Occurrence of enteric viruses causing clinical diarrhea in small ruminants in northern Indian plains: a reverse transcription PCR based molecular study

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Keywords
Bovine coronavirus, Group A rotavirus, Group B rotavirus, One-step Reverse transcription PCR, Sequencing and phylogenetic analysis.

Summary
Neonatal diarrhoea is a major threat responsible for high mortality in neonates particularly during the first week of life. In goat kids besides *E. coli*; Group A, Group B Rotavirus (GARV/GBRV), Bovine Corona virus (BCoV), Cryptosporidium parvum and Clostridium perfringens are frequently involved as causative agents of diarrhoea. It requires highly sensitive and specific assays to diagnose the disease at field level. Detection of GARV/GBRV and BCoV was done by one-step RT-PCR (osRT-PCR). In the present investigation, diarrhoeal(n=254) and non-diarrhoeal (n=50) faecal samples and necropsy tissue samples (n=17) of goat kids and lambs(n=22) were collected from different outbreaks and screened for GARV, GBRV and BCoV by a conventional osRT-PCR. The prevalence of rotavirus small ruminants was recorded as 14.57% for GARV, 7.48% for BCoV and 1.18% for GBRV. The prevalence of GARV in lambs was recorded as 22.7%. While non-diarrheic samples (n=50) obtained from asymptomatic kids showed 3 samples positive for GARV (6%) and 1 sample positive for BCoV (2%) while none of them were detected for GBRV. Sequencing and phylogenetic analysis revealed two major branches, where CIRG F2 strain was closely associated with bovine and human GARV strains, indicating the relevance of genetic re-assortment and its zoonotic potential. Two more strains viz., CIRG 1873 and CIRG1841 were placed in a clade genetically close to porcine GARV isolates. This shows the dynamic nature of the circulating strains of GARV.

Introduction

Livestock wealth plays a significant role in the up-liftment of the farmers by providing sustainable livelihood security.

Neonatal enteritis is an important disease complex or syndrome that causes deaths of animals before 3 weeks of age, and results in significant economic losses. Viral agents can predispose the neonates to secondary infections in the gastrointestinal tract, especially in lambs and goat kids younger than 21 days due to nascent immune system. Although the neonatal diarrhoea is common in young ones of large ruminants, there is dearth of knowledge about pathology, pathogenesis and immune histochemical localization of viral agents that cause neonatal enteritis their small ruminant counterparts. In this study, we carried out investigations with the aim of detecting rotavirus and coronavirus leading to viral enteritis in goat kids and lambs.

Neonatal diarrhoea is a major threat responsible for high mortality in neonates particularly during the first week of life. In goat kids *E. coli*, *Cryptosporidium parvum*, *Coccidia like Eimeria* spp. and *Clostridium*

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perfringens types A, B and C are frequently involved as causative agents of neonatal kid diarrhoea (Van Metre DC 2000). Viral agents can predispose the neonates to secondary infections in the gastrointestinal tract, especially in lambs and goat kids younger than 21 days due to nascent immune system. Rotavirus gastroenteritis is a worldwide disease affecting primarily infants, young children and young ones of wide variety of mammalian and avian species. Very few reports are available from the Indian sub-continent regarding the association of enteric viruses with clinical enteritis in small ruminants. Diarrhoea in goat kids is most frequently found associated with GARV (Dey *et al*. 2007), Group B rotaviruses cause adult diarrhoea and are reportedly geographically confined, having been first identified in a large waterborne epidemic in China in 1983. This virus, designated as adult diarrhoea rotavirus (ADRV) has a unique RNA pattern on polyacrylamide gel electrophoresis and is antigenically distinct from group A rotaviruses (Tao *et al*. 1983) and another enteric pathogen bovine coronavirus (BCoV) is a major viral pathogen associated with neonatal calf diarrhoea (NCD) (Mebus *et al*. 1973). Coronaviruses are known to infect and cause remarkably diverse disease syndromes in wide range of mammalian and avian species. Variable tissue tropism and their capability to quickly adapt to new hosts and ecological niches is well-documented (Vlasova and Saif 2021). In this study, we carried out investigations with the aim of detecting rotavirus and coronavirus leading to associated with viral enteritis in goat kids and lambs.

