



VETERINARIA RIVISTA DI SANITÀ PUBBLICA VETERINARIA **ITALIANA**

Paper



Molecular detection and epidemiological features of picobirnavirus in sheep and goat herds from southern Brazil.

Nathalia Leontino Scherpinski¹, Joice Aparecida Andrade¹, Caroline do Nascimento Ferreira¹, Petronio Pinheiro Porto², Liza Ogawa², Francielle Gibson da Silva Zacarias², Elisabete Takiuchi^{1*}

¹Federal University of Paraná - BR

²State University of Northern Paraná - BR

*Corresponding author at: Federal University of Paraná - BR

E-mail: e.takiuchi@ufpr.br

Veterinaria Italiana, Vol. 62 No. 4 (2026) DOI: 10.12834/VetIt.4087.42979.2

Abstract

Picobirnaviruses (PBVs) are bisegmented double-stranded RNA viruses that have been detected in faecal samples from diverse hosts, but their epidemiology in small ruminants remains poorly understood. This study investigated PBV occurrence and epidemiological factors associated with detection in 405 animals (355 sheep and 50 goats) from nine herds in southern Brazil. Faecal samples were analysed by polyacrylamide gel electrophoresis (PAGE) and RT-PCR targeting PBV genogroup I (PBV-GI). PBV was detected in all nine herds and by either method in 173/405 (42.7%) animals, including 134/355 (37.7%) sheep and 39/50 (78.0%) goats. Epidemiological analyses were restricted to sheep. After mutual adjustment, faecal consistency was not associated with PBV detection (adjusted odds ratio [aOR] = 0.73, 95% CI: 0.44–1.19; $P = 0.207$), whereas farming system showed an overall association ($P = 0.012$), with higher odds in subsistence than extensive systems (aOR = 2.68, 95% CI: 1.39–5.21). Leave-one-herd-out analyses supported the stability of the faecal-consistency result but showed that the farming-system association was sensitive to herd composition. Sequencing of 15 selected RT-PCR amplicons confirmed their identity as PBV, and BLAST analysis revealed heterogeneous closest sequence matches. To our knowledge, this is the first multi-herd epidemiological investigation of PBV in small ruminants in Brazil, demonstrating widespread viral detection and providing new insights into factors associated with PBV detection in sheep.

Keywords

epidemiology, PAGE, RT-PCR, small ruminants

Introduction

Picobirnaviruses (PBVs) are bisegmented double-stranded RNA viruses belonging to the *Picobirnaviridae* family. Segment 1 (2.4–2.7 kb) encodes the capsid protein precursor, whereas segment 2 (1.7–1.9 kb) encodes the RNA-dependent RNA polymerase (RdRp), which is widely used for PBV detection and genogroup classification (Delmas et al., 2019). Based on RdRp sequence analysis, three major PBV genogroups (GI–GIII) have been recognised. GI and GII are predominantly associated with vertebrates, whereas GIII has been mainly identified in invertebrates (Boros et al., 2018; Malik et al., 2014; Perez et al., 2021; Ullah et al., 2022).

PBV-GI is the most frequently reported genogroup in animals and has been detected in faecal samples from a wide range of mammals and birds (Atasoy et al., 2022; Ganesh et al., 2011; Huaman et al., 2021; Hutton et al., 2026; Pereira et al., 2024; Qian et al., 2024; Takiuchi et al., 2016). Although PBV detection has been reported in association with diarrhoea and gastroenteritis (Ganesh et al., 2014; Malik et al., 2014; Qian et al., 2024), its occurrence in faecal samples from non-diarrhoeic animals is also well documented, indicating that the aetiological role of PBVs in enteric disease remains uncertain (Gallagher et al., 2017; Ganesh et al., 2014; Masachessi et al., 2015; Takiuchi et al., 2016).

Despite the broad detection of PBVs across animal species, molecular epidemiological studies specifically addressing

PBVs in sheep and goats remain scarce. Following an early incidental detection of PBV in a diarrhoeic lamb in Spain (Muñoz et al., 1996), only three molecular investigations in small ruminants have been reported worldwide (Karayel-Hacioglu et al., 2022; Kunz et al., 2018; Malik et al., 2018).

Given the limited epidemiological data available, additional investigations are warranted to improve our understanding of PBV circulation in small-ruminant populations. The present study investigated the occurrence of PBV in sheep and goat herds from the state of Paraná, southern Brazil. In sheep, we additionally evaluated whether faecal consistency and farming system were independently associated with PBV detection using multivariable analysis. Selected PBV-GI RT-PCR amplicons were also sequenced to confirm their identity and assess their nucleotide similarity to PBV sequences available in public databases.

