Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 956-960

Received: 04-05-2019
Accepted: 06-06-2019

UM Tumlam
Department of Veterinary Microbiology and Animal Biotechnology Nagpur Veterinary College, MAFSU, Seminary Hills Nagpur, Maharashtra, India

VC Ingle
Department of Veterinary Microbiology and Animal Biotechnology Nagpur Veterinary College, MAFSU, Seminary Hills Nagpur, Maharashtra, India

Dhruv Desai
Department of Veterinary Microbiology and Animal Biotechnology Nagpur Veterinary College, MAFSU, Seminary Hills Nagpur, Maharashtra, India

SR Warke
Department of Veterinary Microbiology and Animal Biotechnology Nagpur Veterinary College, MAFSU, Seminary Hills Nagpur, Maharashtra, India

Correspondence
UM Tumlam
Department of Veterinary Microbiology and Animal Biotechnology Nagpur Veterinary College, MAFSU, Seminary Hills Nagpur, Maharashtra, India

Molecular characterization and phylogenetic analysis of rotavirus of human infants, calves and piglets

UM Tumlam, VC Ingle, Dhruv Desai and SR Warke

Abstract
Rotavirus has been registered as one of the common and worldwide prevalent causative agent of enteric illness in newborn of most mammals and poultry species. The infection brings about severe symptoms of diarrhea in lambs, pigs and calves. In the present study out of 58 cattle & buffaloes calves fecal sample 04 (6.89%), 50 human infants stool samples 18 (36.00%), 71 piglets fecal samples 07(9.85%) were screened by rapid antigen detection kit. It was observed that out of 4 faecal samples from calves, 1 sample and out of 18 human stool samples, 07 samples out of 7 piglets 3 samples were found positive for RNA-PAGE. Representative PAGE positive fecal and stool samples, were selected for RT-PCR. The VP4 & VP7 genes of one calve sample were successfully amplified by RT-PCR with expected size of1011bp & 864bp. out of seven only six human infants samples amplify for VP7 gene, 3 out of 7 human infant sample amplify for VP4 gene and 3 out of 3 piglet samples amplify for VP4 & VP7 gene with the expected size of 1062 bp & 876 bp were confirmed positive for Rotavirus infection. One sample from calve and one from human infants, one from piglets were sent for sequencing. The phylogenetic analysis was carried out and it was confirmed that the positive samples were showing relatedness with the Rotavirus sequences taken from genebank and after sequence analysis, it was found that the piglets positive sample showed its relation with human rotavirus. This is indicative of trans-species transmission of rotavirus can occur. This work indicates that the migration of rotavirus throughout the country is possible.

Keywords: Rotavirus, human infants, calve, piglets, phylogenetic analysis

Introduction
Diarrheal diseases are mainly attributed to enteric viruses like *rotavirus, norovirus, astrovirus, adenovirus* and bacteria like *Salmonella, E. coli and Campylobacter*. Among the enteric viruses, *rotavirus* has been identified as the most common cause of severe gastroenteritis in children and young ones of animals. According to World Health Organization estimates 5.27 million children aged below 5 years die every year from vaccine-preventable rotavirus infections, most of these children are from low-income countries (WHO, 2012) [13] Rotavirus is the most common cause of severe gastroenteritis in animals and is recognized as the single most significant cause of severe gastroenteritis, malnutrition and diarrhea, affecting a wide range of mammalian and avian species (Estes MK and Kapikian AZ, 2007) [2] The situation remains stern in India with rotavirus diarrhea leading to an estimated 1.22–1.53 million deaths, 4.57–8.84 million hospitalizations and 2 million hospital visits in children below 5 years of age, every year. India pays out Rs.2.0–3.4 billion annually to treat rotavirus infections (Jacqueline *et al.*, 2009) [5] Likewise, rotavirus-associated enteritis is a major problem in young calves. Besides causing infection in cattle and buffalo calves, rotaviruses also affect piglets, foals, lambs, and young ones of pet animals and poultry. The virus affects young animals resulting in huge economic loss to farmers in terms of cost of treatment and mortality. Genus Rotavirus belongs to the family Reoviridae. Rotaviruses are non-enveloped double-stranded RNA viruses about 70 nm in diameter and possess icosahedral symmetry. The genome consists of 11 segments of dsRNA of molecular weight ranging from $2.0 \times 10^5$to 0.2×10^6, that code for 6 structural proteins (VP1, VP2, VP3, VP4, VP6 and VP7) and 6 non-structural (NSP1-NSP6) protein. To date, at least 27 G-types, 35 P-types and 42 different G–P type combinations have been detected. Matthijnssens *et al*., 2011) [9] Therefore, the present study was conducted considering the zoonotic importance of Rotavirus, targeting VP4 & VP7 gene and phylogenetic analysis of the positive PCR samples of calves and human infants, piglets was performed and thereby confirmed Rotavirus infection.