RNA-PAGE based electropherograms are usually used to identify the rotaviruses, but it may not be enough to identify the strain variations from a geographical location. In this study, we carried out investigations with the aim of detecting rotavirus and coronavirus associated with viral enteritis in goat kids and lambs and performed PCR based sequencing of GARV associated with the clinical diarrhoea in goat kids, and deduced the phylogenetic relationships with the other GARV strains available from NCBI database.

Materials and methods

Collection of Samples

A total of 4 goat farms (Breedwise- Jamunapari, Barbari, Jakhrana and Sirohi) and one sheep farm (Muzaffarnagari) were sampled during the current study. The farms are located in the Mathura district of Uttar Pradesh which is of semiarid region with an annual rainfall less than 700mm with very hot summers and cool winters. Faecal samples were collected from 254 diarrhoeic goat kids, 50 non-diarrheic goat kids and 22 lambs below 12 weeks of age from various outbreaks in field and organized farms. The outbreak samples were collected from goat kids below 12 weeks of age from unorganised farmer's flocks (n=10 flocks) that are mostly non-descript goat breeds which were clinically affected by diarrhoea. Each flock on an average contained 3-4 numbers of adult doe and 5-6 numbers of goat kids. The flock is dynamic, as the adult animals are sold during festive seasons. The outbreak attended for the current was observed during December (2020)-January (2021) months in <3 month old kids showing acute to sub-acute diarrhoea. At least 50% of goat kids were showing clinical diarrhoea at a given point of time. At least 3 kids per farmer's flock showing clinical diarrhoea (total n=29) were sampled for the study. Faecal samples were collected from goat kids and lambs by using sterile swabs (Himedia) which were subsequently stored in ice. Further necropsy samples from the intestine parts including jejunum and ileum were also collected and preserved in ice for RNA extraction.

A 10% suspension of each faecal sample was prepared in phosphate buffered saline (PBS), pH 7.2 and centrifuged at 6,000g for 30 min at 4°C. The supernatant was either stored at -20°C or processed for RNA extraction.

Extraction of RNA

Viral RNA extraction suited for double stranded RNA was optimized in the current study using TRIzol® reagent (Cat# 15596026, Invitrogen, Thermofisher) as per the manufacturer's protocol with slight modifications as described below. About 1 ml of TRIzol reagent was mixed with 50-100 mg of fresh or frozen faecal sample and then homogenized well. Similarly 100mg tissue samples (parts of ileum/jejunum) are minced and homogenized using micro-pestle in a 1.5ml sterile nuclease free microfuge tube and mixed with 1ml TRIzol® reagent. The homogenate-reagent mixture was incubated for 5 min. It was followed by addition of 0.2 ml chloroform and vigorous shaking for 15 sec. The resulting mixture was incubated at room temperature (RT) for 2-3 min, followed by centrifugation at 12,000 g for 15 min at 4°C. The colourless upper aqueous phase was transferred to fresh centrifuge tube, to which 0.5 ml of 100% isopropanol was added for RNA precipitation. The mixture was incubated at room temperature for 10 min and centrifuged at 12,000 g for 10 min at 4°C. The supernatant obtained was discarded and the RNA pellet was washed with 1 ml ethanol (75%) by centrifugation at 7500 g for 5 min at 4°C. The ethanol wash was discarded and the RNA pellet was air dried for 5-10 min. The RNA pellet was resuspended in 30 µl nuclease free water, followed by incubation in water bath at 55-60°C for 10-15 min. For double stranded RNA viruses denaturation step

is essential before synthesis of cDNA or one-step RT-PCR. Hence we treated approximately 500ng of total fecal/tissue RNA with 1µl of DMSO in a total volume of 10 µl at 95ºC for 5 mins. The processed RNA was then used for downstream application or stored at –20ºC till further use.

Primers

Table I. *Primers used for amplification of target genes for GARV, GBRV and BCoV.*

Virus	Target gene	Primer	Sequence (5′ → 3′)	Product length	Reference
GARV	VP6	GEN_VP6F	GGCTTWAACGAAGTCTTC	928bp	Matthijnssens *et al*. (2008)
		GAR VP6-928R	GGYGTCATATTYGGTGG		
GBRV	VP7	9B3F	CAGTAACTCTATCCTTTACC	281bp	Matthijnssens *et al*. (2008)
		9B4R	CGTATCGCAATACAATCCG		
BCoV	Nucleocapsid	BCoV-N-F	GCCGATCAGTCCGACCAATC	407bp	Tsunemitsu *et al*. (1999)
		BCoV-N-R	AGAATGTCAGCCGGGGTAT		

