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Research Article

Prevalence of Rotavirus Infection in Children Up To Five Years Presenting With Diarrhea, In Tertiary Care Hospital, Jamnagar, Gujarat (India)

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Abstract: Rotavirus is an intestinal virus belonging to the reoviridae family. Rotavirus is spread through the faecal-oral route by contaminated hands, water or food. The major clinical symptoms are severe diarrhoea and vomiting, including fever. This study, which was conducted in tertiary care hospital, Jamnagar. In my study children less than five years with watery diarrhoea from ward were included. About 15-20 ml of stool specimen was collected screened by Rotavirus Ag (stool) ELISA Kit. Total 170 stool samples from patient of acute diarrhoea were tested for rotavirus antigen and the prevalence of rotavirus diarrhoea was 33.5%. Among positive cases, prevalence was higher in male children (52.6%) than female children (47.4%). Maximum positive cases were found among 7-12 month (51%), followed by 25-60 month (19.3%), followed by 13-18 month (14%), followed by 0-6 month (10.5%), followed by 19-24 month (5.2%). Maximum positive cases (87.71%) of rotavirus were found in top feed children as compare to exclusive breastfeed (12.29%). Maximum positive cases (38.59%) were found in winter season (November to February) although cases were recorded throughout the year. This study revealed that rotavirus infection is the most common cause of acute diarrhoea in infants and young children and it may cause some to severe dehydration in acute diarrhoea in an unvaccinated child. And Study reveal exclusive breastfeed given protection against rotavirus infection.

Keywords: Rotavirus, Watery diarrhoea, Ag ELISA, breastfeed.

INTRODUCTION:

Rotavirus is an intestinal virus belonging to the reoviridae family (Dennehy, P.H. 2000). This type of virus was first described in the 1970s. Rotavirus can cause autumn diarrhoea. Seven types of rotavirus have been described, designated A to G. Among these types, group A causes 90% of the infections observed in human infants and children. Therefore, almost every child <5 years old has been infected by a rotavirus at least once (Velazquez, F.R. *et al.*, 1996). However, a single virus infection strengthens the immunocompetence of an individual and will lower the chance of future infections (Linhares, A.C. *et al.*, 1988).

Diarrheal disorders account for an estimated 1.5 million deaths globally every year making it the second leading cause of child mortality (Ahmad. B, Z. 2011). The burden of diarrhoeal disease is greatest in low- and middle income countries with the highest incidence rates found among children <12 months of age (Liu, L. *et al.*, 2015). Apart from high morbidity and mortality associated with diarrhoeal diseases, diarrhoea also present an economic burden for the developing countries, with more than a third of the hospital beds for children being occupied by patients with diarrhoea, often involving the use of expensive intravenous fluids and drugs (WHO, UNICEF: 2005).

Rotavirus infection affects 95% of children under the age of 5 years regardless of the socio-economic or environmental conditions and leads more frequently to dehydration than other aetiologies (World Health Organisation. 2000-2011; Banerjee, I. *et al.*, 2006; & Chakravarti, A. *et al.*, 2010). Rotavirus infection results in a significant disease burden and economic effect of direct medical costs, loss of work, quality of life and mortality. Vaccination is the only control measure likely to have a significant impact on the incidence of severely dehydrating rotavirus disease (Dennehy, P.H. 2008). Studies estimated that 90,000-

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1,53,000 children die from rotavirus infection in India each year (Jain, V. *et al.*, 2001; Parashar, U. D. *et al.*, 2009).

The major clinical symptoms are severe diarrhoea and vomiting, including fever. However, infections can also be asymptomatic, especially in neonates, older children and adults (Lundgren, O., & Svensson, L. 2001). Cases of asymptomatic infections in older children and adults are probably due to active immunity. Usually all children have become infected several times during the first twenty-four months of life and by the time they reach 5 years of age most children have had repeated infections and developed a life-long lasting immunity to rotavirus disease (Lundgren, O., & Svensson, L. 2001).

