexico	KJ137693.1
ORFV 05 MT 05	Sheep	Brazil	JN613809.1
ORFV 93	Goat	Brazil	JN613810.1
PCPV	Bovine	Finland	JF773695.1
ORFV 04	Sheep	USA	AY424970.1

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 3, pp: (24-31), Month: May - June 2019, Available at: www.noveltyjournals.com

Figure 1

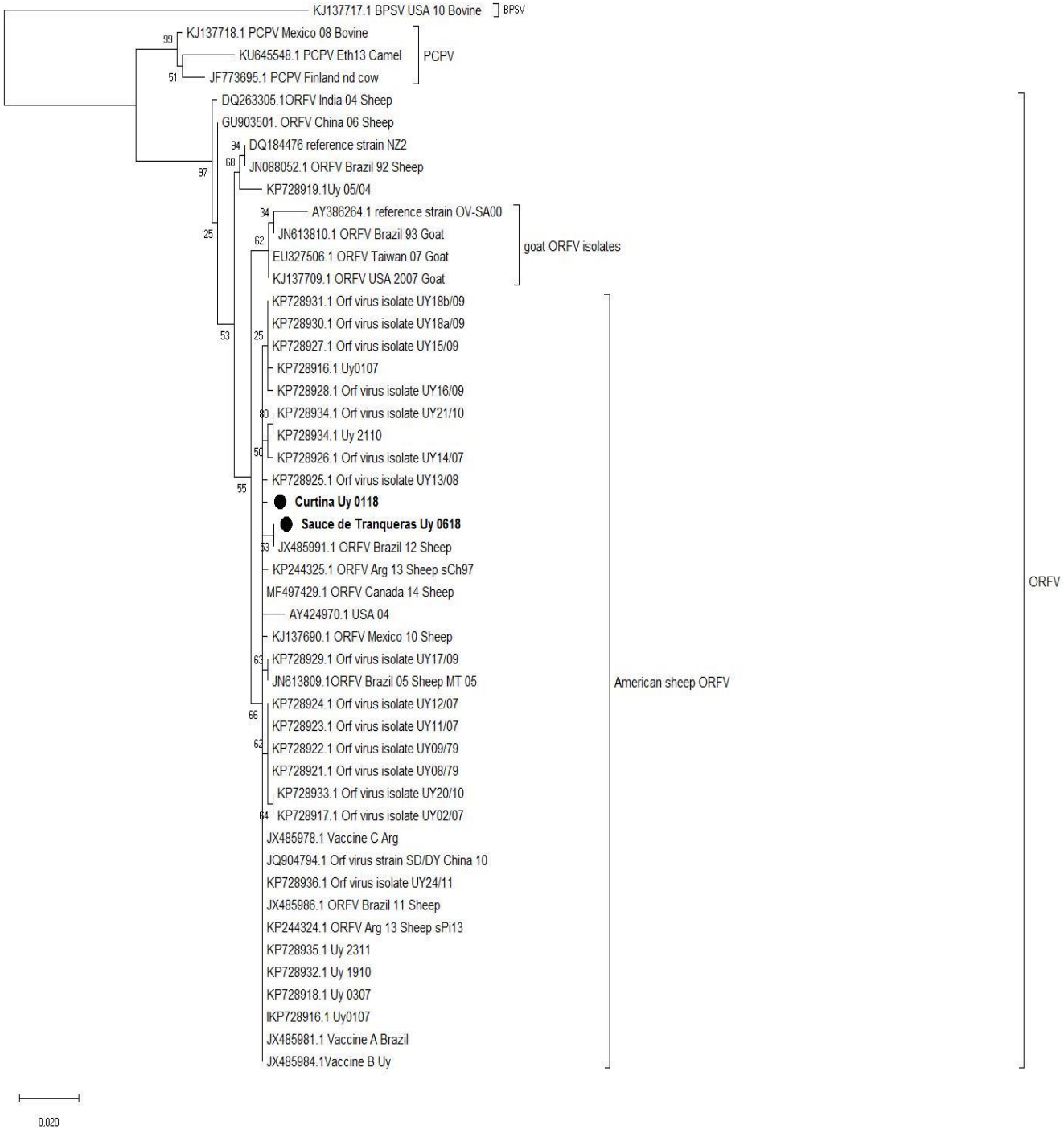

Figure 1: Phylogenetic relationships among ORFV, based on the partial nucleotide sequence of the B2L gene (ORF011). 1000 bootstrap replicates were used. 2018 Uruguayan outbreaks isolates are indicated in boldface circles. The phylogenetic relationship was constructed by the Neighbor-Joining algorithm using MEGA X software. Numbers at nodes represent % of one thousand bootstrap replicates. The scale bars are expressed in relative nucleotide sequence difference.