Materials and Methods

Sample collection

Bovine fecal samples of cattle calves (180), buffaloes calves (31), human infants (50) and piglets (104) between the age group of 0-1yrs were collected from diarrhoeic animals from different regions of Maharashtra during January 2016 to October 2017. Approximately 5-10gms of sample was collected in a sterile, screw capped containers. At the time of sample collection, date of collection, age, clinical signs, important clinical history was recorded. The samples were transported to the laboratory in container containing ice bag and stored at -20 °C till processing. Each of the fecal samples was suspended in 10% phosphate buffered saline (PBS, pH 7.2), clarified by centrifugation at 8000 x g for 10 min at 4 °C and supernatants were collected and stored at – 20 °C till further use.

Extraction of Rota viral ds RNA

The dsRNA of Rotavirus was extracted from faecal and stool samples using TRIzol method Jadhav *et al. (*2009) [6] as per the manufacturer's protocol. The isolated RNA was then used for further downstream applications.

Polyacrylamide Gel Electrophoresis

The RNA extracted from the faecal sample was subjected to ribonucleic acid-poly acrylamide gel electrophoresis (RNA-PAGE) as per the procedure described by Laemmli (1970) [8] & Sambrook and Russel (2001) [10] in separating gel of 8.0% and stacking gel of 5.0% concentration were used for the detection of rotavirus by RNA-PAGE.

Silver staining of dsRNA in polyacrylamide gel

For rapid detection of sample positive for Rotavirus, the typical pattern of migration of 11 different segments of rotavirus RNA were observed via silver staining as described by Svensson *et al.* (1986) [11] The gel was constantly run for 15 min at 60V followed by 110 V for approximately 4 hours.

Reverse transcriptase-polymerase chain reaction

The dsRNA was subjected to reverse transcription as per the protocol given by Isegawa *et al.,* (1993) [4] The synthesized cDNA was stored at -20C till further use. For the detection and confirmation of group A rotavirus, amplification of partial length VP4 &VP7 gene was carried out. The reverse transcriptase polymerase chain reaction (RT-PCR) was conducted for PAGE positive samples. The cDNA synthesis was carried as per the following protocol using cDNA synthesis kit from High-Capacity cDNA Reverse Transcription Kits of Applied Biosystems. The cyclic conditions for VP7 &VP4 gene were Initial denaturation at 95 ^{0}C for 8 min 1cycle respectively followed by 35 cycles of denaturation at 94 ^{0}C for 45 sec, Annealing at 52^{0}C for 45 sec, extension at 72 ^{0}C for 90 sec and one cycle of the final extension at 72 ^{0}C for 10 min 1cycle. The samples were hold at 4 ºC. Initial denaturation at 94 ^{0}C for 4 min 1 cycle 1 cycle respectively followed by 35 cycles of denaturation at 94 ^{0}C for 1 min, Annealing at 48 ^{0}C for 2 min, extension at 72 ^{0}C for 2 min and one cycle of the final extension at 72 ^{0}C for 10 min cycle. The PCR products were stored at 4 ºC and run on agarose gel for the checkup of the amplicon size and amplicon quality. The sequences and nucleotide position of oligonucleotide primers are shown in Table 1.

Table 1: The sequences and nucleotide position of oligonucleotide primers

Primer	designation Sequences (5'-3')	Amplicon size	Reference
Bov9Com3 (+)	TGT ATG GTA TTG AAT ATA CCA C	1011 bp	Isegawa *et al.* (1993) [4]
Bov9Com5 (-)	TCA CAT CAT ACA ACT CTA ATC T		
Bov4Com5	5´TTCATTATTGGGACGATTCACA 3´	864bp	
Bov4Com3	5´CAACCGCAGCTGATATATCATC 3´		
BEG9 1-28	5′GGCTTTAAAAGAGAGAATTCCGTCTGG3′	1062bp	Gentsch *et al.* (2005) [3]
END9 106-136	5′GGTCACATCATACAATTCTAATCTAAG3′		
Con3 11-32	5′TGG CTT CGC CAT TTT ATA GAC G3 3′	876bp	
Con2 868-887	5′ATT TCG GAC CAT TTA TAA CC 3′		