Materials and methods

Herds and animals

The study was conducted between 2016 and 2021 in nine small ruminant herds (H1 to H9) located in different municipalities of Paraná State, southern Brazil. The animals were raised for meat production under semi-intensive, extensive, or subsistence farming systems. A total of 405 animals were sampled, comprising 355 sheep, predominantly Santa Inês × Dorper crossbreds, and 50 Boer goats. The characteristics of the herds included in the study are summarised in Table I.

Herd	Species	Production system	Animals sampled (n)	Municipality
H1	Sheep	Semi-intensive	83	Bandeirantes
H2	Sheep	Semi-intensive	74	Andirá
H3	Sheep and goats	Extensive	85 (73 sheep; 12 goats)	Santo Antônio da Platina
H4	Sheep	Extensive	36	Ribeirão do Pinhal
H5	Sheep	Subsistence	62	Santo Antônio da Platina
H6	Sheep	Subsistence	17	Barra do Jacaré
H7	Sheep	Semi-intensive	10	Santa Mariana
H8	Goats	Semi-intensive	30	Nova Santa Rosa
H9	Goats	Subsistence	8	Palotina

Table I. Characteristics of the small-ruminant herds included in the study, according to animal species, production system, number of animals sampled, and municipality of origin.

Faecal samples

Faecal samples were collected from all 405 animals and classified according to faecal consistency as pasty (n = 134) or solid (n = 259); this information was unavailable for 12 samples. Samples were prepared as 10% or 20% (w/v) suspensions in Tris-calcium buffer (0.01 M Tris-HCl; 1.5 mM CaCl₂; pH 7.3) for solid and pasty faeces, respectively, and centrifuged at 3,000 × g for 10 min. The resulting supernatants were used for viral nucleic acid extraction.

Nucleic Acid Extraction

Aliquots of the faecal suspensions were treated with 1% sodium dodecyl sulphate (SDS), incubated at 56 °C for 30 min, and subjected to a combined phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidine isothiocyanate extraction method, as described by Alfieri et al. (2006). The extracted nucleic acids were analysed by polyacrylamide gel electrophoresis (PAGE) and by RT-PCR for PBV-GI detection.

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE was performed using a 7.5% polyacrylamide gel, followed by silver nitrate staining to visualise the characteristic electrophoretic migration pattern of the two dsRNA genome segments of PBV (Herring et al., 1982; Pereira et al., 1988).

Detection of Picobirnavirus Genogroup I by RT-PCR

RT-PCR was performed using the primer pair PicoB25 (5'-TGG TGT GGA TGT TTC-3') and PicoB43 (5'-A(GA)T G(CT)T GGT CGA ACT T-3'), originally described by Rosen et al. (2000), which amplifies a 201 bp fragment of the RdRp gene of PBV genogroup I. The amplification conditions were as described by Takiuchi et al. (2016). Briefly, 6 µL of the extracted RNA was mixed with 2 µL of primer PicoB25 (20 pmol), heated at 94 °C for 5 min, and immediately chilled on ice for 5 min. Reverse transcription was performed in a 25 µL reaction containing 1× RT buffer, 0.1 mM of each dNTP, 120 U of M-MLV Reverse Transcriptase (Promega), and nuclease-free water. The reaction was incubated at 37 °C for 50 min, followed by enzyme inactivation at 70 °C for 5 min. PCR amplification was performed in a final volume of 50 µL containing 1× PCR buffer, 2 mM MgCl₂, 0.2 mM of each dNTP, 20 pmol each of primers PicoB25 and PicoB43, 2.5 U of Platinum Taq DNA Polymerase (Invitrogen), and 25 µL of the cDNA. Cycling conditions consisted of an initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. Amplification products were separated by electrophoresis in 2% agarose gel stained with ethidium bromide (0.5 µg/mL) and visualised under ultraviolet illumination.

Genetic Sequencing and Sequence Analysis

Fifteen PCR amplicons were selected for purification and subjected to bidirectional Sanger sequencing on an ABI 3500 Genetic Analyzer (Applied Biosystems), using the same forward and reverse primers employed for amplification. Sequence quality was assessed using Phred software, and nucleotide sequence identities were determined by BLASTn comparison with sequences available in the GenBank database. Because the amplified region comprised only a short fragment of the RdRp gene, sequence comparisons were used primarily to confirm PBV-GI identity and describe the closest nucleotide matches available in GenBank.