One step Reverse transcription polymerase chain reaction (osRT-PCR)

RT-PCR amplification of target genes for GARV, GBRV and BCoV was done directly from faecal RNA using specific SuperScript® III One Step RT –PCR system with Platinum® Taq High Fidelity kit (Invitrogen, USA) as per the manufacturer's instructions. For positive controls, standard commercially available vaccine strains were used, whereas negative controls were kept as no-template controls. The PCR tube (0.2 ml) containing the reaction mixture was tapped thoroughly with finger and then flash spun in a microcentrifuge to settle reactants at the bottom. The DNA amplification reaction was performed in Master Cycler Gradient Thermocycler (Eppendorf, Germany) with a pre-heated lid. The cycling conditions for PCR included an initial denaturation of DNA at 98°C for 2 min, followed by 30 cycles each of 30 sec denaturation at 98°C, 90 sec annealing at 55°C and 40 sec extension at 72°C, and a final extension of 10 min at 72°C. The resultant PCR products were stored at –20°C until further analysis by agarose gel electrophoresis.

Sequencing and phylogenetic analysis of GARV

Sequencing

PCR sequencing was done for the native GARV positive amplicons obtained from the current study. GARV samples for VP6 gene sequencing were selected based on a representative, one from each of the three groups of goat kids viz., clinically diarrheic, non-diarrheic and necropsied. The VP6 gene amplicon was amplified, gel-eluted and sequenced by Sanger's dideoxy sequencing using Big dye®terminator kit for both the strands of the genome. The raw data generated as chromatogram file was proof-read and aligned (Clustal W) using BioEdit® software and the aligned data were analyzed for phylogenetic analysis along with the other reference strains of GARV using MEGA 6.0 software. The aligned sequences were graphically transformed to remove the gaps to plot the sequence identity of nucleotide composition in the open reading frame of the VP6 gene of GARV.

Phylogenetic Analysis

The phylogenetic analysis was conducted by minimum evolution (ME) tree analysis. For this, the evolutionary distances were computed using PISSON correction method based on the number of amino acids substitution per site. Neighbour joining (500 replicates) algorithm was used to generate initial tree.

The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei 1992). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches (Felsenstein 1985).

The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*. 2004) and were in the units of the number of base substitutions per site.

The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar 2000) at a search level of 1.

The Neighbor-joining algorithm (Saitou and Nei 1987) was used to generate the initial tree. T

he analysis involved 11 nucleotide sequences from NCBI based on the representatives from bovines, porcine and humans across various parts of the globe. Codon positions included were 1st+2nd+3rd+Noncoding.

All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*. 2013).

Results

In the current study, a total of 304 faecal samples, 50 were non -diarrhoeic faecal samples from goat kids, diarrhoeic goat kids samples (n=232) and diarrhoeic lambs (n=22) from different outbreaks in field goats and organized farms and 17 diarrhoeic tissue samples from necropsies conducted on goats were screened for presence of GARV, GBRV and BCoV.

Conventional RT-PCR was employed for identification of GARV, GBRV and BCoV using the published primers (Table I).

A much higher proportion, viz., 14.57% of the samples from diarrheic neonatal goat kid were positive for GARV, while GBRV was detected in 1.18% samples, which was much lesser than the incidence of GARV (Table II).

The incidence of BCoV was found 7.48% in the faecal samples collected from the neonates affected with diarrhoea (Table II).

Interestingly the non-diarrheic samples(n=50) obtained from asymptomatic kids also showed 3 samples positive for GARV (6%) but not for GBRV, while one sample detected positive for BCoV (2%) (Table III).

Among the necropsied goat kids (n=17) the GARV were detected in 7 samples (41.18%), GBRV in 2 samples (11.76%) and BCoV in 4 samples (23.53%) (Table IV).

