Detection of picobirnavirus and rotavirus in diarrhoeic faecal samples of cattle and buffalo calves in Mumbai metropolis, Western India

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Summary
In this study 113 diarrhoeic faecal samples obtained from buffalo (n = 68) and cattle (n = 45) calves under 1 years of age were analysed in order to determine the presence of rotavirus infection and the frequency of picobirnavirus excretion. Eleven (9.73%) samples positive for group A rotavirus were identified through RNA-polyacrylamide gel electrophoresis (RNA-PAGE), while 4 (3.53%) samples showed a bisegmented genome with a typical picobirnavirus pattern. This is the first report of picobirnavirus in cattle and buffalo calves from Western India.

Presenza di picobirnavirus e rotavirus in campioni fecali diarroici di vitelli bovini e bufalini nella città di Mumbai, India occidentale

Parole chiave
India,
Mumbai,
Picobirnavirus,
RNA- polyacrylamide gel electrophoresis,
Rotavirus.

Riassunto
Questo articolo descrive i risultati di uno studio condotto su 113 campioni di feci prelevati da bufali (n = 68) e bovini (n = 45) di età inferiore ad 1 anno e analizzati per rotavirus e picobirnavirus. Dei 113 campioni testati mediante RNA-polyacrylamide gel electrophoresis (RNA-PAGE), 11 (9.73%) sono risultati positivi per il ceppo A del rotavirus mentre 4 (3.53%) hanno mostrato un genoma bisegmentato tipico del picobirnavirus. È la prima volta che picobirnavirus è stato rilevato in bufali e bovini in India occidentale.

Introduction

Picobirnaviruses (PBV) are a group of small viruses whose genome is composed of 2 segments of double-stranded RNA (dsRNA), ranging in size from 2.69 kbp to 2.36 kbp for the larger segment and from 2.36 to1.58 kbp for the smaller segment (7). The virion is non-enveloped, with a diameter of 33 to 35 nm and has icosahedral symmetry. Picobirnavirus has large and small genome profile depending on the size of 2 segments of double-stranded RNA. It was first described by Pereira *et al.* (17), who detected two bands of bisegmented double-stranded RNA genome by polyacrylamide gel electrophoresis (PAGE) in faecal samples from children. Since then, PBV has been detected in faecal samples from different animals including rats (17), avian (1, 13), guinea-pigs (18), pigs (9), rabbits (8), cattle (3, 12, 24) and giant anteaters (*Myrmecophaga tridactyla*) (10). Picobirnavirus has been identified in both normal and diarrheic faeces so its pathogenicity is still unclear. Rotaviruses are the major causal agents of acute viral gastroenteritis in young animals as well as in children, as such they are responsible for significant economic losses throughout the world. The group A is the most frequently isolated in the case of rotaviral diarrhoea. The rotavirus belongs to the genus *Rotavirus* under the family *Reoviridae* and its genome can be separated into 11 discrete segments by RNA-PAGE. This study reports the detection of PBV and rotavirus in diarrhoeic faecal samples of cattle and buffalo calves in Mumbai metropolis, Western India.

Materials and methods

Samples

A total of 113 faecal samples including 68 from buffalo and 45 from cattle were collected from different farms in Mumbai. All sampled animals were under 1 year of age and with diarrhoea. The diarrhoeic faecal samples were collected between October 2008 and October 2009.

A 10% suspension of each faecal sample was prepared in lysis buffer [3 M sodium acetate, pH 5.2, 10% sodium dodecyl sulphate (SDS)]. The suspension was vortexed for 10 min, followed by centrifugation at 10,000 × g (10,381 rpm) for 15 min at 4°C to remove coarse particles and cellular debris. The clarified supernatant was transferred into sterilised vials and stored at -20°C or processed for RNA extraction.

Viral RNA extraction

Picobirnavirus-positive samples were detected using the protocol used for rotavirus genome extraction. Viral RNA was isolated with the guanidinium isothiocyanate using the lysis method (6). In brief, 500 µl of the clarified 10% faecal suspension was vortex-mixed with an equal volume of GIT lysis buffer and 0.1 volume of 2 M sodium acetate, pH 4.6 and kept on ice for 15 min. A mixture containing an equal volume of phenol, chloroform and isoamyl (PCI) alcohol (25:24:1) was added, vortexed, kept on ice for 15 min and centrifuged at 12,000 g for 10 min at 4°C. The supernatant was transferred into sterile tubes (Ratiolab GmbH, Dreieich, Germany). The PCI extraction was repeated twice. The aqueous phase was transferred into fresh tubes, with a mixture containing an equal volume of chloroform and isoamyl alcohol, vortexed and centrifuged at 12,000 g for 10 min at 4°C. Again, the aqueous phase was placed in fresh tubes and 0.1 volume of 3 M sodium acetate, pH 5.2 was added and vortexed. To this, an equal volume of isopropanol was added; tubes were inverted 4-5 times and kept at -20°C overnight for precipitation. RNA was pelleted by centrifugation at 12,000 g for 10 min at 4°C and the pellet was washed with pre-chilled 70% ethanol. The pellet was air dried, dissolved in 30 µl of nuclease free water and stored at -20°C until further use.