Statistical Analysis

Descriptive analyses were performed for all 405 animals, with PBV detection summarised according to species, herd, and detection method. A sample was considered PBV-positive when positive by PAGE and/or RT-PCR. Epidemiological analyses were restricted to sheep (n = 355) because the small number of goats (n = 50 from three herds) and their uneven distribution across the epidemiological variables precluded reliable multivariable analysis.

In sheep, associations of PBV detection with faecal consistency (solid or pasty) and farming system (extensive, semi-intensive, or subsistence) were evaluated using binary logistic regression. Solid faeces and extensive farming were the reference categories. The association between the two explanatory variables was assessed using Pearson's chi-square test. Univariable models were followed by a multivariable model including both predictors, and results are reported as crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs). Overall effects were assessed using likelihood-ratio tests, and pairwise comparisons among farming systems were performed using estimated marginal means with a Tukey adjustment.

Because farming system was defined at the herd level and only seven sheep herds were sampled, the robustness of the estimates was evaluated by a leave-one-herd-out sensitivity analysis, sequentially refitting the multivariable model after excluding each herd. Model adequacy was assessed by examining multicollinearity, residuals, leverage, and influential observations. Statistical significance was set at $P < 0.05$. Analyses were performed in R 4.6.1 using the emmeans and performance packages.

Results

PBV was detected in all nine investigated herds. Of the 405 samples analysed, six (1.5%) were positive exclusively by PAGE, 161 (39.8%) exclusively by RT-PCR, and six (1.5%) by both methods. Thus, 12/405 (3.0%) samples were PAGE-positive, 167/405 (41.2%) were RT-PCR-positive, and 173/405 (42.7%) were positive by at least one method. Overall PBV detection by either method was 134/355 (37.7%) in sheep and 39/50 (78.0%) in goats (Table II).

PBV-Positive samples					
Sheep herd	No. of animals evaluated	PAGE only, n (%)	RT-PCR only, n (%)	Both methods, n (%)	Total positive, n (%)
H1	83	5 (6.0%)	32 (38.6%)	5 (6.0%)	42 (50.6%)
H2	74	0	18 (24.3%)	0	18 (24.3%)
H3	73	0	18 (24.7%)	0	18 (24.7%)
H4	36	0	11 (30.6%)	0	11 (30.6%)
H5	62	1 (1.6%)	27 (43.5%)	0	28 (45.2%)
H6	17	0	14 (82.4%)	0	14 (82.4%)
H7	10	0	3 (30.0%)	0	3 (30.0%)
Total	355	6 (1.7%)	123 (34.6%)	5 (1.4%)	134 (37.7%)

PBV-Positive samples					
Goat herd	No. of animals evaluated	PAGE only, n (%)	RT-PCR only, n (%)	Both methods, n (%)	Total positive, n (%)
H3	12	0	10 (83.3%)	1 (8.3%)	11 (91.7%)
H8	30	0	23 (76.7%)	0	23 (76.7%)
H9	8	0	5 (62.5%)	0	5 (62.5%)
Total	50	0	38 (76.0%)	1 (2.0%)	39 (78.0%)

Table II. Detection of picobirnavirus by PAGE and RT-PCR in sheep and goat herds. Note: "PAGE only" and "RT-PCR only" indicate samples positive exclusively by the respective method; "Both methods" indicates samples positive by both PAGE and RT-PCR; and "Total positive" indicates detection by at least one method. Percentages were calculated based on the number of samples analysed in each herd po

In sheep, PBV was detected in 95/222 (42.8%) samples with solid faeces and 39/133 (29.3%) samples with pasty faeces. According to farming system, detection frequencies were 29/109 (26.6%) in extensive, 63/167 (37.7%) in semi-intensive, and 42/79 (53.2%) in subsistence systems. Faecal consistency and farming system were associated with each other (Pearson $\chi^2 = 52.92$, $df = 2$, $P < 0.001$), supporting their joint evaluation in the multivariable model.