Agarose gel electrophoresis

The PCR amplified products were analyzed on agarose gel electrophoresis (AGE). The amplified products were analyzed by electrophoresis on 1% agarose, and analyzed and photographed by a gel documentation system (BIO-RAD, USA). RT- PCR positive, one calve sample & one human infants sample, one piglet sample was sent for sequencing. The sequences were analyzed using BLAST (Basic Local Alignment Search Tool) and the Clustal-W (CLUSTAL2.1multiple sequence alignment) to generate sequence alignment reports. Molecular Evolutionary Genetic Analysis (MEGA) version 7.0 was used for construction of phylogenetic tree. The bootstrapped phylogenetic tree was constructed using Neighbor-joining method.

Results and discussion

In the present study out of 58 cattle & buffaloes calves fecal samples screened, 04 (6.89%) were found to be positive. The per cent positivity of 10.26% (4/39) was recorded in early days of 0-1 month and Out of 50 human infants stool samples tested 18 (36.00%) found to be positive for rota virus infection. The per cent positivity of 34.88% (15/43) was recorded in infants of more than 6 months and Out of 71 piglets fecal samples screened, 07(9.85%) were screened by rapid antigen detection kit. It was observed that on RNA PAGE analysis of samples revealed one sample of calves and 7 samples of human infants, 3 sample of piglets to be positive with typical migration pattern of 11 segments of rotavirus RNA.

All the positive samples showed a typical migration pattern of 4:2:3:2 type, which is of group A rotavirus, with segments 2, 3 and 4 migrating close together, segments 7, 8 and 9 migrating as a triplet and segments 10 and 11 apart. The VP4&VP7 genes of one calve were successfully amplified by RT-PCR with expected size of1011bp &864bp (Fig 1) and out of seven only six human infants samples amplify for VP7 gene, 3out of 7 human infant sample amplify for VP4 gene and 3 out of 3 piglet samples amplify for VP4 &VP7 gene with the expected size of 1062 bp &876 bp for both species(Fig 2&3). Similar findings were reported by Wani *et al.,* (2004) [12] who screened 10 diarrheic fecal samples from four to forty-five-day old calves positive for group A

rotavirus. Of them 6 rotavirus were successfully amplified using VP7 genes based RT-PCR of the expected size (1,011 bp). Ahmed *et al.,* (2017) [1] found that 36.22% (71/196) of fecal samples of both diarrheic and non-diarrheic calves with age group up to 4 months from different places of Assam, India found to be positive for both VP7 and VP4 genes of group A bovine rotavirus by RT-PCR. Kumar *et al.,* (2011) [7] reported an increased false positive rate with RNA-PAGE (40) as compared to only (15) for RT-PCR when tested fecal samples of bovine calf, children, piglets and pigs in together. But overall result indicated that it is tough to put RT-PCR in direct detection method as compared to RNA-PAGE simply because of large product size and sequence variations in rotavirus for VP7 gene. The PCR product of one human infants and one calve representative samples were sent for sequencing and sequences were brought. (fig.4) Using BLAST, the sequences of the both samples (one human and one calve) obtained were confirmed and compared to the sequences available in the GenBank database. The resulted sequences were also deposited in the GenBank database with the Accession numbers LC377483, LC377479, LC377480, LC377484, LC377485, LC377486 respectively.

