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Research Article

MOLECULAR DETECTION OF ROTAVIRUSES IN HUMAN AND ANIMALS IN WESTERN MAHARASHTRA

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ABSTRACT

Neonatal diarrhoea caused by rotavirus results in noteworthy economical losses due to extraordinary morbidity, mortality, treatment cost along with reduced growth rate of diseased animals. In present research work, a total of 245 animal faecal samples were collected from calves, buffalo calves, lambs, kids and piglets suffering with clinical signs of diarrhoea typical to the rotavirus infection from five different districts of Western Maharashtra region. Similarly, 61 stool samples from human infants and children up to 5 years with a history of yellow to greenish diarrhoea, a typical symptom of rotavirus infection were also collected from the same area of research work. By using RNA-PAGE technique, overall occurrence of rotavirus infection in humans and animals was noted to the tune of 49.18% (30/61) and 4.08% (10/245). After screening the cattle and buffalo samples, the occurrence was observed as 15% (09/60) and 1.81% (01/55), respectively. The experiment revealed highest percentage of overall prevalence in animal species in Solapur (9.3%) district followed by Satara (8.82%) and Kolhapur (4.47%) districts, while no positivity was observed in Sangli and Pune districts. In case of human samples processed, high positivity was observed in Satara (70%) district, after that 50% occurrence was noted in Sangli, Solapur and Pune districts each, followed by Kolhapur district with 35.29% positivity. While, no significant sex-wise difference was observed in rotavirus prevalence exhibited in human and animal species. However, the current study found a significant prevalence of rotavirus infection (49.18%) in children of Western Maharashtra, highlighting a concerning scenario from the public health point of view and requiring a significant preventive action in the study area.

Keywords: Neonatal diarrhoea, RNA-PAGE, Rotavirus, Western Maharashtra

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Rotavirus infections are extremely common and have been identified in almost all mammalian and bird species on the planet. Besides, it is the most common cause of acute dehydrating diarrhoea, particularly affecting infants and young children (Luchs and Timenetsky, 2016). Moreover, bovine neonatal diarrhoea (BND), which primarily affects calves in their first month and causes high economic loss due to costs associated with pharmaceutical treatments, decreased growth averages in calves, and high morbidity and mortality in cattle, is caused by rotavirus infection, one of the main agents involved in the development of the disease (Jenny *et al.*, 1981; Cashman *et al.*, 2010). The pathogen is transmitted mostly by the faecal - oral and oral routes, after faecal traces or other contaminated material enters in to the digestive tract of vulnerable hosts. Group A rotaviruses are the utmost responsible cause of acute gastroenteritis amongst children less than 5 years of age and young ones of different mammalian species. About 78,000 deaths per year in Indian children under the age of five are attributed to rotavirus, which is a significant cause of severe diarrhoea requiring hospitalization. This fact is suggestive of the highly virulent nature of the pathogen (Kumar *et al.*, 2020). Previously, Monica *et al.* (2007) reviewed studies conducted in India between 1990 and 2005 and determined that 20.8% of hospitalised children with diarrhoea had rotavirus illness. Following a rotavirus surveillance, Kumar *et al.* (2018) reported the occurrence of two rotavirus strains in cattle calves from two different geographical locations in India that were closely related to or resembled artiodactyl or human rotavirus strains, suggesting the potential role of interspecies transmission and reassortment events. Ribonucleic acid based polyacrylamide gel electrophoresis (RNA-PAGE) was regarded as the gold standard because it had a number of benefits, including as high sensitivity, the ability to detect minute amounts of virus, clear results, and the ability to differentiate between various rotaviruses (El-Ageery *et al.*, 2020). These facts served as the impetus for designing and carrying out the current research project, which aims to conduct a molecular surveillance study of rotavirus infections in humans and domestic animals simultaneously to look for any interspecies transmission in the Western region of Maharashtra state of India, a region that has been largely understudied in this context so far.

