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Detection and Molecular Analysis of Picobirnavirus and Rotavirus from Animal Faecal Samples

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ABSTRACT

Background: Picobirnavirus (PBV) is an enteric virus with potential for interspecies transmission, affecting various animal species and possibly humans. This study aimed to investigate the prevalence and molecular characterization of PBV in faecal samples from various animal species in Jabalpur, Madhya Pradesh, India. Diarrhoeic and non-diarrhoeic samples from calves (cattle and buffalo), goats, pigs, dogs and poultry were collected for analysis.

Methods: A total of 150 faecal samples, including diarrhoeic and non-diarrhoeic samples, were collected from calves (cattle and buffalo), goats, pigs, dogs and poultry. Faecal suspensions were prepared and RNA extraction was performed. Both RNA-PAGE (Polyacrylamide Gel Electrophoresis) and PCR techniques were employed to detect PBV, with PCR also used for Rotavirus detection. Various primer sets targeting the RdRp gene of segment 2 were utilized for PBV detection, while specific primers were applied for Rotavirus detection. Sequencing and phylogenetic analysis were conducted on selected samples to characterize viral genetic similarity.

Result: Among the 150 faecal samples, 4.0% (6/150) were positive for PBV by RNA-PAGE, with three buffalo and three dog samples displaying a three-band pattern. RT-PCR using different primer sets revealed a 7.33% (11/150) prevalence of PBV, with buffalo and goats showing the highest rates. Co-infection of PBV and Rotavirus was observed in some samples, suggesting possible interactions between these viruses. Nucleotide identities of the goat isolate S14 (GenBank OP866965) with other PBV sequences in GenBank ranged from 93.40% to 95.73%.

Key words: Interspecies transmission, Picobirnavirus, RNA-PAGE, Rotavirus, Three-band pattern.

INTRODUCTION

The high rates of morbidity and mortality, the reduction in growth and the treatment of neonatal diarrhoea cause substantial economic losses in both animals and humans. Moreover, diarrhoeic animals may harbour, incubate and act as a source of infection to healthy animals and humans (Holland, 2014). Neonatal diarrhoea is caused by a variety of infectious agents, including viruses, bacteria and protozoa. Acute gastroenteritis is caused by viruses such as enteric Coronaviruses, Bocavirus, Kobuvirus, Rotavirus as well as later recognized viruses such as Picobirnavirus (PBV) (Kattoor *et al.*, 2016). PBVs are genetically distinct, rapidly evolving and spreading worldwide. PBVs may have ambiguous clinical implications, but they may pose a public health risk (Kumar *et al.*, 2020). Genetic relatedness between human and animal PBV warrants a thorough investigation of the potential for zoonotic transmission. As a result, continual surveillance is required to detect the emergence of new PBVs in a different host and to determine their origin, diversity and public health implications (Malik *et al.*, 2018).

This study was designed to molecularly characterize Picobirnavirus (PBV) in fecal samples collected from various animal species in and around Jabalpur. RNA-PAGE (Polyacrylamide Gel Electrophoresis) and PCR techniques were employed for the detection of PBV, followed by molecular characterization and phylogenetic analysis on selected samples. Both PBV and rotavirus were simult

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aneously screened from the clinical samples, with rotavirus detected in some cases.