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 6, Issue 3, pp: (24-31), Month: May - June 2019, Available at: www.noveltyjournals.com

Figure 2

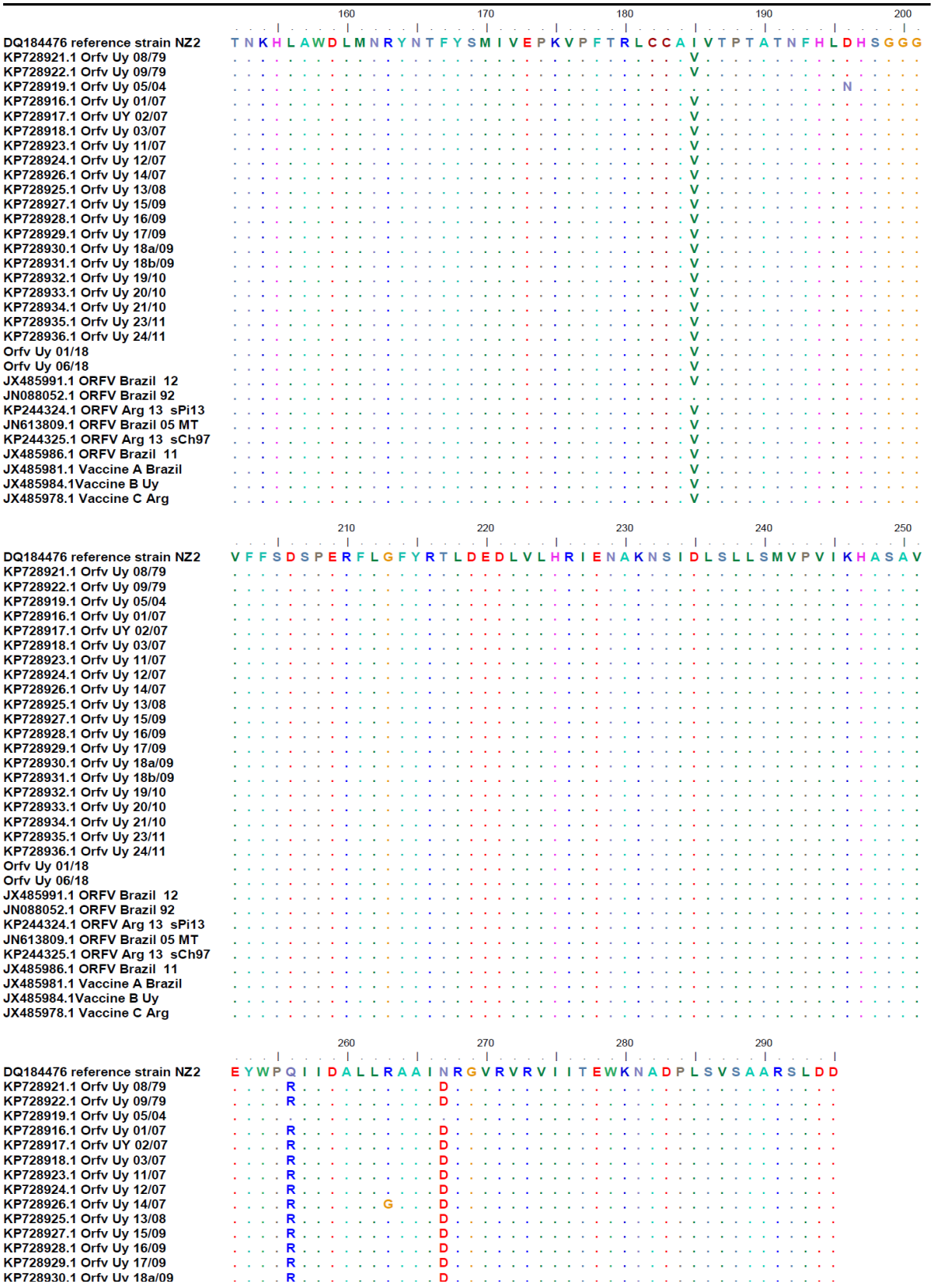

Figure 2: Alignment of the deduced amino acid sequences for the B2L gene from South American strains. The strain NZ2 was used as a reference sequence (Accession Number DQ 184476).