Brain & Development 41 (2019) 19–28

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Neonatal seizures and white matter injury: Role of rotavirus infection and probiotics

Jung Sook Yeom [a,b], Ji Sook Park [a,b], Young-Soo Kim [c], Rock Bum Kim [d], Dae-Sup Choi [e], Ju-Young Chung [f], Tae-Hee Han [g], Ji-Hyun Seo [a,b], Eun Sil Park [a,b], Jae-Young Lim [a,b], Hyang-Ok Woo [a,b], Hee-Shang Youn [a,b], Chan-Hoo Park [b,h,*]

[a] Department of Pediatrics, Gyeongsang National University School of Medicine, Jinju, South Korea
[b] Department of Gyeongsang Institute of Health Science, Gyeongsang National University School of Medicine, Jinju, South Korea
[c] Department of Neurology, Gyeongsang National University School of Medicine, Jinju, South Korea
[d] Department of Preventive Medicine, Gyeongsang National University School of Medicine, Jinju, South Korea
[e] Department of Radiology, Gyeongsang National University School of Medicine, Jinju, South Korea
[f] Department of Pediatrics, Sanggyepaik Hospital, Inje University College of Medicine, Seoul, South Korea
[g] Department of Diagnostic Laboratory Medicine, Sanggyepaik Hospital, Inje University College of Medicine, Seoul, South Korea
[h] Department of Pediatrics, Changwon Gyeongsang National University Hospital, Changwon, South Korea

Received 29 December 2017; received in revised form 29 June 2018; accepted 2 July 2018

Abstract

Background: Recent reports associate rotavirus infection with neonatal seizures of distinctive white matter injury (WMI) pattern, but evidence is lacking. We examined this association prospectively and analyzed factors related to occurrence of seizures and WMI pattern in neonates with rotavirus infection.

Methods: We prospectively included 228 neonates (≥34 gestational weeks) who were admitted to a regional neonatal intensive care unit between February 2015 and April 2016 and underwent rotavirus antigen testing using stool samples. Patients with neonatal seizures of other etiologies were excluded.

Results: Seventy-eight (34.2%) neonates were rotavirus-positive. Otherwise-unexplained seizures were more frequently observed among rotavirus-positive than among rotavirus-negative neonates (20.5% vs. 4.0%, $p < 0.001$). Rotavirus infection increased the risk of seizures (odds ratio [OR], 6.19; $p < 0.001$), even after adjustment for confounders (OR, 4.46; $p = 0.007$). After stratification according to probiotic administration immediately after birth, rotavirus infection remained a significant risk factor only in patients without probiotic medication (OR, 4.83; $p = 0.01$ vs. OR, 2.44; $p = 0.49$). The WMI pattern was observed in 9 of 22 neonates with seizures, and this subgroup was characterized by rotavirus infection (100% vs. 53.8%, $p = 0.004$) and seizure onset on days 4–6 of life (66.7% vs. 15.0%; $p = 0.02$). G9P[8] was the most common genotype in this subgroup but was also commonly detected in neonates without seizures.

Conclusion: Rotavirus infection is an independent risk factor for neonatal seizures, and associated with the WMI. Immediate administration of probiotics after birth may reduce rotavirus-associated neonatal seizures.

Keywords: Rotavirus; Newborn; Seizures; White matter; Probiotics

* Corresponding author at: Department of Pediatrics, Changwon Gyeongsang National University Hospital, 642-120, Samjeongja-ro, Seongsan-gu, Changwon, Gyeongnam, South Korea.
E-mail address: aroma@gnu.ac.kr (C.-H. Park).

https://doi.org/10.1016/j.braindev.2018.07.001

1. Introduction

While afebrile seizures following rotavirus infection are widely recognized in infants and young children [1–4], rotavirus has been largely overlooked as a potential causative pathogen in neonatal seizures. However, recent Korean studies have suggested an association between rotavirus infection in neonates presenting with seizures and a distinctive pattern of white matter injury (WMI) [5–7]. Cystic evolution and neurological sequelae have been reported in these neonates [5–7]. Considering the relatively high prevalence of rotavirus infection in neonatal intensive care units (NICUs) [8], pediatricians should be aware of this issue. However, the worrisome conclusions from these previous studies were based on limited evidence because of the retrospective study design and absence of systematic assessment of neonatal seizures. In addition, it remains unclear which factors are involved in rotavirus-related brain injury in newborns. We aimed to verify the relationship between rotavirus infection and neonatal seizures, and to determine significant factors potentially associated with seizures and with the WMI pattern seen in rotavirus infections.