Table II. *Prevalence of GARV, GBRV and BCoV in small ruminants*

Goat kids and lambs sampled farm-wise and field outbreaks	Sample (*n*)	Positives % (*n*)		
		GARV	GBRV	BCoV
Barbari	101	14.8% (15/101)	1.9% (2/101)	12% (12/101)
Jamunapari	64	12.5 (8/64)	0 (0/64)	6.25 (4/64)
Jakhrana	18	0 (0/18)	0 (0/18)	0 (0/18)
Sheep	22	22.7% (5/22)	0 (0/22)	0 (0/22)
Sirohi	20	20% (4/20)	5% (1/20)	15% (3/20)
Outbreaks (non-descript goat breeds)	29	17.2% (5/29)	0 (0/29)	0 (0/29)
Total	**254**	**14.57% (37/254)**	**1.18% (3/254)**	**7.48% (19/254)**

Table III. *Prevalence of GARV, GBRV and BCoV in small ruminants in necropsied samples*

Farmed goat kids animals Necropsied breed-wise	Sample (*n*)	Positives % (*n*)		
		GARV	GBRV	BCoV
Barbari	4	75% (3/4)	25% (1/4)	25% (1/4)
Jamunapari	5	40% (2/5)	20% (1/5)	60% (3/5)
Jakhrana	4	25% (1/4)	0% (0/4)	0% (0/4)
Sirohi	4	25% (1/4)	0% (0/4)	25% (1/4)
Total	**17**	**41.18% (7/17)**	**11.76% (2/17)**	**23.53% (4/17)**

Table IV. *Prevalence of GARV, GBRV and BCoV in small ruminants in necropsied samples*

Farmed non diarrheic goat kids sampled breed-wise	Sample (*n*)	Positives % (*n*)		
		GARV	GBRV	BCoV
Barbari	13	7.69% (1/13)	0% (0/13)	7.69% (1/13)
Jamunapari	13	7.69% (1/13)	0% (0/13)	0% (0/13)
Jakhrana	12	0% (0/12)	0% (0/12)	0% (0/12)
Sirohi	12	8.33% (1/12)	0% (0/12)	0% (0/12)
Total	**50**	**6% (3/50)**	**0% (0/50)**	**2% (1/50)**

Occurrence of enteric viruses in neonatal goat kids and lambs

Lambs showed a little higher incidence of 22.7% of GARV infection compared to goat kids. However, sheep with clinical diarrhea didn't show either GBRV or BCoV infection, which may be due to the lower sample size (n=22).

One interesting observation noted here is that the Jakhrana breed, showed very less observations of diarrhea (n=18) for the whole study period and completely free of any of the enteric viruses targeted during the current study.

GARV was screened using RT-PCR based on an amplicon size of 928 bp for VP6 gene and were 37 (14.57%) were positive 37 in all the detected clinically diarrheic samples. Similarly GBRV was detected by VP7 based RT-PCR with an amplicon size of 281bp which showed was only 3 cases (1.18%) positive for GBRV. Among the clinically positive goat kids 19 (7.48%) detected positive for BCoV based on nucleocapsid gene RT PCR which has an amplicon size of 407 bp (Fig. 1, 2, 3, 4 and 5).

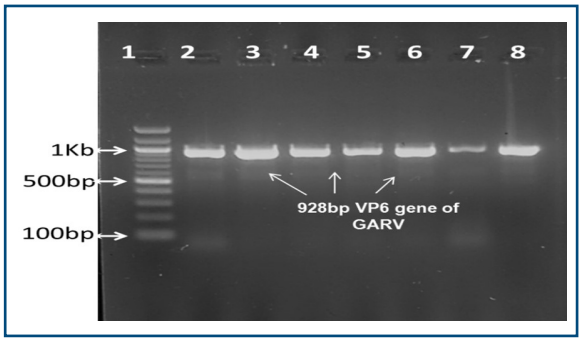

Figure 1. *Gel electrophoresis of reverse transcription PCR (RT-PCR) targeting VP6 gene of Group A rota virus (GARV). Lane1- 100bp DNA ladder, Lane 2- GARV positive control RNA, Lanes 3-5 – Field fecal samples of diarrheic goat-kids collected from farmers flock, Lane 6-fecal samples of diarrheic goats collected from organized livestock units of CIRG, Lane 7- NTC, Lane 8 – fecal samples of diarrheic lamb collected from Muzaffarnagari sheep unit of CIRG.*

Sequencing and molecular characterization of GARV isolates from diarrheic goat kids

Three GARV isolates viz., *CIRG F2*, *CIRG 1873* and *CIRG 1841* obtained from caprine neonatal diarrhea from CIRG farms were sequenced for VP6 gene.

There were two major branches with each having two sub-branches, clades and multiple taxa. CIRG F2 strain of GARV was placed in a sub-branch of unique clade, and was closely related to the bovine and human GARV strains.