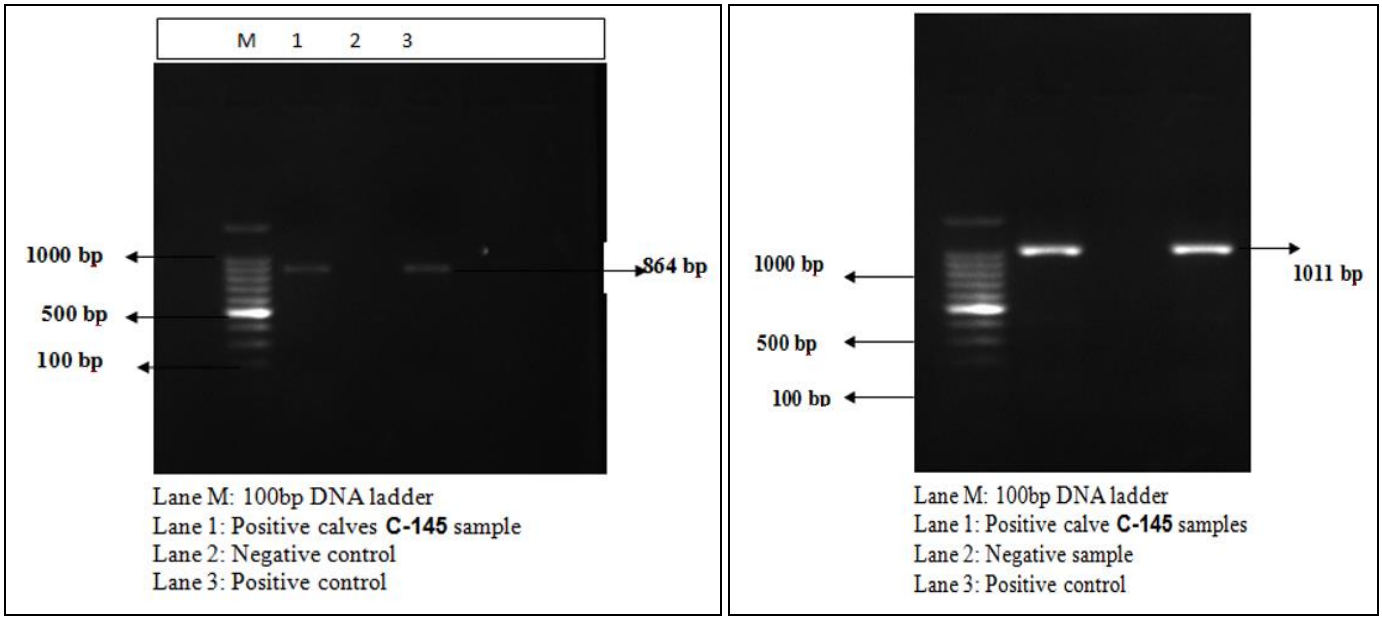

Fig 1: VP4&VP7 RT-PCR showing positive cattle calve sample resolved on agarose gel along with molecular weight marker

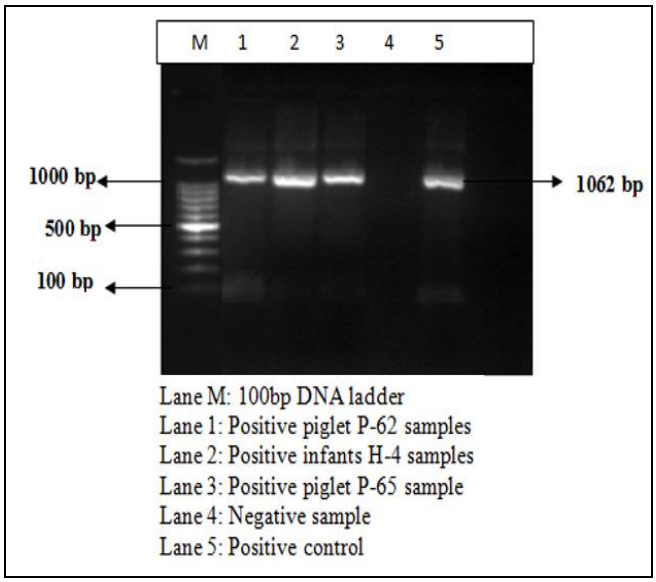

Fig 2: VP7 RT-PCR showing positive Piglets and Human infants sample resolved on agarose gel along with molecular weight marker

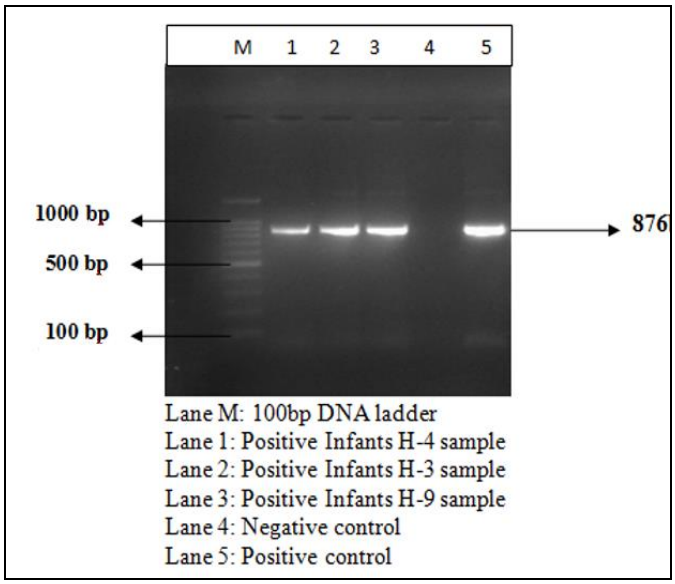

Fig 3: RT-PCR showing VP4 positive Human infants sample resolved on agarose gel along with molecular weight marker