Rotavirus is spread through the faecal-oral route by contaminated hands, water or food (Viral Gastroenteritis. 2003). The amount of rotavirus shed in faeces has been shown to be 1010 virus particles/gram of stool (Greenberg, H.B., & Estes, M.K. 2009). There are few studies of infectivity but those indicate that only 10 or less particles are needed for an infection (Greenberg, H.B., & Estes, M.K. 2009; Graham, D.Y. *et al.*, 1987). Probably, as the very infectious norovirus, causing "the winter vomiting disease", rotavirus may also be spread by aerosol through vomits, since droplet spread of aerosolized rotavirus has been shown experimentally, using a mice model (Prince, D. S. *et al.*, 1986).

Very few studies on the prevalence of rotavirus among children have been conducted in tertiary care hospital. This study aimed to identify the proportion of children with acute gastroenteritis infected with rotavirus through systematic sampling over a one year period in a tertiary care Government hospital.

MATERIAL AND METHODS:

Study Area

This study, which was conducted in tertiary care hospital for a period from November 2017 to September 2018.

Study Population

In my study 170 children less than five years with watery diarrhoea from ward were included.

Inclusion Criteria:

i. Children under five years with acute gastroenteritis.
ii. Children below five years with diarrhoea alone.

Exclusion Criteria:

- Children under five years with bloody diarrhoea.
- Children less than five years who acquired diarrhoea during hospitalization (Healthcare Associated Infection/Nosocomial infection)
- Children below five years with chronic diarrhoea (diarrhoea >14 days)
- Children under five year with immuno compromised state.

Clinical History:

Detailed history was taken from the parents/guardian of the patient and entered in the proforma as follows:

Name, age, gender, geographical area (urban/rural), Antenatal history- full term/preterm, birth weight, immunization, Onset and duration of diarrhoea, whether blood stained or not, vomiting, abdominal pain, and fever, Feeds- breast fed/bottle fed, Physical examination of the child to detect signs of dehydration-consciousness, whether the baby taking feeds, active, skin turgor. Macroscopically examination of stool specimen – watery, colour, whether blood stained or not.

Sample collection, transport and storage:

About 15-20 ml of stool specimen was collected in a sterile wide mouthed universal container during acute diarrhoea. Unpreserved samples should be kept at 4 c and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 c until used. Freezing the specimens does not adversely affect the test however, avoid respected freeze/thaw cycles. All dilutions must be made with the diluted wash buffer.

Preparation of Sample:

Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4 Ml of diluted wash buffer, mix well and allow the heavy particulates to settle. For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution).

Procedure (Rotavirus Ag ELISA):

Rotavirus Ag (stool) ELISA Kit was used. ELISA plate reader capable of reading at 450/620-650 nm was used. Ensure all samples and reagents are at room temperature before use. Frozen samples must be thawed completely before use. The rotavirus Ag(stool) sandwich ELISA is an in vitro procedure for the qualitative determination of rotavirus antigen in feces.

Break the number of wells needed (number of samples plus 2 for controls) and place in strip holder. Add 100 µl of the negative and positive control to respective well. Add 100 µl of the stool supernatant to the appropriate test well. Incubate at room temperature for 30 minutes, then wash. Add 2 drops of Reagent 1 (blue solution) to each well. Incubate at room temperature for 5 minutes, then wash. Add 2 drops of Reagent 2 (red solution) to each well. Incubate at room temperature for 5 minutes, then wash. Add 2 drops chromogen to each well. Incubate at room temperature for 5 minutes. Add 2 drops of stop solution to each well. Mix wells by gently tapping the side of the strip

holder with index finger. Read results visually or using an ELISA plate reader.

RESULTS AND ANALYSIS:

The present study, which was conducted in tertiary care hospital for a period from November 2017 to September 2018.

Table 1: Prevalence of rotavirus infection

Sr. No.	Total Samples	Positive Samples	Prevalence
1.	170	57	33.5%

In this study, total of 170 stool samples were taken during study period from November-2017 to September-2018. Among this, 57(33.5%) were found to be rotavirus positive shown in Table 1.