The other sub-branch of the major branch contained multiple taxa of various clades containing bovine and porcine GARV strains, where CIRG 1873 and CIRG 1841 taxa were placed in a separate clade. The second major branch contained two clades predominantly from human and bovine GARV isolates. This shows that the CIRG F2 might be in a different genotype compared to the other two CIRG isolates, and requires complete genotype mapping to identify their origin (Fig. 7).

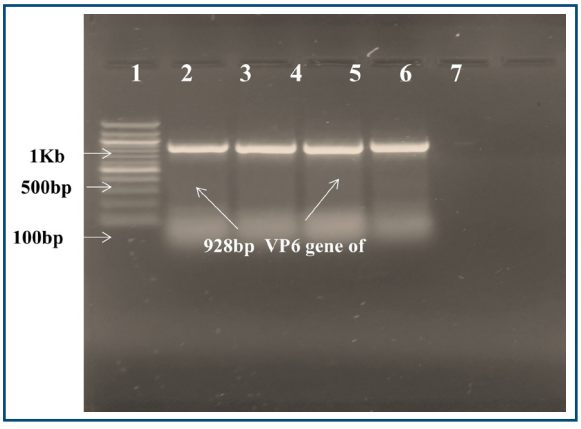

Figure 2. *Gel electrophoresis of reverse transcription PCR (RT-PCR) targeting VP6 gene of Group A rota virus (GARV). Lane 1- 100bp DNA ladder, Lane 2- GARV positive control RNA, Lanes 3-4 – Field diarrheic samples of goat kids collected from farmers flock, Lanes 5-6 – fecal swabs of goat kids collected from organized livestock units of CIRG, Lane 7- No template control (NTC).*

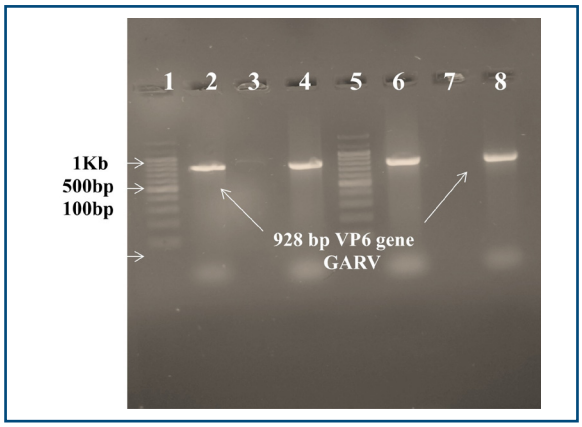

Figure 3. *Gel electrophoresis of reverse transcription PCR (RT-PCR) targeting VP6 gene of Group A rota virus (GARV) from necropsied intestine samples. Lane 1 & 5 100bp DNA ladder, Lane 2- GARV positive control RNA, Lanes 3-4 – Jejunum of affected goats showing positive for VP6 gene of GARV, Lanes 6&8 – Ileal samples of necropsied goat kids suspectedly died of GARV showing positive for VP6 gene of GARV, Lane 7- NTC.*

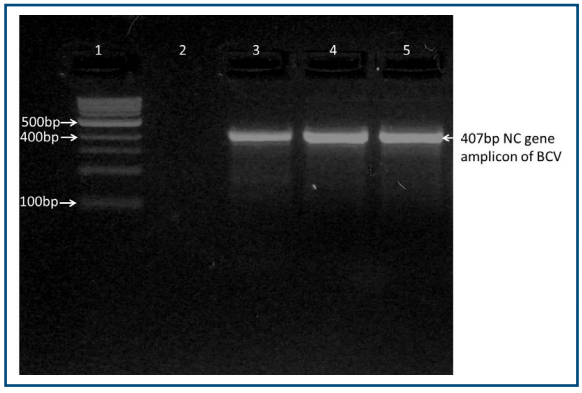

Figure 5. *Gel electrophoresis of reverse transcription PCR (RT-PCR) targeting Nucleocapsid protein gene of Bovine Corona virus (BCoV). Lane 1- 100bp DNA ladder, Lane 2- No template control, 3-4 - Field diarrheic samples of goat kids collected from farmers flock , Lane 5 – positive control RNA of BCoV.*

Sequencing and molecular characterization of GARV isolates from diarrheic goat kids

Three GARV isolates viz., CIRG F2, CIRG 1873 and CIRG 1841 obtained from caprine neonatal diarrhea from CIRG farms were sequenced for VP6 gene. There were two major branches with each having two sub-branches, clades and multiple taxa. CIRG F2 strain of GARV was placed in a sub-branch of unique clade, and was closely related to the bovine and human GARV strains. The other sub-branch of the major branch contained multiple taxa of various clades containing bovine and porcine GARV strains, where CIRG 1873 and CIRG 1841 taxa were placed in a separate clade. The second major branch contained two clades predominantly from human and bovine GARV isolates. This shows that the CIRG F2 might be in a different genotype compared to the other two CIRG isolates, and requires complete genotype mapping to identify their origin (Fig. 6).