MATERIALS AND METHODS

Collection of faecal and stool samples: From October 2018 to April 2019, a total of 245 faecal samples were collected from calves (60), buffalo calves (55), lambs (40), kids (40) and piglets (50) suffering with watery diarrhoea in to sterile 20 ml sample collection vials, from the local

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dairy farms, animal farms and the government animal hospitals of villages of different Tehsils of Western Maharashtra region (Table 1). Besides, 61 stool samples of children suffering with diarrhoea were also collected from civil hospitals, primary health centres and district civil hospitals (Table 2). The samples were transferred to the laboratory on ice and were stored at -20º C until processing. The sex-wise distribution of sources of all samples is illustrated in Table 2.

Sample Processing: The 10% suspension of faecal and stool samples were prepared in 0.06M phosphate buffered saline (PBS) of pH 7.2, followed by centrifugation at 12000 RPM for 30 mins to remove coarse particles and debris. Then supernatant was stored at -20ºC until further use.

Extraction of RNA: The extraction of RNA for rotavirus was carried out by TRIzol method as per Gill *et al.* (2017) and Gentsch *et al.* (2009). The adopted protocol was as follows in brief, initially the 10% faecal/stool suspension was vortexed and allowed to settle at room temperature for 30-60 minutes before use. The stool suspension was clarified by centrifugation at 5000 rpm in a mini centrifuge for 5 min at room temperature. The 250µl supernatant of the stool and faecal sample was transferred to a sterile 1.5 ml Eppendorf tube and 750 µl of TRIzol reagent was added to it. The tube was vortexed for 30 sec and incubated at room temperature for 5 min. After the incubation, 200µl of chloroform was added to each sample and vortexed for 30 sec., followed by incubation for 3 min. further centrifugation was at 12000 rpm for 5 minutes at 40°C for separation of phases. The 450µl clear, upper aqueous phase was transferred to sterile new Eppendorf tube avoiding white interface and pink organic phase. To the above mixture 700 µl of ice cold isopropanol (Isopropyl alcohol) was added and gently mixed 4-5 times by turning the tube upward and downward followed by incubation at -20° C for 20 minutes. The tube was then centrifuged at 12000 rpm at 40° C for 15 minutes to obtain the pellet of double-stranded (ds) RNA. The supernatant was discarded very carefully and pellet was air dried at room temperature. Further, the pellet was resuspended in 20µl of diethyl pyrocarbonate (DEPC) treated RNase free water. The samples were stored at -20° C for further use.

RNA- Polyacrylamide Gel Electrophoresis (RNA-PAGE): The segmented RNA genome was analysed by RNA-PAGE discontinuous buffer system as described by Herring *et al.*, (1982) with slight modifications. The glass plates were cleaned with soap and water and then wiped with Isopropyl alcohol (IPA). IPA was allowed to evaporate. The glass plates were then assembled for gel casting according to manufacturer's instructions. The top level of resolving gel was marked with pen. The stacking gel was loaded top of the resolving gel. The comb was properly inserted into a stacking gel and the gel was allowed to polymerize for 15-30 min. After polymerization of the gel comb was removed and the glass plates were assembled on electrophoresis apparatus. Running buffer was added to tank and glass plates were inserted in to the vertical gel assembly. The wells were filled with 1x Tris-glycine buffer and air was removed.

Electrophoresis: The extracted dsRNA was dissolved in 1X RNA-PAGE sample loading buffers and loaded into the well. Tris-Glycine buffer 1X was used in the Electrophoresis. The gel was run at 120V for 1-2 hrs till the dye reached at lower third of the gel.

Silver staining of the gel: The gel was stained by silver nitrate staining method (Herring *et al.*, 1982). Properly stained gel was sealed in a plastic bag. It was then gently put on the light source for the photography. After staining procedure, 11 bands of the rotavirus depicted in control sample were compared with the test samples. The pattern in control sample was 4:2:3:2 i.e. first six bands (1 to 6) clearly visualized and a single band comprising of 7^{th}, 8^{th} and 9^{th} followed by two separate bands i.e. 10^{th} and 11^{th} indicating the eleven segmented rotavirus.