In univariable analysis, pasty faeces were associated with lower odds of PBV detection than solid faeces (OR = 0.55, 95% CI 0.35–0.87; global likelihood-ratio $P = 0.011$). After adjustment for farming system, this association was attenuated and no longer supported statistically (aOR = 0.73, 95% CI 0.44–1.19; global $P = 0.207$). Farming system remained associated with PBV detection in the adjusted model (global $P = 0.012$). Compared with extensive farming, the adjusted odds were 1.53 (95% CI 0.89–2.66) for semi-intensive farming and 2.68 (95% CI 1.39–5.21) for subsistence farming (Table III). Tukey-adjusted pairwise comparisons indicated a difference between subsistence and extensive systems ($P = 0.009$), whereas the comparisons between semi-intensive and extensive ($P = 0.281$) and between semi-intensive and subsistence ($P = 0.114$) were not statistically significant.

Leave-one-herd-out (LOHO) sensitivity analyses showed that the adjusted association between faecal consistency and PBV detection remained non-significant regardless of the herd excluded. In contrast, estimates for farming system

varied according to herd composition. Exclusion of H6 attenuated the association between subsistence and extensive farming, with the aOR decreasing from 2.68 to 1.91 and the 95% CI including unity, whereas exclusion of H5 markedly increased the corresponding estimate.

Variable	Category	Positive, n/N (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude global P	Adjusted global P
Faecal consistency	Solid	95/222 (42.8)	Reference	Reference	0.011	0.207
	Pasty	39/133 (29.3)	0.55 (0.35–0.87)	0.73 (0.44–1.19)		
Farming system	Extensive ^a	29/109 (26.6)	Reference	Reference	0.001	0.012
	Semi-intensive ^{ab}	63/167 (37.7)	1.67 (0.99–2.86)	1.53 (0.89–2.66)		
	Subsistence ^b	42/79 (53.2)	3.13 (1.71–5.83)	2.68 (1.39–5.21)		

Table III. Univariable and multivariable logistic regression analyses of factors associated with PBV detection by PAGE and/or RT-PCR in sheep. Note: Overall P values are from likelihood-ratio tests. Adjusted ORs were obtained from the multivariable model including faecal consistency and farming system simultaneously. Superscript letters indicate Tukey-adjusted pairwise comparisons among farming systems; categories sharing a letter did not differ significantly. OR, odds ratio; CI, confidence interval.

Sequencing confirmed all 15 selected RT-PCR amplicons as PBV. Fourteen sequences showed their closest BLAST matches to PBVs previously detected in other host species, including pigs, humans, foxes, cattle, and birds, whereas only sequence H8-01 showed the highest nucleotide identity to an ovine PBV sequence (Supplementary Table I).

Discussion

The present study expands the currently limited epidemiological knowledge regarding PBV occurrence in small ruminants by demonstrating widespread detection across sheep and goat herds from southern Brazil. PBV was detected in all investigated herds, with overall detection rates of 37.7% in sheep and 78.0% in goats. The higher detection rate obtained by RT-PCR than by PAGE is consistent with the greater analytical sensitivity of molecular amplification methods. Similar findings have been reported in previous studies comparing these diagnostic approaches for PBV detection in different host species, despite differences in sampling strategies and laboratory protocols. Across these studies, positivity rates ranged from 4.6% to 27.0% for PAGE and from 11.2% to 62.0% for RT-PCR (Carruyo et al., 2008; Kunz et al., 2018; Kylla et al., 2017; Reddy et al., 2023; Ribeiro Silva et al., 2014).

Six sheep samples displaying a PBV-compatible electrophoretic profile were not amplified by RT-PCR (Table II). This finding may reflect the extensive genetic diversity of PBVs and the dependence of RT-PCR on sequence complementarity between viral templates and primers (Hutton et al., 2026). The PicoB25/PicoB43 primer pair targets a short region of the RdRp gene of PBV-GI and may therefore fail to amplify highly divergent strains or PBVs belonging to other genogroups. Alternatively, RNA degradation or the presence of PCR inhibitors commonly found in faecal samples may also have contributed to the lack of RT-PCR amplification. In contrast, PAGE enables primer-independent visualisation of bisegmented dsRNA genomes when present at detectable concentrations (Kunz et al., 2018). Overall, the limited overlap between the results obtained by PAGE and RT-PCR indicates that the two techniques provide complementary information, and their combined application may provide a more comprehensive assessment of PBV occurrence by compensating for the inherent limitations of each technique.