RNA-polyacrylamide gel electrophoresis

The genome of PBV was detected and analysed by RNA-polyacrylamide gel electrophoresis (RNA-PAGE) using discontinuous buffer system without SDS as described by Laemmli (14). The PAGE was performed at a constant voltage of 120 V (8-10 V cm) for 3 hrs using 8% resolving and 5% stacking gel. Subsequently, the gel was stained with silver nitrate according to the method described by Svensson *et al.* (22) with some minor modifications. The segment lengths of PBV were estimated by comparison with the length of genome segments of faecal sample positive for rotavirus.

Results

Molecular analysis

RNA-polyacrylamide gel electrophoresis analysis of these samples revealed that 9.73% (11/113) was positive for group A rotavirus, whilst 4 (3.53%) presented a bisegmented genome, the bands of which ranged between segments 3 and 5 compared to the rotavirus segments (Figure 1). Out of 68 faecal samples of buffalo calves tested for viral gastroenteritis, 3 (4.41%) were found positive for picobirnavirus and 8 (11.76%) for rotavirus. Similarly, of 45 faecal samples of cattle calves tested for viral gastroenteritis, 3 (6.66%) were found positive for rotavirus, whereas 1 (2.22%) tested positive for PBV.

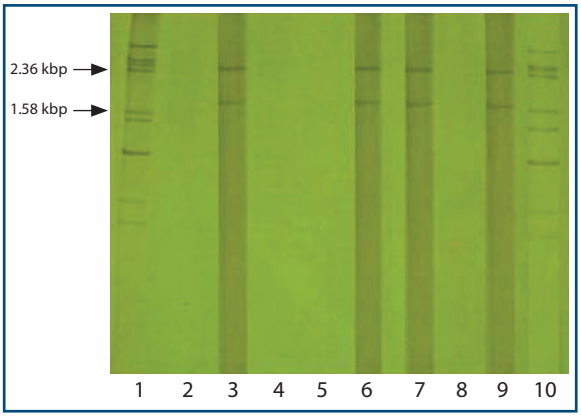

Figure 1. *Electrophoretic analysis of RNA genome segments of rotavirus and picobirnavirus isolated from bovine and buffalo calves.*

Lane 1: cattle rotavirus.
Lane 10: buffalo rotavirus.
Lane 3: cattle picobirnavirus.
Lanes 6, 7, and 9: buffalo picobirnavirus.
Lanes 2, 4, 5, and 8: negative samples.

The size of the segments was estimated to be approximately 2.36 kb and 1.58 kb for the larger and smaller segments, respectively (Figure 1).

Discussion

The detection of PBV has been done by RNA-PAGE, because this method allows for sufficiently fractionating the viral RNA from other nucleic acids in the stool samples showing distinct electropherotypic profiles (21). In Brazil, PBV has been detected using PAGE in faeces of diarrhoeic and non-diarrhoeic calves (3). Malik *et al*. (16) reported 3.67% (5/136) positivity for PBV, showing a typical genomic migration pattern with 2 discrete bands with size of approximately 2.4 and 1.7 kb for the larger and smaller segments, respectively. The overall prevalence of the large PBV genome profile amongst diarrhoeic buffalo calves in Mumbai was 4.67%, whereas earlier studies have reported presence of PBV infections among diarrhoeic and non-diarrhoeic calves as 0.70% (3). Bhattacharya *et al.* (2) reported that the prevalence of the small genome profile PBV amongst cases of diarrhoea in children in Kolkata was 2.47%. Pereira *et al*. (19) reported large genome profile PBV infections among diarrhoeic and non-diarrhoeic human cases at prevalence rate of approximately 0.45%.

Rotaviruses are the most commonly identified viral causes of diarrhoea of neonatal food animals (11). In our study the Group A rotavirus was detected in 9.73% of the diarrhoeic calves, this finding is in agreement with Chitambar *et al*. (5), suggesting that the rotavirus is one of the more important causative agents in neonatal diarrhoea.

Although other studies have also demonstrated the presence of PBV in various hosts with a certain frequency, conclusive data regarding the pathogenicity of this virus are lacking. Some articles tried to associate the presence of PBV with manifestations of gastroenteritis (15, 17). However, since PBV has also been detected in animals without clinical signs, its true role in this clinical manifestation remains to be defined (4, 9, 15). Finally, we would like to highlight that, to the best of our knowledge, this is the first report of a PBV identified in cattle and buffalo calves in Western India.

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