2. Methods

2.1. Study design and participants

The prospective observational study was conducted at the Gyeongsang National University Hospital (GNUH) NICU from February 2015 to April 2016. All neonates (≥34 gestational weeks) who were admitted to the NICU for over 3 days were recruited. Informed consent was obtained from the parents. Stool samples were collected from the neonates and tested using a rotavirus enzyme-linked immunosorbent assay (ELISA) at days 3 and 7 of life for in-born neonates and on the day of admission for out-born patients. The test was repeated weekly until the patients were discharged, as well as whenever the neonates demonstrated symptoms associated with rotavirus infection. Based on detection of rotavirus antigens in stool specimens, the neonates were divided into rotavirus-positive and -negative groups. Symptoms associated with rotavirus infection included fever (≥38 °C), vomiting (2-fold increase in frequency), diarrhea (2-fold increase in the frequency of watery vs. normal stool), seizures, and apnea/bradycardia. Seizures were defined as clinically distinctive paroxysmal phenomena observed by physicians at least twice during the NICU stay, accompanied by abnormal findings on 1-hour electroencephalography (EEG), with definitive responses to antiepileptic drugs. Apnea/bradycardia was defined as an episode of breathing cessation lasting ≥20 s, or shorter if associated with bradycardia or cyanosis, and only if the diagnostic criteria for seizures were not met.

We excluded neonates with other obvious causes of seizures, such as intracerebral hemorrhage, epileptic syndrome, and metabolic encephalopathy. We also excluded neonates with perinatal asphyxia, meningitis due to pathogens other than rotavirus, persistent hypoglycemia (<30 mg/dL, requiring high levels of glucose support >12–16 mg/kg/min) [9], hyponatremia (<120 mEq/L), hypocalcemia (<6.0 mg/dL) [10], and any inborn errors of metabolism, regardless of seizure occurrence, in order to avoid underestimation of seizures in neonates with such conditions. Perinatal asphyxia was defined if meeting at least one of the following criteria: fetal distress at delivery and/or immediately preceding delivery, requirement for resuscitation at birth, and 5-min Apgar score ≤5 [11]. Meningitis was defined as confirmed presence of pathogens in the cerebrospinal fluid (CSF), with or without CSF pleocytosis (≥25 white blood cells/mm^3). Screening for inherited metabolic disorders was performed using tandem mass spectrometry at least 72 h after birth. The independent ethics committee of GNUH approved the study.

2.2. Clinical data

The following clinical data were obtained for all patients: gestational age; sex; presence of rotavirus antigen in stool; symptoms during rotavirus testing; birth weight; age of symptom onset; Apgar score; obstetric history, including parity and delivery method; in-born (born in same hospital) versus out-born (born elsewhere and transferred) birth; maternal obstetric complications including maternal fever, diabetes mellitus, hypertension, placenta abruption, and uterine rupture; meconium aspiration syndrome; perinatal asphyxia; meningitis; and probiotic administration within 24 h of birth. Laboratory data obtained at the time of the symptoms. Data on seizure type, duration, frequency, time of occurrence, and antiepileptic drugs were obtained for patients with seizures.

2.3. Viral diagnosis and genotype analysis of rotavirus

Rotavirus infection diagnosis was based on positivity of stool samples, determined using a rotavirus ELISA kit (VIDAS rotavirus, bioMérieux Vitek, France). In patients with seizures, an array of PCR tests using fresh CSF samples and additional PCR analyses of rotavirus, parechovirus, and enterovirus using frozen samples (CSF, serum, or stool) were performed as in our previous study (see Supplementary material S1) [5]. The frozen samples had been stored at –70 °C at the National Biobank of Korea. Genotypic analyses of rotavirus were performed as described by Han et al (see Supplementary material S2) [12].

2.4. Magnetic resonance imaging (MRI) investigations

Neonates presenting with seizures underwent brain MRI within 5 days of symptom onset. MRI findings of the seizure group were reviewed by a neuroradiologist blinded to the patients' clinical information. The distinctive WMI pattern on diffusion-weighted imaging (DWI) was defined as extensive and symmetrical areas of restricted diffusion in the periventricular white matter, deep white matter, and white matter tracts, including the corpus callosum [5–7].