Chart-1

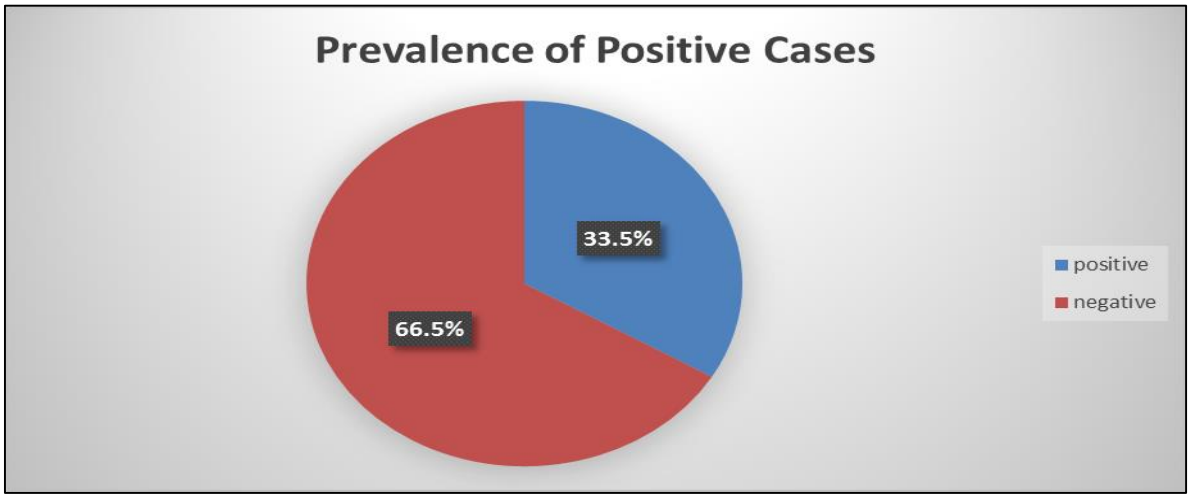


Table 2: Gender wise distribution of rotavirus positive cases

Sr. No.	Gender	Total	Positive cases	Percentage
1.	Male	97	30	52.6%
2.	Female	73	27	47.4%
	Total	170	57	100%

Out of 57 positive rotavirus cases, majority of them were male children 30(52.6%) and 27(47.4%) were female children, showing in Table 2.

Chart-2

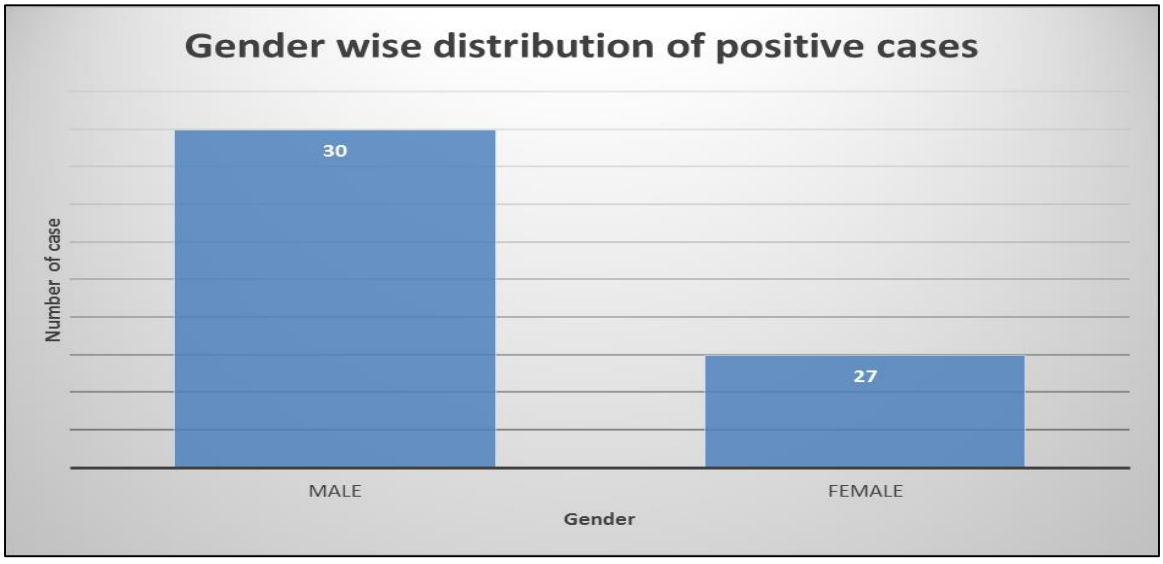


Table 3: Age wise distribution of rotavirus positive cases

Sr no.	Age (in month)	Positive cases	Prevalence
1	0-6	06	10.5%
2	7-12	29	51%
3	13-18	08	14.0%
4	19-24	03	5.2%
5	25-60	11	19.3%
	Total	57	100%