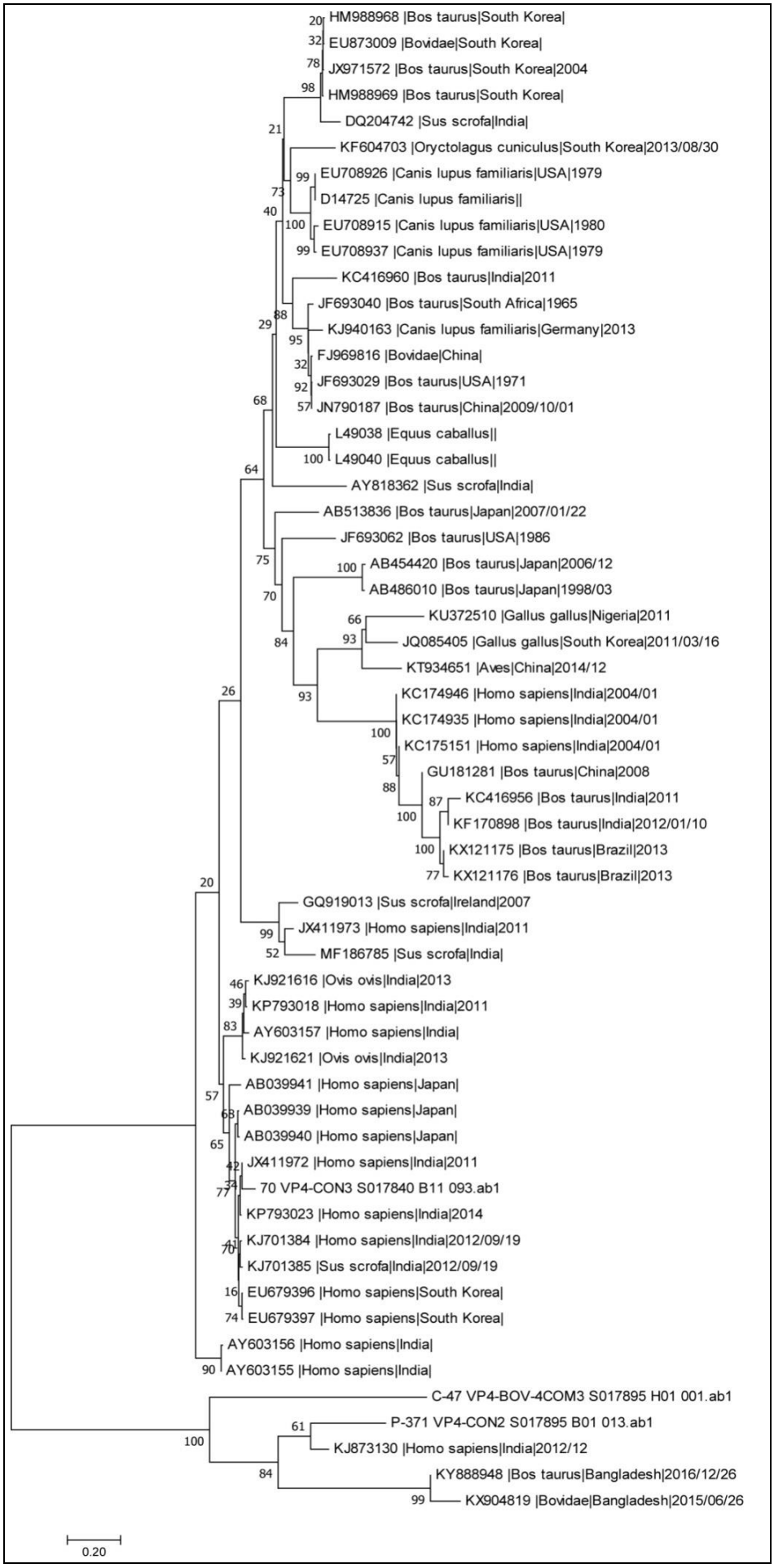

Fig 4: Phylogenetic tree of Partial gene sequence of Calf, Piglets &Human infants