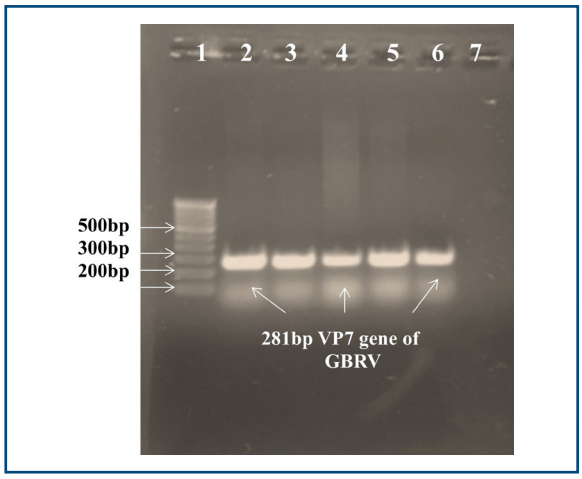

Figure 4. *Gel electrophoresis of reverse transcription PCR (RT-PCR) targeting VP7 gene of Group B rota virus (GBRV). Lane 1-100bp DNA ladder, Lane 2- GBRV positive control RNA, Lanes 3-4 – Field diarrheic samples of goat kids collected from farmer's flock, Lanes 5-6 – faecal swabs of goats collected from organized livestock units of CIRG, Lane 7- NTC.*

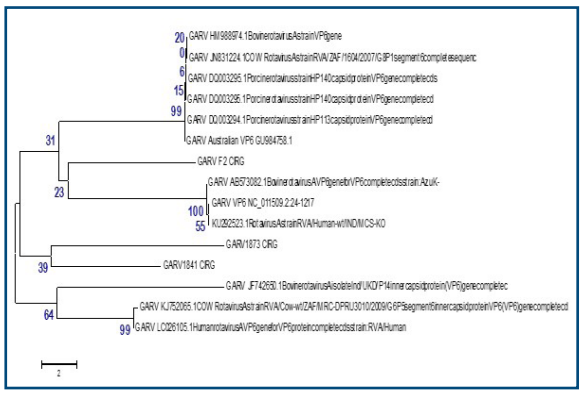

Figure 6. *Phylogenetic analysis of three caprine isolates having GARV VP6 gene with other major GARV isolates from Human and other species.*

Discussion

Neonatal enteritis is an important disease that causes deaths of animals before 3 weeks of age, and results in significant economic losses. Viral agents can predispose the young animals to the secondary infections in the gastrointestinal tract, especially in lambs and goat kids younger than 21 days. There is still little knowledge about enteric viruses and their effects including the prevalence and geographic distribution in small ruminants. Hence, in the current study molecular diagnostics were used as a tool to carry out epidemiological investigations with the aim of detecting rotavirus and coronavirus presence goat kids and lambs and its association with viral enteritis.

In the present study, a much higher proportion, viz., 14.57% of faecal samples from diarrheic neonatal goat kids were positive for specifically for GARV41.18% animals positive in necropsied cases,

while 6% positive in asymptomatic goat kids.

Dey *et al*. (2007) reported rotavirus in 8.68% of the faecal specimens in Black Bengal goats affected with diarrhoea, which is lower than that of our findings. GBRV in the present study was detected in 1.18% samples in diarrheic animals, 11.76% in necropsied goat kids and no positivity in healthy goat kids screened by VP7 gene based osRT-PCR. The positivity for GBRV was much lesser compared to GARV, but a comparative association of GBRV against GARV with the pathological manifestations is important to understand rotaviral group-specific differences in pathogenesis of enteritis in goat kids.. Also there is no study available that specifically provides data on GBRV incidence in goats based on antigen based diagnostic assays. A few addressed in goats earlier but that too only in GARV like Reddy *et al*., 2014, studied on prevalence of group A rota virus (GARV) in diarrhoeic goat kids by RNA-PAGE and RT- PCR technique. The overall prevalence rate of GARV was found to be 15.0% and 13.33% by RT-PCR and RNA-PAGE respectively. The study revealed that there was high prevalence of GARV in diarrhoeic goats which can pose threat to animals as well as humans.