Few epidemiological studies have investigated PBV infection in sheep and goats worldwide. The first report originated from an incidental finding during a survey of enteric pathogens involving 230 sheep and 135 goats in Spain, in which PBV was detected by PAGE in only a single faecal sample obtained from a lamb (Muñoz et al., 1996). Despite the passage of nearly three decades since this first description, epidemiological data in small ruminants remain scarce. To date, only three studies specifically investigating PBV in sheep and/or goats have been published (Karayel-Hacioglu et al., 2022; Kunz et al., 2018; Malik et al., 2018).

Kunz et al. (2018) provided the first evidence of PBV in sheep from Brazil, detecting the virus by RT-PCR in 62.0% (62/100) of faecal samples collected from a single meat sheep flock. The study demonstrated the circulation of PBV-GI within a single herd, with positive samples identified across all production categories, including lambs, ewes, rams, breeding females, and debilitated animals destined for culling.

The first comprehensive epidemiological study of PBV in sheep and goats was conducted by Malik et al. (2018) in India. Among the 400 animals evaluated (196 goats and 204 sheep), PBV was detected in 35.8% of them, comprising 83 goats and 60 sheep. More recently, Karayel-Hacioglu et al. (2022) expanded previous investigations by screening

sheep and goats for both PBV GI and GII. However, the study included only 66 diarrhoeic animals from four herds, and PBV was detected in only four sheep, with all detected viruses belonging to genogroup I. The present study expands the findings of Kunz et al. (2018), whose study was also conducted in the state of Paraná, Brazil, by including multiple sheep and goat herds from different municipalities across the state. Together with the study by Malik et al. (2018), it represents one of the largest epidemiological investigations of PBV in small ruminants reported to date and broadens the current understanding of PBV circulation across different populations, production systems, and geographic regions in southern Brazil.

Although PBV detection was higher in goats (78.0%) than in sheep (37.7%), this difference should be interpreted descriptively rather than as evidence of a species effect. Only 50 goats from three herds were included, compared with 355 sheep from seven herds, and the distribution of goats across the epidemiological variables was markedly unbalanced. Therefore, goats were retained in the descriptive assessment of PBV occurrence but were not included in the multivariable epidemiological analysis.

In sheep, PBV detection was more frequent in solid than in pasty faeces in the univariable analysis, consistent with the findings of Kunz et al. (2018), who also observed a higher frequency of PBV detection in sheep with solid faeces. However, faecal consistency and farming system were associated with each other, and the association between faecal consistency and PBV detection was attenuated after adjustment for farming system and was no longer statistically supported. This result remained stable throughout the leave-one-herd-out (LOHO) analyses, indicating that the crude association did not represent an independent relationship with PBV detection. These findings further support the evidence that PBV detection is not consistently associated with altered faecal consistency. Moreover, faecal consistency alone should not be regarded as equivalent to clinical health status, and the present results do not provide evidence either for or against a causal role of PBV in diarrhoea.

PBV detection in sheep also differed according to farming system. In the multivariable model, farming system remained associated with PBV detection, with higher adjusted odds in subsistence than in extensive systems. To our knowledge, the association between PBV detection and farming system has not previously been evaluated in small ruminants. However, given the limited number of sheep herds and the herd-level definition of farming system, the observed association may partly reflect herd-specific characteristics rather than farming system alone, as supported by the leave-one-herd-out (LOHO) sensitivity analyses. Notably, H6, a small subsistence flock, showed the highest PBV detection frequency among sheep herds (14/17; 82.4%), and its exclusion attenuated the association between subsistence and extensive farming. Farming system may therefore represent a broader set of unmeasured herd-level characteristics, including differences in animal management, biosecurity practices, animal contact patterns, health status, herd composition, or sampling period, which may have contributed to the observed variation. These factors were not evaluated individually in the present study. Accordingly, the observed association should not be interpreted as evidence that subsistence farming itself increases PBV occurrence, and studies including larger numbers of independently sampled herds within each farming system are needed to clarify this relationship.

In addition, individual information on age, sex, and production category was not systematically available, preventing assessment of their potential association with PBV detection. This is relevant because Kunz et al. (2018) previously detected PBV across different production categories within a sheep flock.