RESULTS AND DISCUSSION

The most significant route of rotavirus infection is transmission through the faecal-oral route, via contact with contaminated hands, surfaces and objects (Butz *et al.*, 1993). After staining procedure, 11 bands of the rotavirus were observed with the pattern of 4:2:3:2 i.e. first six bands (1 to 6) clearly visualized and a single band comprising of 7^{th}, 8^{th} and 9^{th} followed by two separate bands i.e. 10^{th} and 11^{th} indicated the eleven segmented rotavirus. All positive sample recovered were sequenced for further confirmation.

Occurrence of rotavirus in different species: Amongst the total 306 faecal (245) and stool (61) samples processed by employing RNA-PAGE techniques, a total of 9 cattle, 1 buffalo and 30 human stool samples were turned out to be positive for rotavirus (Fig. 1, Table 1 and 3). Based on these results, the overall prevalence of rotavirus infection in humans and animals was noted to the tune of 49.18% (30/61) and 4.08% (10/245), respectively. Amongst cattle and buffalo, it was observed as 15% (09/60) and 1.81% (01/55), respectively (Table 1 and 3). A high prevalence of rotavirus infection in human was revealed in present investigation.

In animals, in present study an occurrence of 4.08% (10/245) rotavirus infection was observed which is on lower side when compared with a report from Kolkata which reported a prevalence of 10.52% (10/95) Nataraju *et*

Table 1. Overall district and animal species wise occurrence of rotavirus infection in Western Maharashtra region

Sr.No.	Species	Cattle calves			Buffalo calves			Sheep lamb			Goat kid			Piglet			Total		
	District	Cases	rota +ve	%	Cases	rota +ve	%	Cases	rota +ve	%	Cases	rota +ve	%	Cases	rota +ve	%	Cases	rota +ve	%
1.	Sangli	16	00	00	19	00	00	06	00	00	04	00	00	08	00	00	53	00	00
2.	Solapur	11	03	27.27	12	01	8.33	05	00	00	11	00	00	04	00	00	43	04	9.30
3.	Kolhapur	20	03	15	12	00	00	10	00	00	12	00	00	13	00	00	67	03	4.47
4.	Satara	06	03	50	07	00	00	07	00	00	05	00	00	09	00	00	34	03	8.82
5.	Pune	07	00	00	05	00	00	12	00	00	08	00	00	16	00	00	48	00	00
	Total	60	09	15	55	01	1.81	40	00	00	40	00	00	50	00	00			
	Overall rotavirus infection in animals																245	10	4.08

*+ve- positive, %- percentage

Table 2. Sex-wise type of samples collected in Western Maharashtra

Sr. No.	Species	Cases	Male	Female
1.	Children	61	31	30
2.	Cattle calves	60	28	32
3.	Buffalo calves	55	24	31
4.	Sheep lambs	40	24	16
5.	Goat kids	40	24	16
6.	Piglet	50	30	20

Table 3. Overall district wise occurrence of rotavirus infection in human in Western Maharashtra region

Sr. No.	Species	Children		
	District	Cases	rota +ve	%
1.	Sangli	06	03	50
2.	Solapur	14	07	50
3.	Kolhapur	17	06	35.29
4.	Satara	10	07	70
5.	Pune	14	07	50
Overall Rota virus infection in human		61	30	49.18

*+ve- positive, %- percentage

al. (2009), but comparable to a study in Haryana Manuja *et al.* (2008), which had a prevalence of 4.61% (21/455). The lower occurence of rota virus detected in animal group in present study is in agreement with the results obtained by other researchers (Deshmukh *et al.*, 2018; Murni Trisunuwati and Liao, 2016; Basera *et al.*, 2010), attributable to the resemblance of age group and season of sample collection. Deshmukh *et al.* (2018) reported 10.90% rota viral positivity by RNA-PAGE technique in 211 bovine samples collected from Marathwada region which is an adjacent geographical area of present investigation. Murni Trisunuwati and Liao (2016) carried out a study involving screening of faecal samples collected from bovine animals in Taiwan and reported 5% prevalence in calves. Basera *et al.* (2010) noted 11.81% rotavirus positivity in bovine samples from Dehradun and Pantnagar, India.