Previously, many studies assessed the relevance of BCoV as the primary pathogen in the neonatal diarrhoea and the associated mortality (Ammar *et al*. 2014). In the present study, the occurrence of BCoV was 7.48% clinically diarrheic animals, 23.53% from necropsied goat kids died due to enteritis and 2% from apparently healthy goat kids. As per the previous study, the prevalence of BCoV was 11.76% in the clinical diarrheic calves aged below 3 months (Rai *et al*., 2011). A prevalence rate of 3-20% has been recorded for coronavirus in calf diarrhoea (Mayameei *et al*. 2009).

Gumusova *et al*. 2007 reported 41.12% seropositivity against BCoV in the goat sera from Turkey. In Ghana, the prevalence of BCoV in the entire animal population was 0.3% (4/1,498), in goats 0.2% (3/1,498) and in cattle 0.6% (1/1,498) (Burimuah *et al*. 2020). Faecal samples from clinically diarrheic calves (less than 3 months age) from Northern India when screened for the presence of BCoV revealed an incidence rate of more than 14% (15/101) with ELISA and about 20% (20/101) by RT-PCR (Hansa *et al*. 2012). Using sandwich antigen ELISA Kits, the overall prevalence of the BCoV in calves (below 3 months age) was observed in 0.83% (03/360) cases in Central India and 0.87% (04/456) cases were found positive from North India (Singh *et al*. 2019).

Human origin strains of rotavirus were differentiated from bovine origin strains based on NSP4 geno-group A sequences of AD63, which produced the homologous identity close to 90 per cent and 82 per cent with the compared RV5 and B223 strains, respectively (Rajendran *et al*. 2014). In another gene linkage based study conducted by Itturiza-Gomara *et al*. (2003), it was found that rotaviruses possess either SGI/NSP4A or SGII/NSP4B type gene linkage specificities in human and animal strains and the VP6 sequence of AD63 is clustered with SGI strains of animal origin, while the NSP4 genes are clustered with human-strain based genogroup A. This adds leverage to our point that there is a possibility of a reassortment between rotaviruses of animal and human origin, as identified in the Figure. 4; which clearly shows the associations between our strains (CIRG F2, CIRG 1873 and CIRG 1841) with the human strains and the porcine origin GARV-VP6 gene. To reiterate, in our phylogenetic analysis, two major branches were observed, where CIRG F2 strain was closely associated with bovine and human GARV strains, indicating the relevance of genetic re-assortment and its zoonotic potential. Two more strains viz., CIRG 1873 and CIRG1841 were placed in a clade genetically close to the porcine GARV isolates. This shows the dynamic nature of the circulating strains.

A total of 304 faecal samples were collected, out of which, 254 were diarrheic samples. Among these, 232 were from goat kids, 22 from lambs, 50 from non-diarrheic goat kids and 17 tissue samples from necropsies conducted from different outbreaks and farms. Based on conventional RT-PCR 14.57% were positive for GARV, followed by 7.48% for BCoV and 1.18% for GBRV. While in lambs, the incidence of GARV is comparatively higher with 22.7%, with no incidence of either GBRV or BCoV. Even among the goat kids that suspected died of enteritis based on the gross pathology lesions, 17.2% detected presence of GARV. This highlights the possibility of genetic re-assortment leading to change in the host-specificity or increased host spectrum.

However, there is a dearth in reports of rotavirus typing from animals in north Indian states. So an attempt was made to characterize the isolates from this study in North India and sequence their VP6 encoding genes to determine the phylogenetic relationship and elucidate the zoonotic potential of virus due to possible re-assortment events between human and animal rotaviruses.

To conclude, the current study could reveal some important features of the enteric viral affections in small ruminants. The GARV were found to be the most common enteric pathogen followed by BCoV and lastly GBRV. The GARV emerged as the most important viral enteric pathogen, that was associated with clinical diarrhoeic cases as evidenced by the conventional RT-PCR assay and sequencing based character. This indicates the fact that GARV needs attention in field conditions leading to diarrhea, slower growth rate and mortality in goat kids, which can only be addressed by vaccination and hygiene measures.

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