To further characterise the RT-PCR products, selected amplicons were sequenced and compared with PBV sequences available in GenBank. The predominance of closest BLAST matches to PBVs previously detected in other host species should be interpreted with caution (Supplementary Table I). Because the sequences analysed were derived from a short 201 bp fragment of the RdRp gene, they were used primarily to confirm the identity of the amplified products as PBV rather than to infer phylogenetic relationships or host specificity. Indeed, the approximately 200 nt RdRp region has been shown to provide limited resolution for reliable phylogenetic classification of PBVs (Perez et al., 2021). In addition, the limited number of PBV sequences from small ruminants available in public databases further constrains the assessment of genetic diversity and limits comprehensive comparative analyses. Despite these limitations, previous studies have demonstrated marked genetic heterogeneity among PBVs detected in small ruminants. Malik et al. (2018) described highly heterogeneous PBV strains detected in sheep and goats from India. Likewise, Karayel-Hacioglu et al. (2022) reported that PBVs detected in sheep from Turkey exhibited greater nucleotide identity to sequences derived from swine, equines, and primates than to previously described ovine PBVs.

Importantly, these cross-host sequence similarities should not be interpreted as evidence of host origin, interspecies transmission, or zoonotic potential. Similar sequences recovered from different animal species may reflect the extensive genetic diversity of PBVs, limitations in the representation of host-associated sequences in public databases, or shared environmental sources of exposure or contamination (Wang, 2022; Hutton et al., 2026). More broadly, the biological host range of PBVs remains unresolved. Although PBVs have long been detected in animal-

associated samples, evidence establishing vertebrates as their true cellular hosts remains limited. Genomic features such as conserved prokaryotic ribosome-binding sites have raised the possibility that at least some PBVs may have prokaryotic hosts (Boros et al., 2018; Wang, 2022), while PBV-like genomes using alternative genetic codes have also suggested possible fungal hosts (Ullah et al., 2022; Wang, 2022; Hutton et al., 2026). Consequently, the animal species from which a PBV sequence is recovered should not necessarily be regarded as its true biological host.

Conclusion

This study provides new epidemiological data on PBV occurrence in small ruminants from southern Brazil, demonstrating its detection in both sheep and goats across all herds investigated. In sheep, multivariable analysis indicated that farming system, but not faecal consistency, was associated with PBV detection, although this association was sensitive to herd composition. To our knowledge, this is the first multi-herd epidemiological investigation of PBV involving both sheep and goats in Brazil, providing a broader perspective on PBV detection in small-ruminant populations in the country. Further studies involving larger numbers of independent herds and longer genomic sequences are warranted to better characterise PBV epidemiology, genetic diversity, evolutionary relationships, and host associations in small ruminants.

Ethical approval

The study was conducted in accordance with the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and was approved by the Ethics Committee on the Use of Animals of the Universidade Federal do Paraná under protocol number 33/2016.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualisation: ET; Methodology: ET; Formal analysis: ET, FGSZ, LO, PPP; Investigation: NLS, CNF, PPP, LO, FGSZ; Writing original draft preparation: NLS, JAA; Writing, review and editing: ET, JAA; Visualisation: NLS, JAA, LO, FGSZ; Supervision: ET; Project administration: ET, PPP; Funding acquisition: ET, PPP. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors gratefully acknowledge André Lucio Fontana Goetten, from the Universidade Federal de Santa Catarina (UFSC), Brazil, for his valuable contribution to the statistical analyses.

Funding

N.L.S. and J.A.A. acknowledge the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES/MEC) for the scholarship granted.

References

Alfieri, A. A., Parazzi, M. E., Takiuchi, E., Médici, K. C., & Alfieri, A. F. (2006). Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998-2002. *Tropical animal health and production*, 38(7-8), 521–526. <https://doi.org/10.1007/s11250-006-4349-9>.

Atasoy, M. O., Isidan, H., & Turan, T. (2022). Genetic diversity, frequency and concurrent infections of

picobirnaviruses in diarrhoeic calves in Turkey. *Tropical animal health and production*, 54(2), 127.
<https://doi.org/10.1007/s11250-022-03128-4>.

Boros, Á., Polgár, B., Pankovics, P., Fenyvesi, H., Engelmann, P., Phan, T. G., Delwart, E., & Reuter, G. (2018). Multiple divergent picobirnaviruses with functional prokaryotic Shine-Dalgarno ribosome binding sites present in cloacal sample of a diarrheic chicken. *Virology*, 525, 62–72. <https://doi.org/10.1016/j.virol.2018.09.008>.

Carruyo, G. M., Mateu, G., Martínez, L. C., Pujol, F. H., Nates, S. V., Liprandi, F., & Ludert, J. E. (2008). Molecular characterization of porcine picobirnaviruses and development of a specific reverse transcription-PCR assay. *Journal of clinical microbiology*, 46(7), 2402–2405. <https://doi.org/10.1128/JCM.00655-08>.

