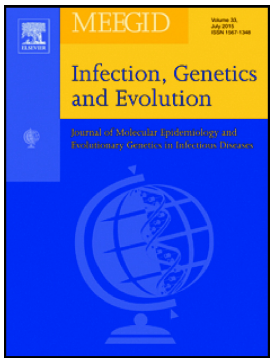

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Detection, risk factors and molecular diversity of norovirus GIII in cattle in Uruguay

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Key words: Bovine norovirus, cattle, diarrhea, genotypes, Uruguay

Abstract: Uruguay is a leading exporter of bovine meat and dairy products, and cattle production is one of the principal economic backbones in this country. A main clinical problem faced by livestock farmers is neonatal calf diarrhea (NCD); however, causes of NCD have not been extensively studied in Uruguay. Bovine norovirus (BoNoV) has been proposed as one of the possible etiologies of NCD as experimentally infected calves developed diarrhea and enteropathy, although limited information is available from field surveys. The aims of this study were to determine the frequency of infection, to investigate possible risk factors, and to determine the molecular diversity of BoNoV in Uruguay. A total of 761 samples of feces or intestinal contents from dairy and beef calves were analyzed through RT-qPCR. The overall frequency of detection of BoNoV was 66.1% with higher frequency in dairy (70.5%) than beef (15.9%) calves ($p<0.01$). BoNoV was detected similarly in diarrheic (78.8%) and non-diarrheic (76.2%) dairy calves ($p=0.50$). Calves ≤2 weeks of age (84%) were infected more often than older (62.7%) calves ($p<0.01$). Phylogenetic analysis confirmed the presence of GIII.1 and GIII.2 genotypes. In addition, we reported the circulation of recombinant strains and the detection of a strain with the recently described novel VP1 genotype. This study represents the first report describing the circulation, the associated risk factors, and the molecular diversity of BoNoV in Uruguay.

Introduction

A main problem that the livestock industry must face is neonatal calf diarrhea (NCD), a complex and multifactorial clinical syndrome of worldwide distribution. Although NCD affects both beef and dairy cattle, it is particularly important in dairy farming as it represents the major cause of mortality of calves before weaning (Urie *et al*., 2018). NCD leads to economic losses to the livestock industry due to a negative impact on animal wellness with short- and long-term effects on production (Waltner-Toews *et al*., 1986; Donovan *et al*., 1998). The causes of NCD outbreaks are poorly known and rarely investigated; also little is known about the prevalence, relative importance, possible interrelationships and pathogenic effects of the numerous microorganisms that have been shown or suggested as a cause of diarrhea (Selman, 1981).

Bovine noroviruses (BoNoVs) have not captured the attention that other pathogens have received and are not included in routine diagnosis for NCD, so their impact on livestock health and production remains unclear (Di Felice *et al*., 2016). BoNoVs were discovered in 1978, and their pathogenicity is mainly due to lesions in the small intestine, including villus atrophy with loss and attenuation of the villus epithelium, inducing diarrhea (Woode and Bridger, 1978; Jor *et al*., 2010; Otto *et al*., 2011; Jung *et al*., 2014).

Classified within the genogroup III (GIII) of the *Norovirus* genus in the *Caliciviridae* family (Scipioni *et al*., 2008), BoNoV are non-enveloped viruses with a single stranded RNA genome of positive polarity of approximately 7.5 kb containing 3 ORFs. Transmission is mainly sustained by the fecal-oral route, and low infectious doses as well as the great diversity of strains increase the risk of infection (Scipioni *et al*., 2008).

Although BoNoV is studied to a much lesser extent than other viruses that are well known causative agents of NCD such as rotavirus and coronavirus, several studies confirm that BoNoVs are widely present in cattle and sometimes at a high frequency in cases of diarrhea in different countries (van Der Poel *et al*., 2000; Deng *et al*., 2003; van Der Poel *et al*., 2003; Milnes *et al*., 2007); they are also detected in non-diarrheic calves (Jor *et al*., 2010). In addition, a serological study indicated that exposure to BoNoV can reach over 99% of the analyzed samples (Deng *et al*., 2003).

There have been recognized three genotypes within GIII, namely GIII.1, GIII.2, and GIII.3, being GIII.1 and GIII.2 associated to bovine norovirus, and GIII.3 to ovine norovirus. In addition, several studies have demonstrated the circulation of recombinant strains, with the recombination breakpoint in the ORF1-ORF2 junction genomic region (Bull *et al*., 2007). Both genotypes GIII.1 and GIII.2, formerly referred to as Jena virus and Newbury-2 virus, respectively, have been shown to be diarrheagenic when inoculated experimentally into calves (Woode and Bridger, 1978; Jor et al., 2010; Otto et al., 2011; Jung et al., 2014).

Uruguay is one of the main exporters of bovine meat (FAO, 2018) and dairy products (IDF, 2013), and cattle production is one of the main economic backbones in this country (DIEA, 2019), however, there are no studies on BoNoV in cattle. The aims of this study were to determine the frequency of infection, to investigate possible risk factors, and to determine the molecular diversity of BoNoV in calves in Uruguay.