Table 3 shows that among the positive cases, 29(51%) cases were from the age group of 7-12 month followed by 11(19.3%) were from 25-60 month, followed by 8(14%) were from 13-18 month, 6(10.5%) were from 0-6 month and 3(5.2%) were from 19-24 month in my study.

Chart-3

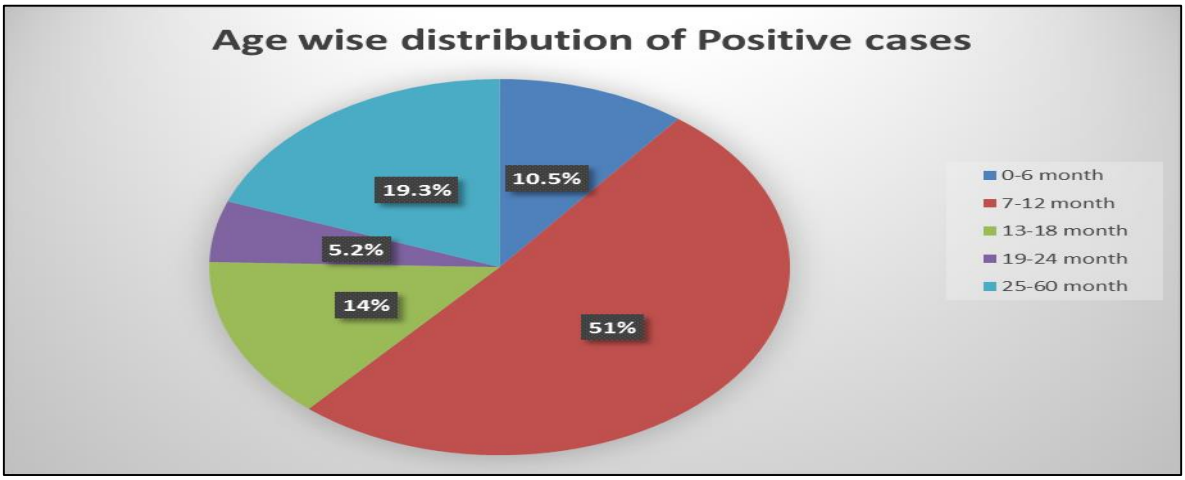


Table 4: Seasonal distribution of rotavirus positive cases

Sr. No.	Month	Positive cases	Prevalence
1	November	07	12.3%
2	December	15	26.3%
3	January	03	5.3%
4	February	02	3.5%
5	March	02	3.5%
6	April	03	5.3%
7	May	02	3.5%
8	June	03	5.3%
9	July	04	7.0%
10	August	05	8.7%
11	September	11	19.3%
	Total	57	100%

Table 4 shows that rotaviral diarrhoea was more prevalent in December month.