Delmas, B., Attoui, H., Ghosh, S., Malik, Y. S., Mundt, E., Vakharia, V. N., & ICTV Report Consortium. (2019). ICTV Virus Taxonomy Profile: Picobirnaviridae. *Journal of general virology*, 100(2), 133–134.
<https://doi.org/10.1099/jgv.0.001186>.

Gallagher, C.A., Navarro, R., Cruz, K., Aung, M.S., Ng, A., Bajak, E., Beierschmitt, A., Lawrence, M., Dore, K. M., Ketzis, J., Malik, Y. S., Kobayashi, N., & Ghosh, S. (2017). Detection of picobirnaviruses in vervet monkeys (*Chlorocebus sabaeus*): molecular characterization of complete genomic segment-2. *Virus research*, 230, 13–18.
<https://doi.org/10.1016/j.virusres.2016.12.021>.

Ganesh, B., Banyai, K., Masachessi, G., Mladenova, Z., Nagashima, S., Ghosh, S., Nataraju, S. M., Pativada, M., Kumar, R., & Kobayashi, N. (2011). Genogroup I picobirnavirus in diarrhoeic foals: Can the horse serve as a natural reservoir for human infection? *Veterinary research*, 42, 52. <https://doi.org/10.1186/1297-9716-42-52>.

Ganesh, B., Masachessi, G., & Mladenova, Z. (2014). Animal picobirnavirus. *Virus disease*, 25(2), 223–238.
<https://doi.org/10.1007/s13337-014-0207-y>.

Herring, A. J., Inglis, N. F., Ojeh, C. K., Snodgrass, D. R., & Menzies, J. D. (1982). Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *Journal of clinical microbiology*, 16(3), 473–477. <https://doi.org/10.1128/jcm.16.3.473-477.1982>.

Huaman, J. L., Pacioni, C., Sarker, S., Doyle, M., Forsyth, D. M., Pople, A., Hampton, J. O., Carvalho, T. G., & Helbig, K. J. (2021). Molecular epidemiology and characterization of picobirnavirus in wild deer and cattle from Australia: Evidence of genogroup I and II in the upper respiratory tract. *Viruses*, 13(8), Article 1492.
<https://doi.org/10.3390/v13081492>.

Hutton, A. L. K., Grigson, S., Bartle, L., Papudeshi, B., Mallawaarachchi, V., Tarasenko, A., Mitchell, J. G., & Edwards, R. A. (2026). Picobirnavirus: how do you find where it's hiding? *Critical reviews in microbiology*, 52(2), 339–349.
<https://doi.org/10.1080/1040841X.2025.2560918>.

Karayel-Hacioglu, I., Gul, B., & Alkan, F. (2022). Molecular characterization of picobirnaviruses in small ruminants with diarrhea in Turkey. *Virus genes*, 58(3), 238–243. <https://doi.org/10.1007/s11262-022-01894-9>.

Kunz, A. F., Possatti, F., de Freitas, J. A., Alfieri, A. A., & Takiuchi, E. (2018). High detection rate and genetic diversity of picobirnavirus in a sheep flock in Brazil. *Virus research*, 255, 10–13. <https://doi.org/10.1016/j.virusres.2018.06.016>.

Kylla, H., Dutta, T. K., Roychoudhury, P., Malik, Y. S., Mandakini, R., & Subudhi, P. K. (2017). Prevalence and molecular characterization of porcine Picobirnavirus in piglets of North East Region of India. *Tropical animal health and production*, 49(2), 417–422. <https://doi.org/10.1007/s11250-016-1210-7>.

Malik, Y. S., Kumar, N., Sharma, K., Dhama, K., Shabbir, M. Z., Ganesh, B., Kobayashi, N., & Banyai, K. (2014). Epidemiology, phylogeny, and evolution of emerging enteric Picobirnaviruses of animal origin and their relationship to human strains. *BioMed research international*, 2014, Article 780752. <https://doi.org/10.1155/2014/780752>.

Malik, Y. S., Sircar, S., Dhama, K., Singh, R., Ghosh, S., Bányai, K., Vlasova, A. N., Nadia, T., & Singh, R. K. (2018). Molecular epidemiology and characterization of picobirnaviruses in small ruminant populations in India. *Infection, genetics and evolution*, 63, 39–42. <https://doi.org/10.1016/j.meegid.2018.05.011>.