Materials and methods

Samples and fecal suspensions

A total of 761 samples of feces (699) or intestinal contents (62) were collected from dairy (717) and beef (44) calves in Uruguay. The intestinal contents were collected

from diarrheic calves that died naturally, and all 699 fecal samples were from live calves. Risk factors for BoNoV infection were only evaluated in dairy calves, as insufficient data was available from beef calves. Fecal samples from dairy calves were categorized as diarrheic (208) and non-diarrheic (235) at the time of sampling (this information was missing for 443 samples); 209 samples came from calves in dairy herds that reported vaccinating the dams against NCD, while 203 came from calves in dairy herds where vaccination against NCD was not practiced (for 412 samples this information was unavailable). Regarding the age of the dairy calves, 127 were up to 1 week old, 180 were in the second week of life, 96 were in the third week of life, 28 were in the fourth week of life, and 10 were more than 4 weeks old. Samples were collected in 2015 (n=39), 2016 (n=490), 2017 (n=185), and 2018 (n=47).

Viral RNA extraction and reverse transcription

From all samples, suspensions were obtained after diluting 1:10 (v:v) in phosphate-buffered saline solution, and supernatants were collected after centrifugation at 3,000 g for 20 minutes at 4°C. Viral RNA was extracted using QIAamp® cador® Pathogen Mini Kit (Qiagen®), following the manufacturer's instructions. Reverse transcription (RT) was carried out with RevertAid® Reverse Transcriptase (Thermo Fischer Scientific®) and random hexamers primers (Qiagen®), following manufacturer's instructions. All RNAs and cDNAs were stored at -80°C until further viral analyses.

BoNoV screening and sequencing

Screening of the samples for GIII BoNoV identification was carried out through a real time polymerase chain reaction (qPCR) targeted to the junction between the

ORF1 and ORF2, which is a highly conserved genomic region. Primers, probe and real time PCR conditions were used as described elsewhere (Wolf *et al*., 2007).

In order to determine the genotypes circulating in the Uruguayan calves, 50 qPCR-positive samples were selected randomly and subjected to amplification of a 517-bp fragment (Wolf *et al*., 2007). Briefly, 12.5 μL of MangoMix™ (Bioline®), 5 μL of cDNA, 4.5 μL of nuclease-free water, 1 μL of dimethyl sulfoxide, 1.0 μL of 10 μM SW GIII forw primer and 1.0 μL of 10 μM NVGIIIrseq primer were mixed in 0.2 mL PCR tubes. PCR products were visualized in 2% agarose gels and positive samples were purified using PureLink™ Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen®) according to the manufacturer's instructions; both DNA strands were sequenced at Macrogen Inc. (Seoul, South Korea). Sequences were deposited in GenBank with accession numbers: MT227833-MT227846, and MT765190-MT765209.

Phylogenetic analysis

A phylogenetic analysis was performed in order to determine the BoNoV genotypes circulating in Uruguay. Database sequences were downloaded using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequences alignment was obtained with ClustalW in MEGA 7 software (Kumar *et al*., 2016). The nucleotide substitution model that best fit our data and the maximum likelihood tree was obtained with IQ-TREE (Trifinopoulos *et al*., 2016).

Recombination analysis

The SimPlot program was used to determine the presence of evidence of recombination in the sequences, with a window size of 50 and a step size of 25. Jena and Newbury2 strains were used as reference sequences for GIII.1 and GIII.2

genotypes, respectively. In order to confirm the evidence, a nucleotide identity matrix and phylogenetic analysis were performed with partial sequences before and after the recombination breakpoint suggested by SimPlot analysis.

Statistical analyses

Categorical data was evaluated with jamovi software (available at https://www.jamovi.org/) using 2x2 contingency tables and through Pearson's Chi-squared test; in multiple comparisons, the Bonferroni correction was applied. Relative risk (RR) and 95% confident intervals (CI) were calculated with jamovi software. Numerical data was evaluated with jamovi software using the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965) and when the null hypothesis was rejected Mann-Whitney U test was used (Mann and Whitney, 1947). In all tests, differences were considered statistically significant if the obtained p-value was ≤ 0.05. Graphics were generated using Microsoft® Office Excel, and the line of tendency that best fit the data (evaluated by the R^2 value) was obtained with the same program.

Results

BoNoV detection and risk factors

Bovine norovirus (GIII) was detected in 66.1% (503/761) of the analyzed samples. The frequency of detection was significantly higher in dairy 70.5% (462/655) than beef (15.9%, 7/44) calves (RR: 2.85, 95%CI: 2.4–3.4; p<0.00001) (Figure 1a). BoNoV was detected in 67.1% (469/699) and 54.8% (34/62) of the samples of feces (live calves) and intestinal contents (deceased calves), respectively, this difference being non-significant (p=0.051) (Figure 1a).