MATERIALS AND METHODS

A total of 150 faecal samples (diarrhoeic and non diarrhoeic) from calves (cattle and buffalo aged between 1-90 days), goat, pig, dog aged between 1-6 month and poultry (broiler) aged between 1-45 days were collected from different farms located in Jabalpur, Madhya Pradesh (subtropical part of India) for virus detection and all the experimental procedures were conducted in the Department of Veterinary Microbiology, Nanaji Deshmukh Veterinary Science University in the year 2021-2023. A 10% faecal suspension was prepared with phosphate buffered saline (PBS, pH 7.2) (Himedia) by dissolving one gram of faeces in 10 ml PBS. Centrifugation was done after thorough vortexing at 7500 rpm for 20 min to remove the coarse debris; the supernatant was used for RNA extraction. The viral RNA was extracted according to procedure described by Boom *et al.* (1990) with slight modifications. RNA-PAGE was used to detect PBV and Rotavirus by observing 2 and 11 genome segments, respectively and their migration pattern in all faecal samples. For electrophoresis, resolving gel (7.5%) was prepared as per the method of Laemmli (1970). The viral genomic electrophoresis was carried out according to Malik *et al.* (2011). Silver staining of the gel was carried out as described by Sevenson *et al.* (1986) and documented. cDNA synthesis was done using PrimeScript™ 1st strand cDNA synthesis kit (Takara) as per manufacturer's instructions. The cDNA was used as a template for PCR to detect both PBV and Rotavirus. Primer details and conditions used for the PCR amplification of viral RNA from the fecal samples are presented in Table 1.

PCR amplification was conducted using a GeneAmp PCR System 9700 (Applied Biosystems), followed by gel electrophoresis. Two representative samples (bovine and goat) were sequenced at Eurofins Genomics India Pvt. Ltd., using Sanger sequencing.

Sequence and phylogenetic analysis

Amplicon size of 201 bp PCR product of RdRp gene (PBV) of one isolate were subjected to sequencing. Amplified RdRp gene sequence was analyzed for evolutionary lineages with various global isolates of PBV obtained from NCBI database. The sequences were analyzed using BLAST

Table 1: PCR primer details and conditions.

Target Gene	Genogroup	Primer Set	Sequence	PCR Product Size (bp)	PCR Mixture Components (25.0 µl)	Reference
RdRp (PBV)	Genogroup I	PicoB25+/ PicoB43-	TGGTGTGGATGTTTC/ A(GA)TG(CT)TGGTCG AACTT	201	12.5 µl 2X PCR Master Mix, 1.5 µl Forward Primer (10 pM), 1.5 µl Reverse Primer (10 pM), 4.0 µl c DNA, 5.5 µl Nuclease-Free Water	Rosen *et al.* (2000)
RdRp (PBV)	Genogroup II	Pico23+ / Pico24-	CGGTATGGATGTTTC/ AAGCGAGCCCATGTA	369	12.5 µl 2X PCR Master Mix, 1.5 µl Forward Primer (10 pM), 1.5 µl Reverse Primer (10 pM), 4.0 µl cDNA, 5.5 µl Nuclease-Free Water	Rosen *et al.* (2000)
RdRp (PBV)	Genogroup I	PBV 7F/ PBV 7R	GCNTGGGTTAGCATGGA/ CAYGGNATGGSATSBGG	275	10.0 µl 2X PCR Master Mix, 1.5 µl Forward Primer (10 pM), 1.5 µl Reverse Primer (10 pM), 4.0 µl cDNA, 3.0 µl Nuclease-Free Water	Malik *et al.* (2017)
RdRp (PBV)	-	PBV F1143/ PBV R1468	AACCCAAATTCACAGTG TCTTGG/ AGAGGATGGTACTTCA CATTCTC	326	12.5 µl 2X PCR Master Mix, 1.5 µl Forward Primer (10 pM), 1.5 µl Reverse Primer (10 pM), 4.0 µl cDNA, 5.5 µl Nuclease- Free Water	Kuhar and Jamnikar-Ciglenecki (2019)
VP6 (Rotavirus)	-	GARV-D-VP6-F/ GARV-F-VP6-R	TTTGATCACTAAYTAT TCACC/ GGTCACATCCTC TCACTA	227	12.5 µl 2X PCR Master Mix, 1.0 µl Forward Primer (20 pM),1.0 µl Reverse Primer (20 pM), 3.0 µl cDNA, 1.0 µl DMSO, 6.5 µl Nuclease-Free Water	Gupta *et al.* (2021)