- Masachessi, G., Ganesh, B., Martinez, L. C., Giordano, M. O., Barril, P. A., Isa, M. B., Paván, G. V., Mateos, C. A., & Nates, S. V. (2015). Maintenance of picobirnavirus (PBV) infection in an adult orangutan (*Pongo pygmaeus*) and genetic diversity of excreted viral strains during a three-year period. *Infection, genetics and evolution*, 29, 196–202. <https://doi.org/10.1016/j.meegid.2014.11.019>.
- Muñoz, M., Alvarez, M., Lanza, I., & Cármenes, P. (1996). Role of enteric pathogens in the aetiology of neonatal diarrhoea in lambs and goat kids in Spain. *Epidemiology and infection*, 117(1), 203–211. <https://doi.org/10.1017/s0950268800001321>.
- Pereira, D., Souto, L. C. S., Guerra, S. F. S., Penha-Júnior, E. T., Lobo, P. S., Soares, L. S., Pinheiro, H. H. C., Chagas, E. H. N., Guimarães, R. J. P. S., Ramos, B. A., Chagas, L. L., Freitas, M. N. O., Furtado, E. C. S., Rodrigues, J. C. P., Casseb, A. R., Martins, L. C., & Mascarenhas, J. D. P. (2024). Occurrence of avian reovirus and picobirnavirus in wild birds from an environmental protection area in the Brazilian Amazon. *Acta Amazonica*, 54(1), Article e54hs23012. <https://doi.org/10.1590/1809-4392202300120>.
- Pereira, H. G., Flewett, T. H., Candeias, J. A. N., & Barth, O. M. (1988). A virus with a bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*) intestines. *Journal of general virology*, 69(11), 2749–2754. <https://doi.org/10.1099/0022-1317-69-11-2749>.
- Perez, L. J., Cloherty, G. A., & Berg, M. G. (2021). Understanding the Genetic Diversity of Picobirnavirus: A Classification Update Based on Phylogenetic and Pairwise Sequence Comparison Approaches. *Viruses*, 13(8), 1476. <https://doi.org/10.3390/v13081476>.
- Qian, L., Zhuang, Z., Lu, J., Wang, H., Wang, X., Yang, S., Ji, L., Shen, Q., Zhang, W., & Shan, T. (2024). Metagenomic survey of viral diversity obtained from feces of piglets with diarrhea. *Heliyon*, 10(4), e25616. <https://doi.org/10.1016/j.heliyon.2024.e25616>.
- Reddy, M. V., Gupta, V., Nayak, A., & Tiwari, S. P. (2023). Picobirnaviruses in animals: a review. *Molecular biology reports*, 50(2), 1785–1797. <https://doi.org/10.1007/s11033-022-08133-2>.
- Ribeiro Silva, R., Bezerra, D. A. M., Kaiano, J. H. L., Oliveira, D. S., Silvestre, R. V. D., Gabbay, Y. B., Ganesh, B., & Mascarenhas, J. D. P. (2014). Genogroup I avian picobirnavirus detected in Brazilian broiler chickens: a molecular epidemiology study. *Journal of general virology*, 95(Pt 1), 117–122. <https://doi.org/10.1099/vir.0.054783-0>.
- Rosen, B. I., Fang, Z. Y., Glass, R. I., & Monroe, S. S. (2000). Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. *Virology*, 277(2), 316–329. <https://doi.org/10.1006/viro.2000.0594>.
- Takiuchi, E., Macedo, R., Kunz, A. F., Gallego, J. C., de Mello, J. L., Otonel, R. A. A., & Alfieri, A. A. (2016). Electrophoretic RNA genomic profiles of Brazilian Picobirnavirus (PBV) strains and molecular characterization of a PBV isolated from diarrheic calf. *Virus research*, 211, 58–63. <https://doi.org/10.1016/j.virusres.2015.09.022>.
- Ullah, K., Mehmood, A., Chen, X., Dar, M. A., Yang, S., & Zhang, W. (2022). Detection and molecular characterization of picobirnaviruses in the wild birds: Identification of a novel picobirnavirus possessing yeast mitochondrial genetic code. *Virus research*, 308, Article 198624. <https://doi.org/10.1016/j.virusres.2021.198624>.
- Wang, D. (2022). The enigma of picobirnaviruses: viruses of animals, fungi, or bacteria? *Current Opinion in Virology*, 54, 101232. <https://doi.org/10.1016/j.coviro.2022.101232>.