The proportion of BoNoV-positive samples was higher in diarrheic 78.8% (164/208) than non-diarrheic 76.2% (179/235) calves, although this difference was not statistically significant (p=0.50) (Figure 1a). Calves born to dams vaccinated against NCD (vaccines include several pathogens but not BoNoV) showed a frequency of detection of 82.8% (173/209) while calves born to dams unvaccinated showed a frequency of detection of 77.3% (157/203), this difference was not statistically significant (p=0.17).

Calves ≤2 weeks of age were more often infected by BoNoV (84%, 258/307) than older calves (62.7%, 84/134), and this difference was statistically significant (RR: 2.06, 95%CI: 1.57–2.69; p<0.00001) (Figure 1a). The frequency of BoNoV detection in calves in their first, second, third, fourth and after the fourth week of life was 82.7% (105/127), 85% (153/180), 68.8% (66/96), 50% (14/28), 40% (4/10), respectively (Figure 1b). The frequency of BoNoV was significantly higher between the age groups: 1vs4, 1vs>4, 2vs3, 2vs4, and 2vs>4, so BoNoV was more frequently detected in calves in their first two weeks of age. This frequency declined with the age, as observed in a line of tendency obtained by a polynomial regression (R^2=1.00) (Figure 1b). Similarly, when the frequency of BoNoV detection was analyzed by the age of the calves in days, a peak between days 7 and 9 was observed (Figure 1c).

The mean age of the positive calves to BoNoV (11.0 days) was significantly lower than the mean age of the negative ones (14.4 days; p<0.001) (Figure 2a); in addition, in the diarrheic calves, the mean age of the positive calves to BoNoV (10.3 days) was significantly lower than the mean age of the negative ones (13.1 days; p<0.001) (Figure 2b). Diarrheic calves (mean age of 10.9 days) were younger than non-diarrheic (mean age of 12.5 days) (Figure 2c), and diarrheic BoNoV-positive calves were younger (mean age of 10.3 days) than non-diarrheic BoNoV-positive calves (mean

age of 11.6 days), but these differences were not statistically significant (p=0.074 and p=0.093, respectively).

The range of the Ct-values was 7.84–40.0 (Figure 3) with a mean Ct-value of 27.8 (SD 7.4) and a median Ct-value of 29.3. Thirty-five samples showed high viral load with Ct-values <15, 237 showed Ct-values between 15 and 30, and 246 showed low viral load with Ct-values >30 (Figure 3). In addition, we compare the Ct values among calf groups; we did not observe differences in the Ct values between diarrheic and non-diarrheic calves, and between alive and deceased calves. On the other hand, we observed lower Ct values in dairy (mean ± SD, 27.7 ± 7.4) than beef (32.2 ± 7.9) calves (p=0.026), and lower Ct values in calves ≤2 weeks of age (26.1 ± 8.0) than older calves (30.2 ± 6.1, p<0.001).

Genetic diversity of BoNoV in Uruguay

Thirty-four sequences were obtained from the 50 subjected to conventional PCR amplification. Twelve strains could not be sequenced because no or very low amplification, and four sequences were excluded from the analysis because showed chromatograms with double peaks, maybe due to coinfection. GIII.1 and GIII.2 genotypes were identified in four and 23 samples, respectively (Figure 4). Three strains clustered within GIII.2, but together with recombinant strains, and one strain clustered together with Chinese strains with evidence of recombination and possessing a novel VP1 genotype (Figure 4). Three sequences were shorter so were excluded from the analysis, but the three were GIII.2 (data not shown).

As some Uruguayan strains clustered together with recombinant strains, we performed several analyses to determine if these were also recombinant. SimPlot analysis showed evidence of recombination in the strains Bo/LVMS3019/2016/UY,

Bo/LVMS3752/2017/UY, Bo/LVMS3970/2017/UY, and Bo/LVMS3974/2017/UY (Figure S1). The recombination breakpoints were observed in the junction ORF1-ORF2 in all the four strains. We confirmed these evidence with nucleotide sequences identity matrices: one performed with the partial 3' end of the polymerase (RdRp, upstream the recombination breakpoints, Table 1), and another with the partial 5' end of the capsid (downstream the recombination breakpoints, Table 1). This was confirmed also by phylogenetic analyses performed with the same two genomic regions used for the identity matrices (Figure S2).

Discussion

The diarrheagenic and enteropathogenic potential of BoNoV have been well established in experimental infections in calves (Woode and Bridger, 1978; Jor *et al*., 2010; Otto *et al*., 2011; Jung *et al*., 2014), but under natural conditions has not yet been thoroughly studied, being relegated behind other pathogens such as rotavirus A, coronavirus, enterotoxigenic and enteropathogenic *Escherichia coli*, *Cryptosporidium parvum*, and *Salmonella enterica*, among others. However, studies have demonstrated its endemicity in several countries (Jor *et al*., 2010; Deng *et al*., 2003; van der Poel *et al*., 2003; Oliver *et al*., 2007; Mauroy *et al*., 2009; Thomas *et al*., 2014) and its role as enteric pathogen in calves (Di Felice *et al*., 2016), endorsing the need for epidemiological surveillance of BoNoV.