Conclusion

The present study records the detection and molecular characterization of VP7 and VP4 genes of group A rotaviruses (GARV) circulating in different animal species viz. cattle and buffalo calves, piglets, and human infants there is evidence of the same strain of this viruses circulating in India. From the phylogenetic study it was confirmed that the phylogenetic analysis of VP4 gene of cattle calf (C- 47) and piglet (P-371) came under same cluster showed 100% homology with human and bovine with Accession Number KJ873130|India|2012/12,KY888948/Bangladesh|2016/12/26, KX904819/Bangladesh|2015/06/26 The phylogenetic analysis of VP7gene of piglet (P-368) VP7-BEG9 in separate node with one cluster showed homology with cattle calf sequence reported from China, India, Japan, South Korea which showed possibility of zoonotic transmission. Phylogenetic analysis showed not much sequence variation between rotavirus from human infant (H-122) and cattle calf (C-145). The piglets and human rotavirus samples were clustered together and after sequence analysis, it was found that the piglets positive sample showed its relation with human rotavirus. This is indicative of trans-species transmission of rotavirus can occur. This work indicates that the migration of rotavirus throughout the country is possible. VP4, VP7 RT-PCR positive samples showed interspecies cross relationship. Sequencing of some of the genotypes and subsequent analysis revealed varying degree of similarity with world and Indian isolates.

Acknowledgement

The authors are grateful to the Associate Dean, Nagpur Veterinary College, Nagpur for providing necessary facilities to undertake the present study**.**

References

1. Ahmed SP, Hazarika RA, Bora DP, Tamuly S. Detection and Genotypic Characterization of Rotavirus from Bovine Calves of Assam, A North Eastern State of India. The Journal of Animal & Plant Sciences. 2017; 27(2):439-445 Issn: 1018-7081.
2. Estes MK, Kapikian AZ. Rotavirus in Fields Virology. 5th edition. 2007; 2:1917-1974.
3. Gentsch JR, Laird AR, Bielfelt B, Griffin DD, Banyai K, Ramachandran M *et al*. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. Journal of Infectious Diseases. 2005; 192:S146-S159.
4. Isegawa Y, Nakagomi O, Nakagomi T, Ishida S, Uesugi S, Ueda S. Determination of bovine rotavirus G and P serotypes by polymerasechain reaction. Molecular and Cellular Probes. 1993; 7:277-284.
5. Jacqueline E, Shobha C, Douglas HE, Rajiv S, Bery G, Sasirekha R *et al*. Disease and economic burdem of rotavirus diarrhea in India. Vaccine 27: 18-24 Ahmed S. P., Hazarika R. A., Bora D. P. And Tamuly S. (2017) Detection And Genotypic Characterization Of Rotavirus From Bovine Calves Of Assam, A North Eastern State Of India. The Journal of Animal & Plant Sciences. 2009; 27(2):439-445 Issn: 1018-7081
6. Jadhav MB. Detection and molecular characterization of bovine and porcine rotaviruses from Nagpur region, M.V. Scthesis, MAFSU, Nagpur, 2009
7. Kumar M, Bhilegaonkar KN, Agarwal RK. Prevalence and characterization of rotavirus from faecal samples of children and animals Indian Journal of Animal Sciences 2011; 81(10):993-999.
8. Laemmli UK. Cleavage of Structural Proteins during the Assembly of Head of Bacteriophage T4. Nature. 1970; 227:680-685.
9. Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Banyai K, Brister JR *et al*. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Archives of Virology. 2011; 156(8):1397-1413.
10. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. 3rd edn. Cold Spring Harbor Laboratory Press, New York, 2001, 2,
11. Sevensson L, Uhnoo I, Garndien M, Wadell G. Taxonomy of Viruses (Eds. Fauquet, C *et al.*). Elsevier, Academic Press, Technology Oie. 1986; 23(3):931-936.
12. Wani SA, Bhat MA, Ishaq SM, Ashrafi MA. Determination of bovine rotavirus G genotypes in Kashmir, India. Rev. Off. Int. Epizoot., 2004; 23:931-936.
13. WHO. Health situation in the south-east Asia region 2011-2012. Report from regional office of SEAR New Delhi, 2012.