Table 4. Sex wise rotavirus prevalence in humans and animals by RNA-PAGE

Sr. No.	Species	Cases	Male	Female	RNA-PAGE positive male	RNA-PAGE positive female
1.	Children	61	31	30	15	15
2.	Cattle calves	60	28	32	6	3
3.	Buffalo calves	55	24	31	0	1
4.	Sheep lambs	40	24	16	0	0
5.	Goat kids	40	24	16	0	0
6.	piglet	50	30	20	0	0

Fig. 1. Electropherotyping of RNA-PAGE positive sample revealing banding pattern of 4-2-3-2 which is typically shown by group-A rotaviruses (human, cattle and buffalo)
Lane 1: Human rota virus (HF294)
Lane 2: Human rota virus (HM247)
Lane 3: Cattle rota virus (CF75)
Lane 4: Negative faecal sample
Lane 5: Buffalo rota virus (BF61)
Lane 6: Negative stool sample

The present investigation noticed that in children, 49.18% (30/61) of diarrhoea was linked with rotavirus infection, which is in agreement with diverse site Indian data reported from 2005 to 2007 (Kang *et al.*, 2013) and comparable to worldwide figures (Widdowson *et al.*, 2009). The high prevalence observed in present study contradicts with the results obtained in the study

conducted by Gill *et al.* (2017) in which, 6.17% of prevalence was reported from 162 stool samples collected from Punjab. This may be attributable mainly to the difference in geographical area and season of sample collection. High percentage of prevalence noted in present investigation concords with the report by Durmaz *et al.* (2014) wherein, they observed 78.2% in 1644 human samples screened by employing RT-PCR. Amongst the samples tested, 38.7% of rota virus positivity was observed in children in the age group of 25 to 36 months. This concordance is attributable to similarity of age group targeted for sample collection. Similar results were also found by Dubal *et al.* (2015) who noted 34% of prevalence of rotavirus in children from Shilong, India.

Occurrence of rotavirus in different districts of Western Maharashtra region: A total of 306 samples (faecal 245, stool 61) from five different districts of Western Maharashtra (viz. Sangli, Solapur, Kolhapur, Satara and Pune) were collected and processed for the detection of rotavirus by RNA-PAGE method. The experiment revealed highest percentage of overall prevalence in animal species in Solapur (9.3%) district followed by Satara (8.82) and Kolhapur (4.47) districts, while no positivity was observed in Sangli and Pune districts. In case of human samples processed, high percentage of occurence was observed in Satara (70%) district, after that 50% prevalence was noted in Sangli, Solapur and Pune districts each. The prevalence of rotavirus in human samples collected from Kolhapur was observed as 35.29%. Although, the district wise sample size of present investigation is on lesser side, a high positivity observed amongst processed human stool samples, raising a question about present scenario of rotavirus infection with public health concern demands an exclusive study to be undertaken with larger sample size in the area of research work under present investigation (Table 1 and 3).

Sex-wise rotavirus prevalence in humans and animals: In present study, on screening of stool samples from human, it was observed that nearly similar number of male (15/31) and female (15/30) children were found positive for rotavirus infection. Amongst animals, 6 out of 28 male calves and 3 out of 32 female calves showed positivity for rotavirus infection. Besides, 1 buffalo calf amongst 31 turned out positive. Based on results obtained in present study, no significant sex-wise difference was observed in number of rotavirus occurrence exhibited in human (Table 4). The current study's findings of no significant gender differences in human rotavirus infection are consistent with the findings of Alam *et al.* (2011), who found rotavirus in 17/35 male and 18/35 female patients from Mymensingh, Bangladesh. Comparatively higher prevalence in male calves than females found in current investigation concords with the results reported by Gill *et al.* (2017) in which, males (8.73%) were observed more susceptible to Group A rotavirus infection than female (6.31%) calves.

CONCLUSIONS

In the current investigation, overall occurrence of rotavirus infection in humans and animals was noted to the tune of 49.18% (30/61) and 4.08% (10/245), respectively in Western Maharashtra. Among cattle and buffalo samples screened from study area the prevalence was observed as 15% (09/60) and 1.81% (01/55), respectively. The RNA-PAGE technique revealed a significant prevalence (49.18%) of rotavirus infection in children in Western Maharashtra, signaling a serious scenario from the public health perspective and demanding attentive prophylactic intervention in the area.

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