(Basic local Alignment Search Tool) and the Clustal-W (CLUSTAL 2.1 multiple sequence alignment) to generate sequence alignment reports. CLUSTAL O program (version 1.2.4) was used to compare nucleotide sequence with Other cognate PBVs and percent identity matrix was created using CLUSTAL 2.1. A BLASTn search confirmed that the sequence of positive isolate was similar to cognate PBVs from GenBank, so the phylogenetic analysis included these sequences. Representative strains and outgroup sequences used were 1-CHN-97 (AF246939) and 4-GA-91 (AF246940) belonging to GI and GII, respectively. Molecular Evolutionary Genetic Analysis (MEGA 6) version was used for construction of phylogenetic tree. The bootstrapped phylogenetic tree was constructed using Maximum-likelihood method and significant bootstrap values with 1000 replicates.

Nucleotide sequence accession numbers

Accession number of the sequence submitted at NCBI for S14 sample (PBV/Goat/India/S14/2021) is OP866965.

RESULTS AND DISCUSSION

In our analysis, we observed species-specific variations in the detection of PBV by RNA-PAGE, as detailed in Table 2. This table outlines the number of samples tested per species, the detection rates of PBV and the presence of VP6 bands in both diarrhoeic and non-diarrhoeic samples.

Among the 25 samples collected from buffalo, 4 were diarrhoeic, with PBV detected by RNA-PAGE in 3 of these diarrhoeic samples (75%), which notably showed two bands on the gel (Fig 1), resulting in an overall PBV prevalence of 12% in buffalo samples. In dogs, PBV was identified in 3 of the 23 diarrhoeic samples (13.04%) *via* RNA-PAGE.

Samples from cattle, goats, pigs and poultry were negative for PBV by RNA-PAGE. This lack of detection may reflect lower viral loads in these species or the labile nature of PBV, which can reduce detection efficiency, especially in stored samples. Previous studies have shown varying PBV detection rates in bovine samples, such as Takiuchi *et al.* (2016) with 8.3% in buffalo and Prasad *et al.* (2018) with 13% in diarrhoeic bovine samples, underscoring the virus's variability across different hosts and sample conditions.

In dog faecal samples, the presence of tripartite bands was detected by RNA-PAGE in 3 out of 23 diarrhoeic samples (13.04%), suggesting the presence of Picotrirnavirus. However, none of these samples were amplified for the RdRp gene by RT-PCR, likely due to the narrow specificity of primers, which may limit the detection of all circulating Picotrirnavirus strains (Malik *et al.,* 2014).

Fecal samples from cattle, goats, pigs and poultry were negative for PBV by RNA-PAGE, possibly due to low viral loads or the instability of PBV. During PBV screening, rotavirus was identified in three buffalo and two pig samples, with characteristic 11-segment RNA migration patterns confirmed by RT-PCR targeting the VP6 gene. Similar mammalian-like electropherotypes (4:2:3:2) in avian rotaviruses have been previously documented, suggesting possible interspecies transmission (Balan *et al.,* 2018).

We used a different set of primers targeting the RdRp gene for molecular detection and characterization. Further molecular characterization was performed using RT-PCR to detect the partial RdRp gene of PBV and VP6 gene of rotavirus. The results of these tests, including the number of positive detections per species, are summarized in Table 3. This study screened 150 fecal samples (25 each from cattle, buffalo, goat, pig, dog and poultry) for PBV using primers targeting the RdRp gene of segment 2. PBV was detected in 11 bovine and goat samples (7.33%). DiagnosticPCR targeting the VP6 gene confirmed the rotavirus-positive samples identified by RNA-PAGE.