The frequency of BoNoV detection of 66.1% in this study was higher than the reported for other enteric viruses such as bovine rotavirus A (57%) (Castells *et al*., 2020), bovine coronavirus (7.8%) (Castells *et al*., 2019a) and bovine astrovirus (26%) (Castells *et al*., 2019b) in cattle in Uruguay, in accordance with studies that have demonstrated that BoNoV could be the most commonly detected pathogen in calves'

feces (Cho *et al*., 2013). In addition, the frequency observed in our study is higher than the reported for BoNoV in other countries including Argentina where the frequency of detection was 3.3% (Ferragut *et al*., 2016), indicating that BoNoV is probably a belittled pathogen involved in calf diarrhea in Uruguay. The detection method used in our work (qPCR), probably has a higher sensitivity and specificity than the conventional PCR used in Argentina (Ferragut *et al*., 2016), which could partially explain this difference.

Despite their diarrheagenic and pathogenic potential under experimental conditions, whether BoNoVs represent a significant or even rare cause of spontaneous disease in cattle has not been widely studied. In our study the frequency of BoNoV detection was similar in diarrheic and non-diarrheic calves, as observed in The Netherlands (van der Poel *et al*., 2003); in USA, a higher frequency was observed in diarrheic calves, suggesting that this virus may be a significant contributor to calf diarrhea (Cho *et al*., 2013). Further investigations are needed to elucidate the clinical significance of the different BoNoV genotypes in spontaneous outbreaks of disease and eventual impact to the livestock industry. Several studies have demonstrated the prolonged shedding of the virus, before and after the manifestation of the diarrhea (Jor *et al*., 2010; Jung *et al*., 2014). Asymptomatic calves can spread the virus and their identification in the field is extremely difficult, which could have influenced the high frequency of detection observed in our study. The management of the calves in intensive systems is even more complicated, because separating only the diarrheic calves may not prevent the virus dispersion at the herd.

The obtained Ct-values ranged from 7.84 to 40.0, similar to the reported in Norway, where Ct-values ranged from 6.9 to 39.7 (Jor *et al*., 2010). Interestingly, we observed a right skewed distribution of Ct-values, as previously reported, which may

reflect prolonged low viral shedding post-clinical illness (Jor *et al*., 2010). Calves can be reinfected, prolonging the viral shedding without clinical signs, acting as reservoirs (Jor *et al*., 2010), which could reflect the high frequency of detection in non-diarrheic calves.

We observed that dairy calves were more often infected by BoNoV and with higher viral loads than beef calves, so studies about possible management conditions, geographic influence, and/or calves' genetic factors influencing this observation are encouraged.

Another factor studied was vaccination against NCD; it is important to clarify that vaccines do not include BoNoV, as there are no commercial vaccines available against this virus. BoNoV detection was higher in calves born to vaccinated dams but the difference was not statistically significant. Studies in humans (where vaccines against rotavirus A –RVA- but not norovirus are available as occurs in cattle) have demonstrated that vaccination against RVA have lead a reduction in RVA detection, leaving norovirus as the main cause of gastroenteritis in children (Hemming *et al*., 2013; Payne *et al*., 2013; Hemming-Harlo *et al*., 2016). In contrast, a study in diarrheic calves in France showed that vaccination against RVA did not promote the emergence of BoNoV (Kaplon *et al*., 2013), which is in concordance with the observed in our study. On the other hand, vaccines seem not to be effective against RVA in cattle (Castells *et al*., 2020), which could have influenced that no difference in BoNoV detection was observed.

The calves' age was analyzed as a factor that could influence the detection of BoNoV, and we observed that BoNoV was most commonly detected in the first two weeks of age, as observed in the USA (Smiley *et al*., 2003; Cho *et al*., 2013), indicating that younger calves are more frequently infected by BoNoV, and in this sense, the mean

age of calves positive to BoNoV was lower than that of calves negative to the virus. In addition, the viral load was higher in calves ≤2 weeks of age than older. Taken together, this data suggests that younger calves are more susceptible to BoNoV infection.

The phylogenetic analysis allowed to confirm that both main genotypes of BoNoV, GIII.1 and GIII.2, were circulating in Uruguay. The predominant genotype was GIII.2, as commonly observed in other countries (Mauroy *et al*., 2009; Kaplon *et al*., 2013; Thomas *et al*., 2014). Unfortunately, the sequence of the tentatively new genotype described in Argentina (Ferragut *et al*., 2016) was not available, thus, we could not determine if this genotype was circulating in Uruguay. The Uruguayan strains clustered in two lineages within GIII.1, in a divergent lineage divided in two sub-lineages within GIII.2, in a lineage together with Chi.P1/GIII.2 strains, and in a lineage together with Chinese strains recently described with a novel VP1 genotype, denoting a high genetic heterogeneity. The region amplified allowed the detection of possible recombinant strains, that were then confirmed by other specific methods. This region, then, is suitable for the detection and analysis of the genetic diversity of BoNoV (Jor *et al*., 2010). However, for a more accurate classification, complete genomes should be obtained.