Chart-4

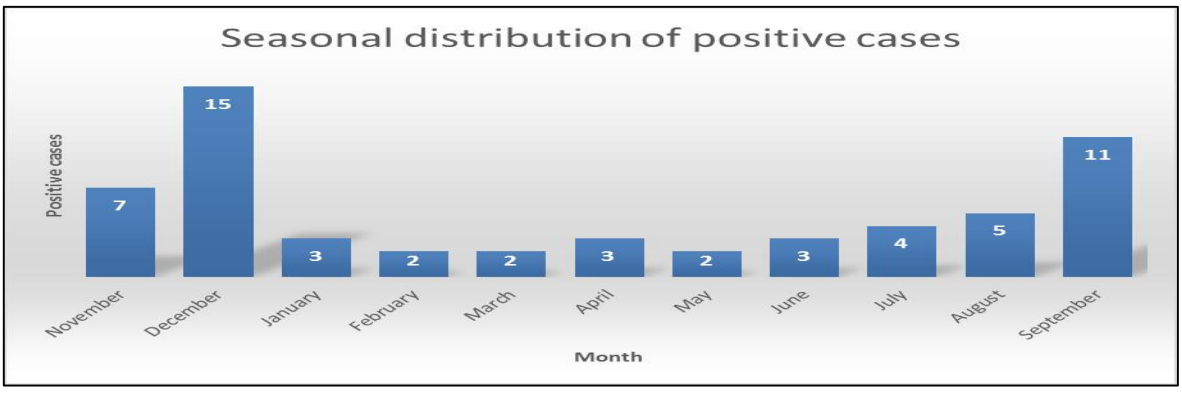


Table 5: Seasonal trends in rotavirus positive cases

Sr. No.	Season	Positive cases	Percentage
1	Winter	27	38.59%
2	Summer	10	12.2%
3	Monsoon	20	35.08%
	Total	57	100%

This table show seasonal trends in rotavirus positive cases. During winter season (November to February) 27(38.59%) cases were found positive which was maximum. During summer (March to June) 10(12.2%) cases were found positive and during monsoon (July to September) 20(35.08%) cases were found positive.

Chart-5

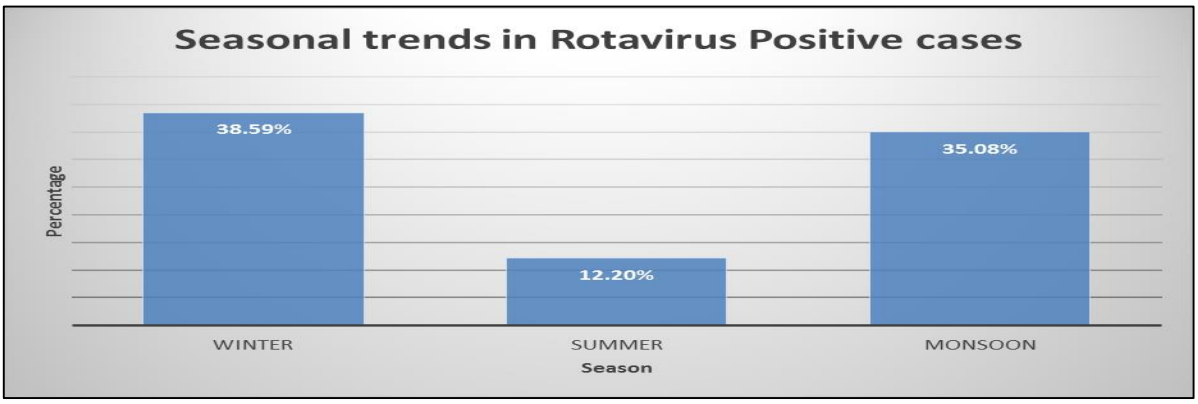


Table 6: Associated multiple clinical findings with watery diarrhoea in rotavirus positive cases

Sr. No.	Clinical feature	Positive case	Percentage
1.	Vomiting	33	57.8%
2.	Abdominal pain	06	10.5%
3.	Fever	23	40.35%
4.	Dehydration	18	31.57%
	Some	14	24.6%
	Severe	04	7.0%

Table 6 shows, associated multiple clinical findings with watery diarrhoea in rotavirus positive cases.