To assess the effectiveness of different primer sets in detecting the partial RdRp gene of PBV, RT-PCR was conducted across a range of samples. The specific outcomes, indicating which primer sets were successful in amplifying the RdRp gene in each sample, are compiled in Table 4. This table provides insights into the genetic diversity of PBV and the challenges associated with its

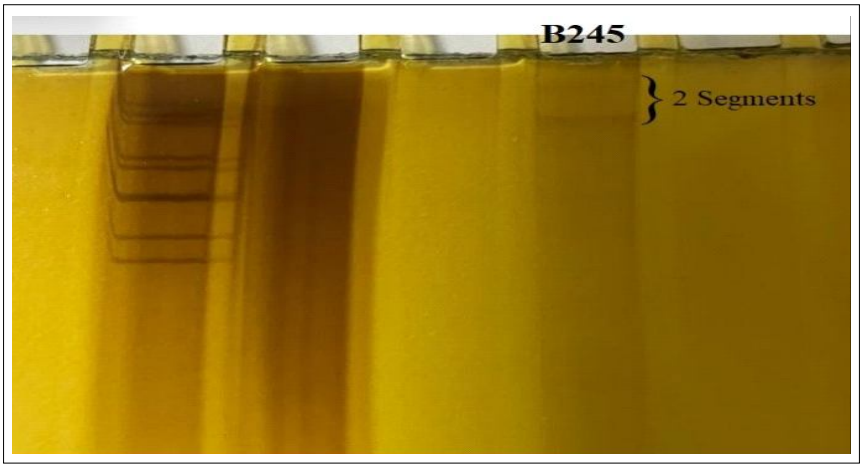


Fig 1: PBV electrophoretic genome pattern (**a**) Electropherogram showing 2 segments of RNA in buffalo sample B245.

Table 2: Species wise distribution of PBV infection detected by RNA-PAGE.

Species	No. of samples			RNA-PAGE positive for PBV			Bands noticed	Percentage positive (Prevalence)	RNA-PAGE positive for VP6	Bands noticed
	Total	Diarrhoeic	Non diarrhoeic	Total	Diarrhoeic	Non diarrhoeic				
Cattle calf	25	02	23	0	0	0	-	0	-	-
Buffalo calf	25	04	21	03	03	0	2	12	3	11
Goat	25	03	22	0	0	0	-	0	-	-
Pig	25	02	23	0	0	0	-	0	2	11
Dog	25	23	02	03	03	0	3	12	-	-
Poultry	25	01	24	0	0	0	-	0	-	-
Total		150			6			04		

detection using a single primer set. In buffalo samples, a 20% PBV prevalence was observed by RT-PCR, consistent with previous findings, such as the 23.4% prevalence in bovine samples reported by Navarro *et al.* (2018). While RNA-PAGE detection was negative in goat samples, RT-PCR revealed a 24% PBV prevalence, aligning with other studies that noted rates from 20% to 70% in small ruminants. For instance, Malik *et al.* (2018) reported a 35.75% prevalence in small ruminants, with higher rates in goats than in sheep.