Four Uruguayan strains showed recombination evidence, confirming the wide circulation of recombinant strains worldwide (Han *et al*., 2004; Oliver *et al*., 2004; Bull *et al*., 2007; Mauroy *et al*., 2009; Jor *et al*., 2010; Di Martino *et al*., 2014; Ferragut *et al*., 2016; Mohamed *et al*., 2018; Karayel-Hacioglu and Alkan, 2019; Wang *et al*., 2019]. This widely distribution of highly similar recombinant strains, may be due to ancestral recombination events that later spread widely in cattle. As for HIV (Reis et al., 2019), widely dispersed recombinant strains should be named BoNoV circulating recombinant forms (BoNoV CRFs), in order to facilitate the classification. A limitation

of this study was the short length of the partial 3' end of the polymerase, so the results related to recombination should be taken with caution. Three of the four recombinant strains were GIII.1/GIII.2, the most widely dispersed CRF. Most of the GIII.1/GIII.2 recombinant clustered together, further supporting a common origin. Interestingly, one strain clustered with recombinant strains with a novel VP1 genotype (Wang *et al*., 2019), which suggests that probably this novel VP1 genotype is widely dispersed, and maybe has been overlooked.

In conclusion, this was the first study on BoNoV conducted in Uruguay, and revealed a high frequency of BoNoV infection in diarrheic and non-diarrheic calves, with higher frequency in dairy than in beef calves. Calves ≤2 weeks of age were infected more often than older calves. Both main genotypes, GIII.1 and GIII.2, were identified, and four recombinant strains were described. Despite no clear association was found with NCD, the results of our study indicate that BoNoV may be a belittled pathogen involved in calf diarrhea.

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Felipe Castells: Resources. **Daniel Castells:** Resources. **Matías Victoria:** Writing - Review & Editing, Supervision. **Franklin Riet-Correa:** Writing - Review & Editing, Supervision. **Federico Giannitti:** Resources, Writing - Review & Editing. **Viviana Parreño:** Writing - Review & Editing, Supervision. **Rodney Colina:** Conceptualization, Resources, Writing - Review & Editing, Supervision.

Conflicts of interest: None

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Table 1. Sequence identity of Uruguayan recombinant strains compared with reference strains

	3' end of RdRp			5' end Capsid		
	Jena (GIII.1)	Newbury2 (GIII.2)	BET-17 (Novel VP1)	Jena (GIII.1)	Newbury2 (GIII.2)	BET-17 (Novel VP1)
MT765208_Bo/LVMS3970/2017/UY	**0.986**	0.934	0.947	0.740	**0.857**	0.784
MT765209_Bo/LVMS3974/2017/UY	**0.986**	0.934	0.947	0.740	**0.857**	0.784
MT227833_Bo/3752/2017/UY	**0.986**	0.934	0.947	0.745	**0.864**	0.774
MT765200_Bo/LVMS3019/2016/UY	0.947	**0.973**	**0.986**	0.808	0.782	**0.876**

Figures

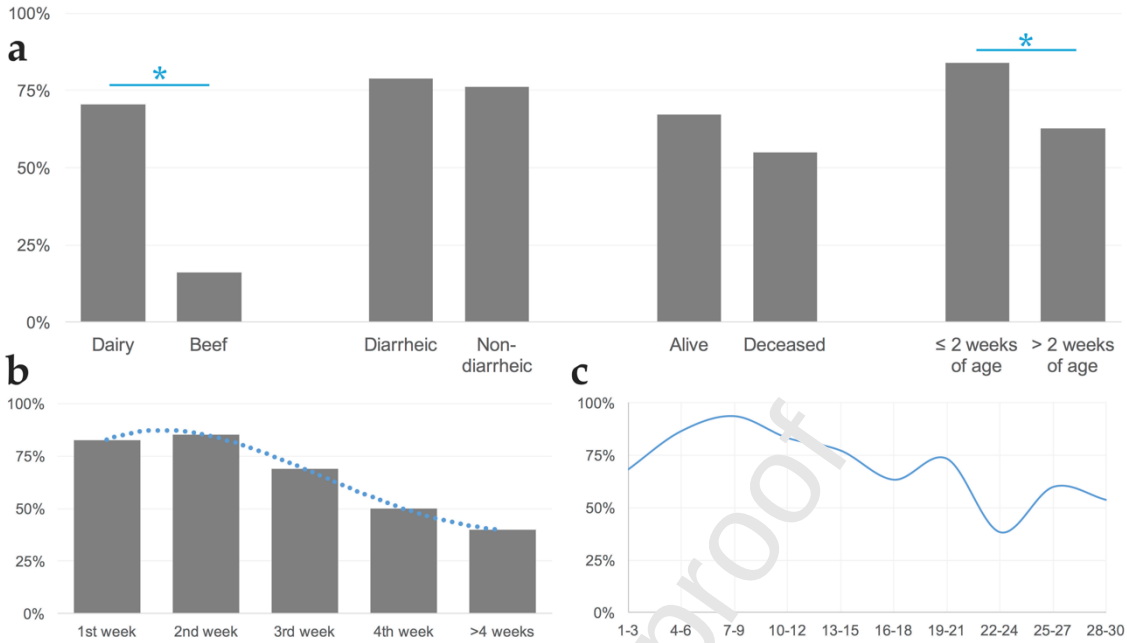

Figure 1. Bovine norovirus (BoNoV) detection. a) Comparison of frequency of BoNoV detection in dairy vs. beef calves, diarrheic vs. non-diarrheic, live vs. deceased calves, and calves ≤ 2 vs. > 2 weeks of age. Comparisons with statistical significant differences are indicated by an asterisk ($p<0.0000$). b) Frequency of BoNoV detection according to the calves age in weeks; a line of tendency (polynomial regression) was adjusted with an $R^2=1.00$. c) Frequency of BoNoV detection according to the calves age in days.