Chart-6

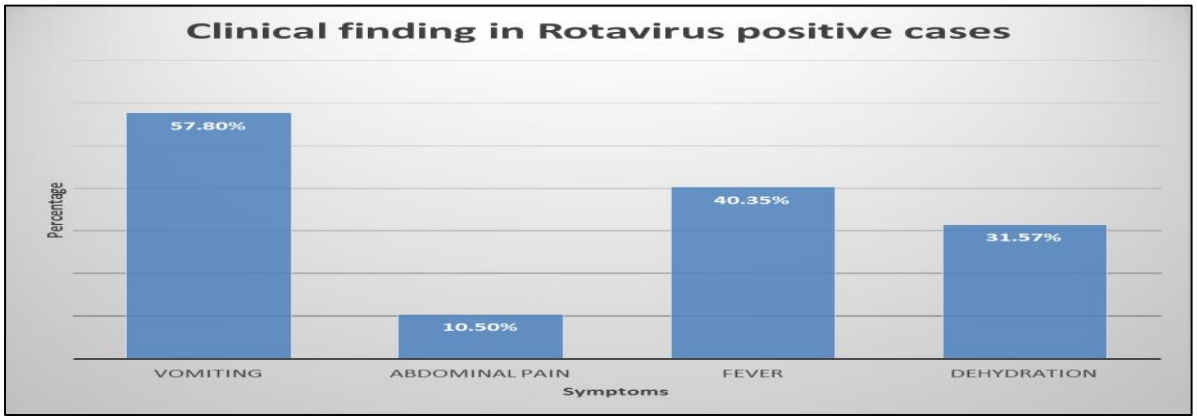
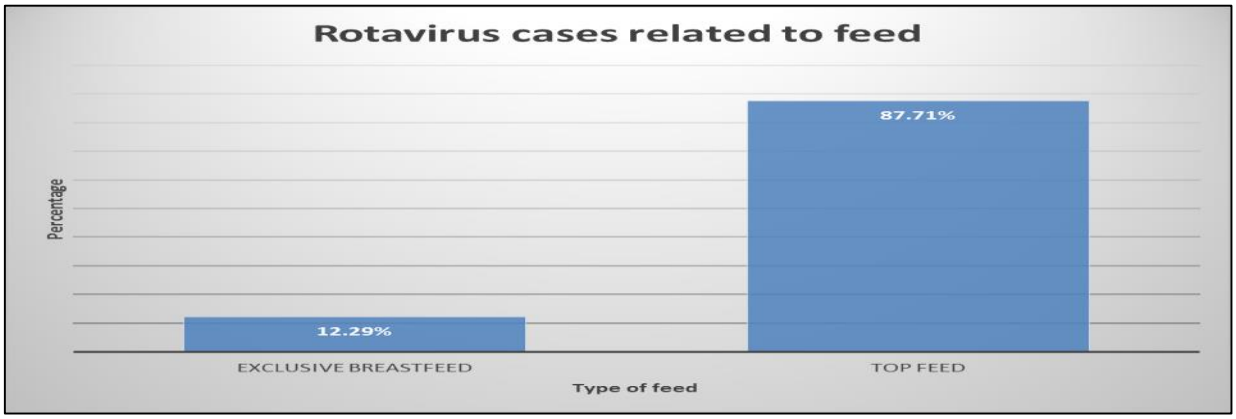


Table 7: Rotavirus positive cases related with feeds:

Sr. No.	Type of Feed	Positive cases	Percentage
1.	Exclusive Breastfeed	07	12.29%
2.	Top Feed	50	87.71%
	Total	57	100%

Table 07 shows that maximum positive cases (87.71%) of rotavirus were found in top feed children as compare to exclusive breastfeed (12.29%)

Chart-07



DISCUSSION:

Rota virus is the most common and deadly cause of severe diarrhoea with dehydration in infants and young children. Even though diarrhoeal deaths have declined considerably through improved access to medical facility, the burden of the diarrhoeal disease continues to remain significant.

Table 8: Comparison of prevalence of rotavirus in different studies

Sr. No.	Study	Prevalence
1.	Present Study	33.5%
2.	Bhatnagar, S., & Srivastava, G. (2017)	40.4%
3.	Ari. A *et al.*, (2016)	29.8%
4.	Bonkoungou *et al.*, (2010)	33.7%
5.	Indrani Banerjee *et al.*, (2006)	27.4%

Table 08 shows comparison of prevalence of rota virus in different studies. In present study prevalence was 33.5%, which was more similar to Bonkoungou *et al.*, (2010) and Ari. A *et al.*, (2016) were respectively 33.7% and 29.8%.