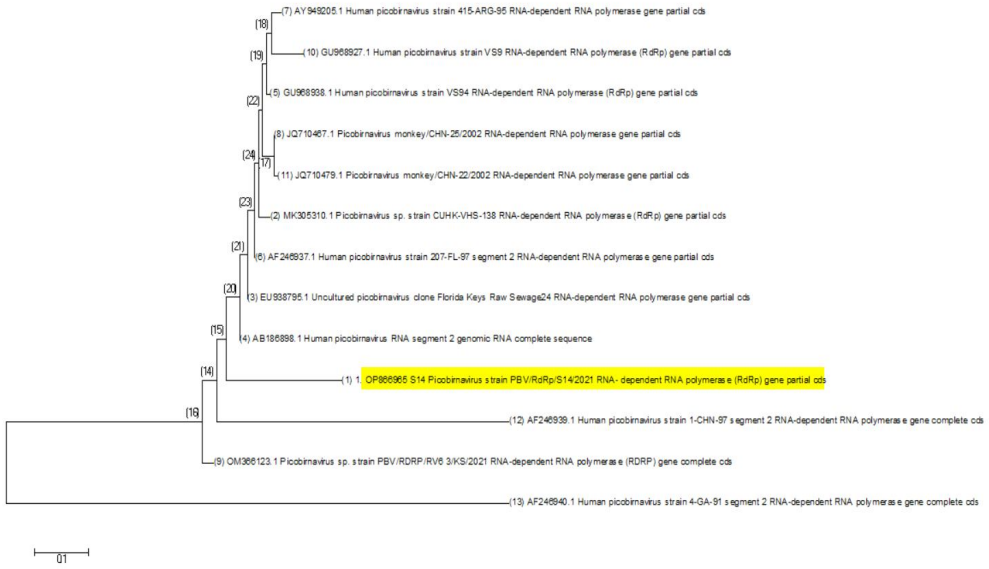
In our study, the presence of tripartite RNA segments was detected in diarrhoeic dog samples by RNA-PAGE, though RT-PCR failed to amplify PBV with any primer set, likely due to low viral load or limited primer specificity. Our analysis identified all PBV-positive samples as genogroup I, with no detection of genogroup II, consistent with GenBank data showing genogroup I as predominant (83.11%). Atasoy *et al.* (2022) similarly found only genogroup I in cattle samples. These findings underscore the importance of using both RNA-PAGE and RT-PCR for PBV detection and suggest that RT-PCR for PBV is limited due to genetic diversity, requiring multiple primers and regular updates to capture emerging strains.

For rotavirus detection, the VP6 gene was targeted using GARV-D-VP6-F and GARV-F-VP6-R primers. Diagnostic PCR identified rotavirus in 4 buffalo, 1 goat and 2 pig samples, while RNA-PAGE confirmed rotavirus with an 11-segment pattern in three buffalo and two pig samples, indicating co-infection with PBV in samples B90, B245, B267 and B539. Similar co-infections of PBV and rotavirus have been observed, such as in Duarte Junior *et al.* (2021) in a toucan, emphasizing the relevance of co-infection screening in various species.

The RT-PCR product of an isolate of goat sample was subjected to nucleotide sequencing through outsourcingat Eurofins Genomics India Pvt. Ltd., Bangalore by Sanger sequencing method using forward and reverse primers for partial RdRp gene. The results of the blast search revealed that the isolate S14 (PBV/Goat/India/S14/2021) had similarity with the isolates of PBV sequences in GenBank ranged from 93.40% to 95.73% belonging to different geographical areas.

Table 3: Species wise overall RT-PCR results for Partial RdRp gene.

Species	No. of Samples	Samples positive for partial RdRp gene (PBV)	Samples positive for VP6 (Rotavirus)
Cattle	25	0	0
Buffalo	25	5	4
Goat	25	6	1
Piglet	25	0	2
Dog	25	0	0
Poultry	25	0	0
	Total	11	8



Phylogenetic distances were measured by the Kimura two- parameter model and the tree was statistically supported by bootstrapping model and the tree was statistically supported by bootstrapping with 1000 replicates. Phylogenetic distances were measured by the Kimura two- parameter model and the tree was statistically supported by bootstrapping model and the tree was statistically supported by bootstrapping with 1000 replicates.

Fig 2: Phylogenetic tree based on partial RdRp gene of PBV of sample. S14 obtained through blast search for S14 sequence PBV/Goat/India/S14/2021 (OP866965 highlighted with yellow colour) similarity in the GenBank.