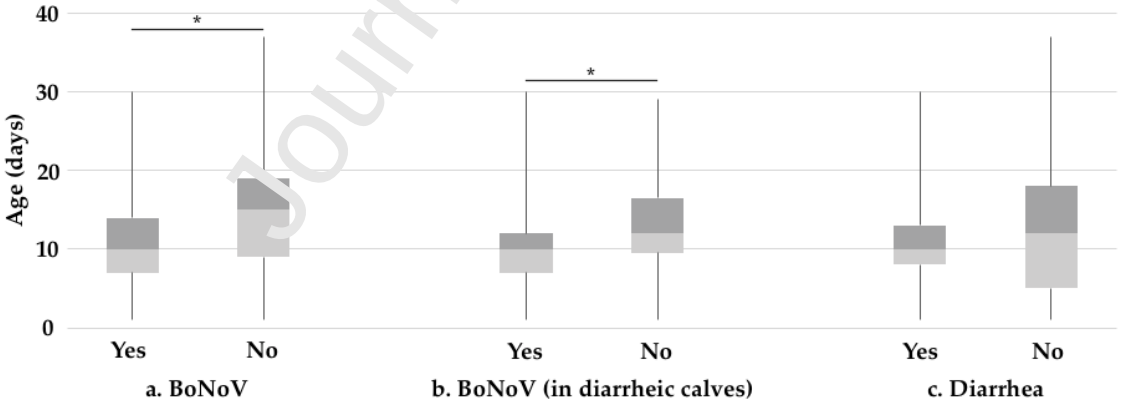

Figure 2. Box and whiskers plots of age (in days) with different outcomes are shown. a) Age in days of BoNoV-positive and BoNoV-negative calves. b) Age in days of BoNoV-positive and BoNoV-negative diarrheic calves. c) Age in days of diarrheic and non-diarheic calves. * indicate statistically significant differences ($p<0.05$).

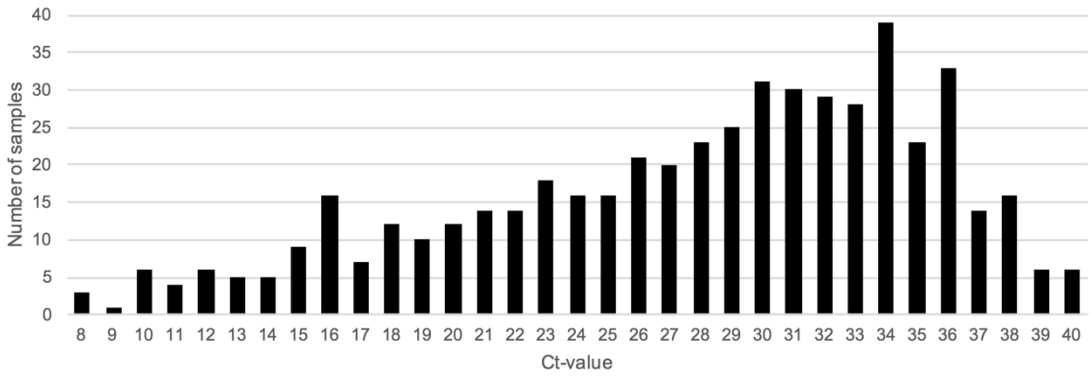

Figure 3. Distribution of Ct-values (rounded to nearest integer value) of the BoNoV-positive samples.