Table 9: Gender wise Comparison of rotavirus in different studies

Sr. no.	Study	Male	Female
1.	Present study	52.6%	47.4%
2.	Ari. A *et al.*, (2016)	72.1%	27.9%
3.	Bonkoungou *et al.*, (2010)	47%	53%
4.	Nakawesi *et al.*, (2010)	58.8%	41.2%
5.	Rerksuppaphol, S., & Rerksuppaphol, L. (2011)	61.4%	38.6%

Table 09 shows gender wise comparison of rota virus in different studies. In present study prevalence was more common in male children as compare to female children respectively 52.6% and 47.4%, which were more similar to Nakawesi *et al.*, (2010) and Rerksuppaphol and Rerksuppaphol *et al.*, (2011).

Table 10: Comparison of age group in rotavirus in different studies

Sr.no	Age (in month)	0-6 month	7-12 month	13-18 month	19-24 month	25-60 month
1.	Present study	10.5%	51%	14.0%	5.2%	19.3%
2.	Ari. A *et al.*, (2016)	18.3%	45.2%	20.2%	6.7%	9.6%
3.	Bonkoungou *et al.*, (2010)	18%	47%	23.2%	6%	5.8%
4.	Indrani Banerjee *et al.*, (2006)	12.9%	42.3%	26.7%	21.1%	-
5.	Wg C B M John *et al.*, (2014)	1.7%	43.3%	35%	18.3%	1.7%

Table 10 shows comparison of age group in rota virus in different studies. In present study prevalence was more common in 7-12 month of age group 51%, which were more similar to bonkoungou *et al.*, (2010) and Ari. A *et al.*, (2016) respectively 47% and 45.2%.

Table 11: Comparison of seasonal trends in rota virus in different studies

Sr. No.	Study	Winter	Summer	Monsoon
1.	Present study	38.59%	12.2%	35.08%
2.	Ari. A *et al.,* (2016)	61.5%	9.7%	28.8%
3.	Bonkoungou *et al.,* (2010)	53.3%	2.9%	43.8%

Table 11 shows the comparison of seasonal trends in rota virus in different studies. In present study prevalence of winter season was 38.59%, which was higher among all seasons followed by 35.08% prevalence in monsoon and 12.2% prevalence in summer, which were similar to study of Bonkoungou *et al.,* (2010) and Ari, A. *et al.,* (2016).

Table 12: Comparison of dehydration in rota virus in different studies

Sr. No.	Study	No	Some	Severe
1.	Present study	68.4%	24.6%	7.0%
2.	Rerksuppaphol and Rerksuppaphol *et al.,* (2011)	63.9%	26.1%	10%
3.	Wg C B M John *et al.,* (2014)	58.8%	30.8%	10.4%

Table 12 shows the comparison of dehydration in rotavirus in different studies. In present study prevalence of severe dehydration was 7% which was similar to study Rerksuppahol and Rerksuppahol *et al.,* (2011), Wg C B M John *et al.,* (2014), respectively 10%, 10.4%. Though approximately 30% patient had dehydration in various study, rotavirus infection had significant impact on morbidity and mortality.

Table-13: Comparison of rotavirus positive cases related with feeds.

Sr. No.	Study	Top feed	Exclusive
1.	Present study	87.71%	12.29%
2.	B M John *et al.,* (2014)	85.72%	14.28%

Table 13 shows that comparison of feeding in Rotavirus positive cases in different study. In present study, maximum cases (87.71%) were found in children who take top feed and minimum cases (12.29%) were found in children on exclusive breastfeeding which was similar to study B M John *et al.,* (2014) in which maximum cases (85.72%) were found in children who take top feed and minimum cases 14.28% found in children on exclusive breastfeeding.

CONCLUSION:

Rotavirus infection is the most common cause of acute diarrhoea in infants and young children and it may cause some to severe dehydration in acute diarrhoea in an unvaccinated child. The peak incidence of rotavirus was noted in winter season. Study reveal exclusive breastfeed given protection against rotavirus infection due to maternal IgA rotavirus antibodies. Children with acute diarrhoea should be monitored for the development of severe dehydration. Introduction of supplementary feed increase the chance of infection unless proper hygienic measure such as a proper sterilization of feeding bottles/bowls, washing hands before preparing/feeding food strictly followed. There were few limitation as this study is hospital based, the prevalence of rotavirus might have been different from the actual prevalence in community.

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