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OM366123.1      GAGGGTGGCCCTAAACCAACAGACGTGAAACAGCGGGTAGTGTGGATGTTTCCTTTCGCT    720
AB186898.1      GAGGGTGGCCCTAAACCAACAGACGTGAAACAGCGGGTAGTGTGGATGTTTCCTTTCGCT    720
MK305310.1      GAGGGTGGCCCTAAACCGAAAGACGTGAAACAGCGAGTAGTGTGGATGTTTCCTTTCGCT    521
OP866965.1      ----------------------------------------TGGATGTTTCCTTTCGCT     18
GU968927.1      ------------------------------------------------------CTTATGCT      8
AY949205.1      -------------------------------------------------------TTTTGCT      7
JQ710467.1      ------------------------------------------------------CTTTCGCT      8
JQ710479.1      ------------------------------------------------------CTTTCGCT      8
GU968938.1      ------------------------------------------------------CTTTCGCT      8
EU938795.1      ----------------------------------------TGGTGTGGATGTTTCCTTTCGCT     23
AF246937.1      ------------------------------------------------------CTTTCGCT      8
                                                                        **  ***

OM366123.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCACTAATCCTTACGTTCCAAAGATTGGGA    780
AB186898.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCACTAATCCTTACGTTCCAAAGATTGGGA    780
MK305310.1      GTTAACATTCGTGAGCTGCAGGTCTATCAGCCATTAATCCTTACGTTCCAAAGATTGGGA    581
OP866965.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCATTAATCCTTACGTTCCAAAGATTGGGA     78
GU968927.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCATTAATCCTTACGTTCCAAAGATTAGGA     68
AY949205.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCATTAATCCTTACGTTCCAAAGATTGGGG     67
JQ710467.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCATTGATCCTTACGTTCCAAAGATTAGGA     68
JQ710479.1      GTTAATATTCGTGAGCTGCAGGTGTATCAACCATTGATCCTTACGTTCCAAAGATTAGGA     68
GU968938.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCATTAATCCTTACGTTCCAAAGATTAGGA     68
EU938795.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCACTAATCCTTACGTTCCAAAGATTGGGA     83
AF246937.1      GTTAATATTCGTGAGCTGCAGGTCTATCAACCACTAATCCTTACGTTCCAAAGATTAGGA     68
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OM366123.1      TTTGTTCCAGCTTGGGTTAGCATGGAACAAGTCGACAGGCGTATCACTAAGATGTTTGAT    840
AB186898.1      TTGGTGCCAGCGTGGGTTAGCATGGAAGCTGTTGATAGGCGTATCACTAAGATGTTTGAT    840
MK305310.1      TTAGTGCCAGCGTGGGTTAGCATGGAAGCTGTTGATAGGCGTATCACTAAAATGTTTGAT    641
OP866965.1      TTAGTGCC----------------------------------------------------     86
GU968927.1      TTAGTGCCTGCTTGGGTTAGCATGGAAGCCGTTGATAGGCGTATCACAAAGATGTTTGAT    128
AY949205.1      TTAGTGCCAGCGTGGGTTAGCATGGAAGCTGTTGATAGGCGTATCACTAAGATGTTCGAT    127
JQ710467.1      TTAGTGCCAGCGTGGGTTAGCATGGAGGCTGTTGATAGGCGTATCACTAAGATGTTTGAT    128
JQ710479.1      TTAGTGCCAGCGTGGGTTAGCATGGAGGCTGTTGATAGGCGTATCACTAAGATGTTTGAT    128
GU968938.1      TTAGTGCCAGCGTGGGTTAGCATGGAAGTTGTTGATAGGCGTATCACTAAGATGTTTGAT    128
EU938795.1      TTGGTGCCAGCGTGGGTTAGCATGGAAGCTGTTGATAGGCGTATCACTAAAATGTTTGAT    143
AF246937.1      TTAGTGCCAGCGTGGGTTAGCATGGAAGCTGTTGATAGGCGTATCACTAAAATGTTTGAT    128
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* represents similarity in the nucleotide among all the sequences. - represents gap.

Fig 3: Comparison of nucleotide sequence of the segment 2 product of goat PBV strain (PBV/Goat/India/S14/2021) with that of sequences obtained through blast search for S14 sequence PBV/Goat/India/S14/2021 (OP866965 indicated with red line) similarity in the GenBank using the CLUSTAL O program (version 1.2.4).

Table 4: RT-PCR result for amplification of partial RdRp gene of PBV (+ = positive, - = negative).