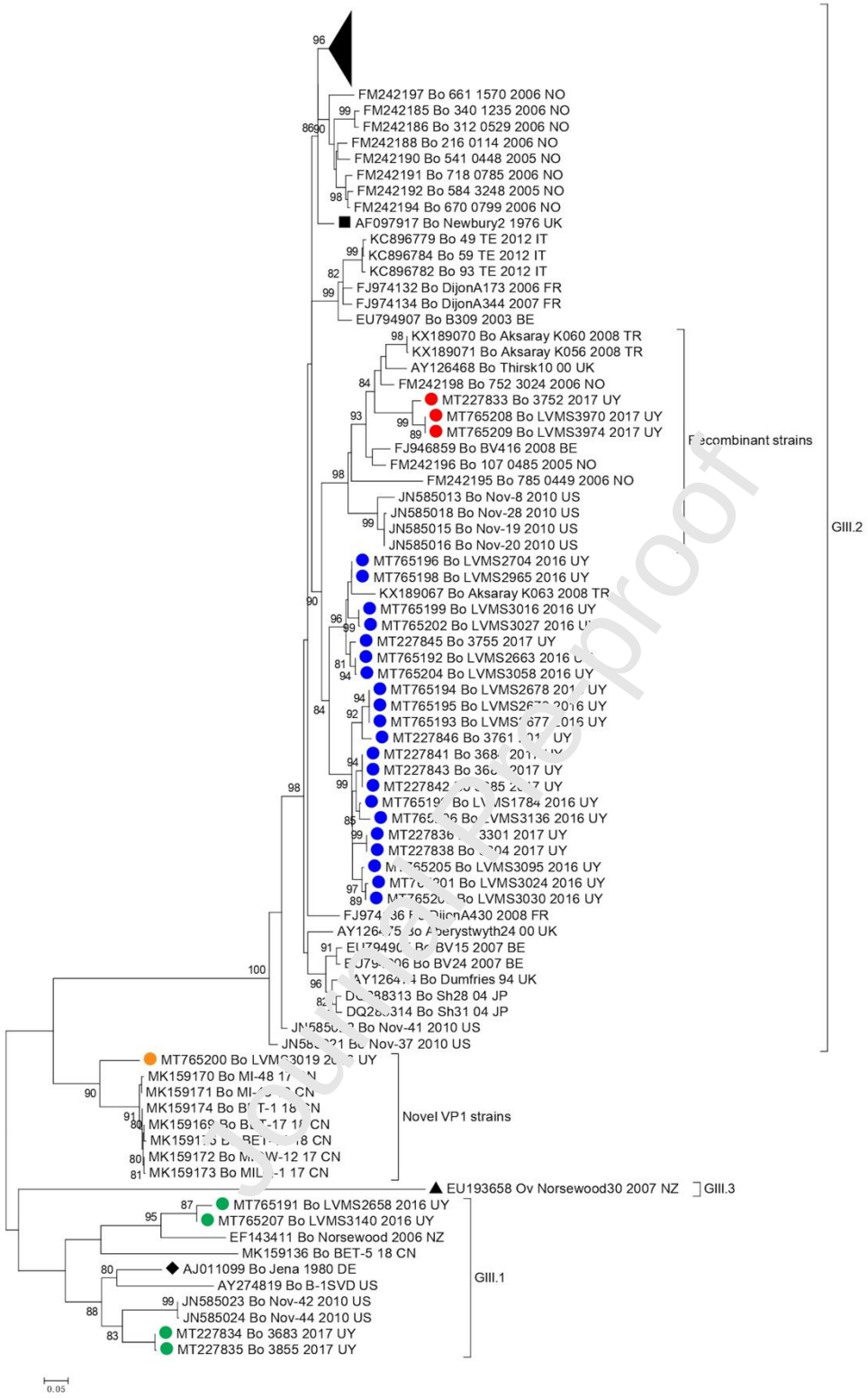

Figure 4. Maximum likelihood tree with the TNe+I+G4 model obtained with IQ-TREE. Reference strains: Jena (GIII.1, black rhombus), Newbury2 (GIII.2, black square), and Norsewood30 (GIII.3, black triangle). Uruguayan strains are indicated with green circle (GIII.1), blue circle (GIII.2), orange circle (novel VP1), and red circle (recombinants GIII.P1-GIII.2). aLRT values higher than 80 are shown.