Sample	Species	RdRp positive using Pico B25+ and PicoB43-	RdRp positive using Pico B23+ and Pico B24-	RdRp positive using PBV 7F and PBV 7R	RdRp positive using F1143 and R1468
B90	Buffalo	+	-	-	+
B245	Buffalo	+	-	-	-
B267	Buffalo	+	-	+	-
B539	Buffalo	+	-	-	-
BI1	Buffalo	-	-	-	+
S10	Goat	+	-	-	-
S11	Goat	+	-	-	-
S14	Goat	+	-	-	-
S16	Goat	+	-	-	-
S20	Goat	+	-	-	-
S386	Goat	+	-	-	-

RT-PCR result for amplification of partial RdRp gene.

On studying the phylogenetic analysis of a goat PBV sample S14, it was found that it had proximity to human PBV strain (Accession no AB186898) and distantly related to monkey picobirnavirus suggesting interspecies transmission. On comparison with prototype strains, it was found closely related to human genogroup I strain 1-CHN-97 and distantly related to human genogroup II strain 4-GA-91 (Fig 2).

PBVs have been detected in both diarrhoeic and clinically healthy animals, as well as in invertebrates and environmental specimens, making their role and transmission mechanisms unclear. This study aimed to investigate PBV infection frequency in diarrhoeic and non-diarrhoeic animals. Most PBV strains identified globally are classified as genogroup I, with reported prevalence rates varying between 0.69% and 23.4%. Our findings align with these trends, showing a 7.33% positivity rate, primarily with genogroup I strains, in agreement with previous studies on PBV distribution.

CONCLUSION

In this study, 150 fecal samples from various animal species were screened for PBV detection and characterization using RNA-PAGE and RT-PCR. Prevalence of PBV in buffalo samples was 12% by RNA-PAGE, similar to previous studies. Detection rates of PBV by RNA-PAGE were generally low (<10%) in other animal species. Multiple primer sets were used for molecular characterization due to the high genomic diversity of PBV. Co-infections of PBV and rotavirus were observed in buffalo and pig samples. PBV prevalence in goat samples was 24% by RT-PCR and none of the samples were positive by RNA-PAGE. Genogroup I PBV strains were predominant in positive samples. The role of PBV in diarrheic and healthy animals remains unclear. PBVs have been detected in invertebrates and environmental specimens, suggesting their presence in gut flora. The study highlights the need for simultaneous use of RNA-PAGE and RT-PCR for PBV detection and the importance of multiple primer sets for molecular characterization.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish,or preparation of the manuscript.

REFERENCES

Atasoy, M.O., Isidan, H. and Turan, T. (2022). Genetic diversity, frequency and concurrent infections of picobirnaviruses in diarrhoeic calves in Turkey. *Tropical Animal Health and Production*. **54:** 1-1. https://doi.org/10.1007/s11250-022-03128-4.

Balan, R., Mini, M., Priya, P.M, Joseph, S. and Sankar, S. (2018). Detection of mammalian-like group a rotavirus in diarrhoeic poultry using RNA-PAGE In Kerala, India. *Indian Journal of Animal Research*. **52(11):** 1654-1655. doi: 10.18805/ijar.B-3415.

Boom, R.C.J.A., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M. and Van der Noordaa, J.P.M.E. (1990). Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology*. **28(3):** 495-503.

Duarte Júnior, J.W.B., Chagas, E.H.N., Serra, A.C.S., Souto, L.C.D.S., da Penha Júnior, E.T., Bandeira, R.D.S., Guimarães, R.J.D.P.S., Oliveira, H.G.D.S., Sousa, T.K.S., Lopes, C.T.D.A. and Domingues, S.F.S. (2021). Occurrence of rotavirus and picobirnavirus in wild and exotic avian from amazon forest. *PLoS Neglected Tropical Diseases*. **15(9):** p.e0 008792.