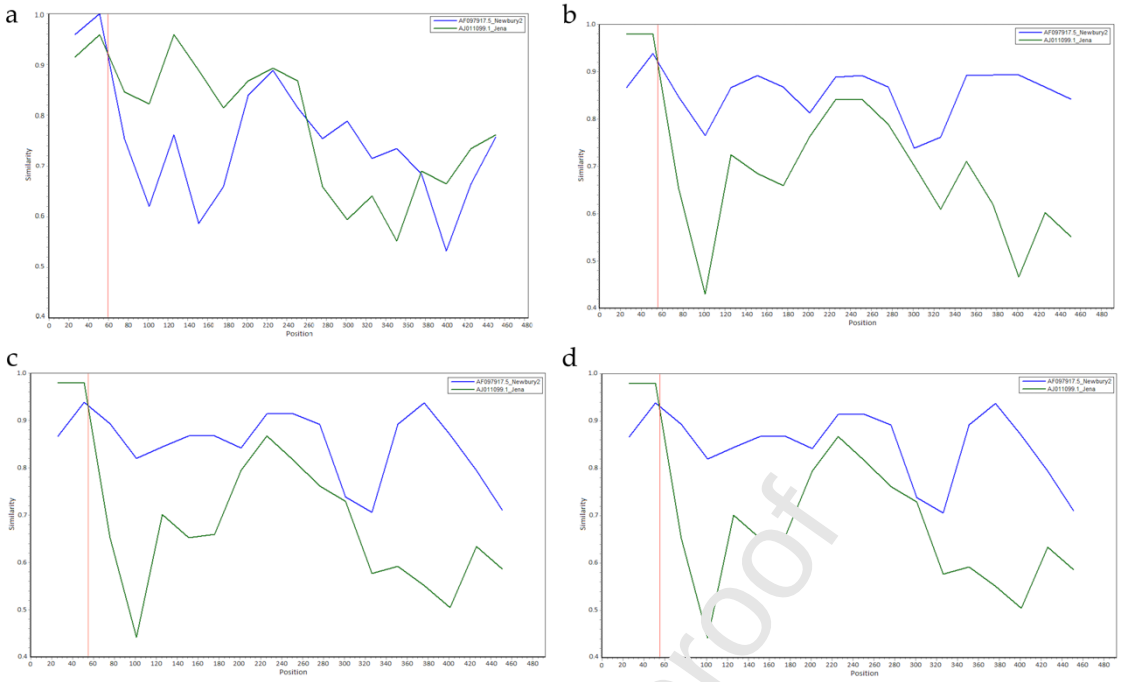

Figure S1. The nucleotide similarity plots for a) Bo/LVMS3019/2016/UY, b) Bo/LVMS3752/2017/UY, c) Bo/LVMS3970/2017/UY, and d) Bo/LVMS3974/2017/UY are shown. Jena and Newbury2 were used as reference strains for the confirmed genotypes GIII.1 and GIII.2, respectively. The recombination breakpoint is shown with a red line.

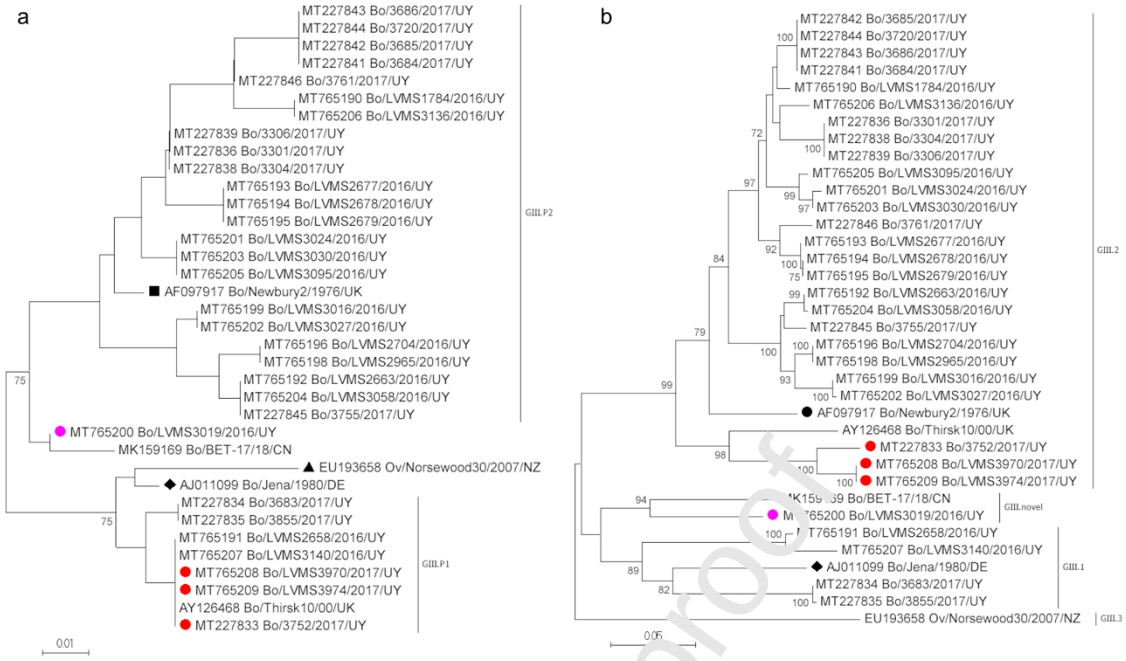

Figure S2. Neighbor joining trees with the K2+G model and 1000 bootstrap replicas were obtained with MEGA7. a) 3'end of RdRp and b) 5'end of VP1. Uruguayan recombinant strains are indicated with red and purple circles. Reference strains of GIII.1, GIII.2 and GIII.3 genotypes are indicated with black square, rhombus, and triangle, respectively.

Conflicts of interest: None

CRediT author statement: Matías Castells: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration, and Funding acquisition. **Rubén Darío Caffarena:** Resources. **María Laura Casaux:** Resources. **Carlos Schild:** Resources. **Felipe Castells:** Resources. **Daniel Castells:** Resources. **Matías Victoria:** Writing - Review & Editing, Supervision. **Franklin Riet-Correa:** Writing - Review & Editing, Supervision. **Federico Giannitti:** Resources, Writing - Review & Editing. **Viviana Parreño:** Writing - Review & Editing, Supervision. **Rodney Colina:** Conceptualization, Resources, Writing - Review & Editing, Supervision.

- Bovine norovirus GIII was detected in diarrheic and non-diarrheic calves
- Bovine norovirus GIII was detected more frequently in dairy than beef calves
- Calves less than 2 weeks of age were infected more often than older calves
- Four recombinant strains were detected
- A strain with a recently described VP1 genotype was detected