Gupta, V., Nayak, A., Swamy, M., Singh, R.V., Gupta, V., Rai, A., Gupta, P. and Pandey, M. (2021). Presence of hum1an G rotavirus genotypes amongst bovine calves and its combination with P genotypes. *Indian Journal of Animal Research*. **1:** 11. doi: 10.18805/IJAR.B-4364.

Holland, R.E. (2014). Some infectious causes of diarrhea in young farm animals. *Clinical Microbiology Reviews*. **3(4):** 345-375.

Kattoor, J.J., Sircar, S., Saurab, S., Subramaniyan, S., Dhama, K. and Malik, Y.S. (2016). Picobirnavirus: A putative emerging threat to humans and animals. *Advances in Animal and Veterinary Sciences*. **4(6):** 327-331.

Kuhar, U. and Jamnikar-Ciglenecki, U. (2019). High detection rate and high genetic diversity of genogroup I Picobirnaviruses from roe deer. *Infection, Genetics and Evolution*. **73:** 210-213.

Kumar, N., Mascarenhas, J.D., Ghosh, S., Masachessi, G., Silva Bandeira, R.D., Nates, S.V., Dhama, K., Singh, R.K. and Malik, Y.S. (2020). Picobirnavirus. In animal-origin viral zoonoses springer, Singapore. pp. 291-312. https://doi.org/10. 1007/978-981-15-2651-0_13.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **227(5259):** 680-685.

Malik, Y.S., Chandrashekar, K.M., Sharma, K., Haq, A.A., Vaid, N., Chakravarti, S., Batra, M., Singh, R. and Pandey, A.B. (2011). Picobirnavirus detection in bovine and buffalo calves from foothills of Himalaya and Central India. *Tropical Animal Health and Production*. **43(8):** 1475- 1478.

Malik, Y.S., Kumar, N., Sharma, K., Dhama, K., Shabbir, M.Z., Ganesh, B., Kobayashi, N. and Banyai, K. (2014). Epidemiology, phylogeny and evolution of emerging enteric Picobirnaviruses of animal origin and their relationship to human strains. *BioMed Research International.* **pp.** 1-13.

Malik, Y.S., Sharma, A.K., Sharma, K., Sircar, S. and Dhama, K. (2017). RNA polymerase gene-based RT-PCR assay with primers update for genus specific detection of picobirnaviruses. *Journal of Animal and Plant Sciences*. **27(2):** 582-588.

Malik, Y.S., Sircar, S., Dhama, K., Singh, R., Ghosh, S., Bányai, K., Vlasova, A.N., Nadia, T. and Singh, R.K. (2018). Molecular epidemiology and characterization of picobirnaviruses in small ruminant populations in India. *Infection, Genetics and Evolution.* **63:** 39-42.

Navarro, J.D.O., Candido, M., De Almeida-Queiroz, S.R., Buzinaro, M.D.G., Livonesi, M.C., Fernandes, A.M. and de Sousa, R.L.M. (2018). Genetic diversity of bovine Picobirnavirus, Brazil. *Virus Genes*. **54(5):** 724-728.

Prasad, M., Brar, B., Ranjan, K. and Misri, J. (2018). Occurrence of neglected picobirna virus in buffalo calves associated with diarrhoea. *The Indian Journal of Veterinary Sciences and Biotechnology.* **13(3):** 16-20.

Rosen, B.I., Fang, Z.Y., Glass, R.I. and Monroe, S.S. (2000). Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. *Virology*. **277(2):** 316-329.

Sevensson, L., Uhnoo, I., Grandien, M. and Wadell, G. (1986). Molecular epidemiology of rotavirus infections in Upsala, Sweden in (1981): Disappearance of a predominant electropherotype. *Journal of Medical Virology*. **18:** 101-111.

Takiuchi, E., Macedo, R., Kunz, A.F., Gallego, J.C., De Mello, J.L., Otonel, R.A.A. and 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.