2.5. Statistical analysis

We first compared demographics and clinical characteristics between rotavirus-positive and -negative groups, using the χ^2 and *t*-tests for categorical and continuous data, respectively. Next, we performed a univariate logistic regression analysis to identify factors related to seizures. The crude odds ratio (OR) with 95% confidence interval (95%CI) of rotavirus infection for seizures was then adjusted for other factors related to seizures, by using backward stepwise multiple logistic regression. Such factors were defined as variables associated with $p < .20$ in univariate analysis but no multicollinearity (variance inflation factor ≥ 5). In addition, we stratified the neonates by probiotic administration, to estimate whether probiotics affect the relationship between rotavirus infection and seizures. Differences between subgroups with and without the WMI pattern were analyzed using the χ^2 and Mann-Whitney *U*-tests for categorical and continuous variables, respectively. *P*-values $< .05$ were considered to indicate statistical significance.

3. Results

During the study period, 418 neonates born after 34 weeks' gestation were admitted for longer than 3 days at this 25-bed regional NICU; of these, 34 (8.0%) were diagnosed with seizures. A total of 171 neonates were excluded due to failure to obtain informed consent (n = 79) or undergo the rotavirus antigen test (n = 92). Thus, stool specimens of 247 neonates (including 31 neonates with seizures) were tested, representing 59.1% of eligible neonates. Nineteen additional patients were also excluded due to other obvious causes of seizures (n = 9) and absence of clinical seizures but presence of predisposing factors for seizures (n = 10) (Fig. 1). Among the 19 neonates excluded, rotavirus infection was observed in 3 patients.

Thus, 228 neonates were ultimately included in this study. The male-to-female ratio was 121:107. The mean gestational age and birth weight were 37.2 ± 2.0 weeks and 2.8 ± 0.6 kg, respectively. The mean age at the time of the rotavirus testing using stool specimens was 5.7 ± 4.1 days. Seventy-eight neonates (34.2%) were rotavirus-positive, while the remaining 150 neonates (65.8%) were rotavirus-negative. Twenty-two neonates with seizures were included, and additional viral testing with PCR was performed on samples of CSF (n = 14), serum (n = 15), and stool (n = 17). MRI was performed in all included patients with neonatal seizures, and WMI pattern was observed in 9 patients (Fig. 2). Among the patients with seizures, there was only one inborn birth that experienced seizures during hospitalization. The remaining 21 patients were outborn births, 20 of whom were immediately transferred from other hospitals or postpartum care facilities due to seizures; only one patient was admitted our hospital after being discharged home from an external facility.

3.1. Seizures were more frequent in the rotavirus-positive patients

Compared with the rotavirus-negative group, the rotavirus-positive group was characterized by older gestational age (37.7 ± 2.1 vs. 36.9 ± 1.9 weeks, $p = 0.003$) and higher birth weight (2.9 ± 0.6 vs. 2.7 ± 0.5 kg, $p = 0.001$) (Table 1). Diarrhea (23.1% vs. 2.0%, $p < 0.001$) and seizures (20.5% vs. 4.0%, $p < 0.001$) were more frequent in the rotavirus-positive group. Probiotic administration at birth was also less common in the rotavirus-positive group (41.0% vs. 66.7%, $p < 0.001$). No significant difference was found between the groups in other variables.

3.2. Other factors related to neonatal seizures

To determine factors potentially associated with neonatal seizures, univariate analysis was applied to compare neonates with seizures ($n = 22$) to those without seizures ($n = 206$) (Table 2). Significant factors related for neonatal seizures included older gestational age (OR, 1.47; 95%CI, 1.15–1.88, $p = 0.002$), higher birth weight (OR, 4.59; 95%CI, 1.96–10.61, $p < 0.001$), and out-born birth (OR, 22.69; 95%CI, 2.99–171.89, $p = 0.003$). In contrast, probiotic administration immediately after birth significantly decreased the risk for seizures (OR, 0.09; 95%CI, 0.02–0.32, $p < 0.001$). No significant multi-collinearity was observed among these potential factors.

3.3. Rotavirus infection as a risk factor for neonatal seizures

Rotavirus infection increased the risk of neonatal seizures (unadjusted OR, 6.19; 95%CI, 2.31–16.57, $p < 0.001$); adjustment for all other factors related to seizures (gestational age, birth weight, out-born birth, and probiotics medication at birth) changed the OR (adjusted OR, 4.46; 95%CI, 1.49–13.35, $p = 0.007$). A

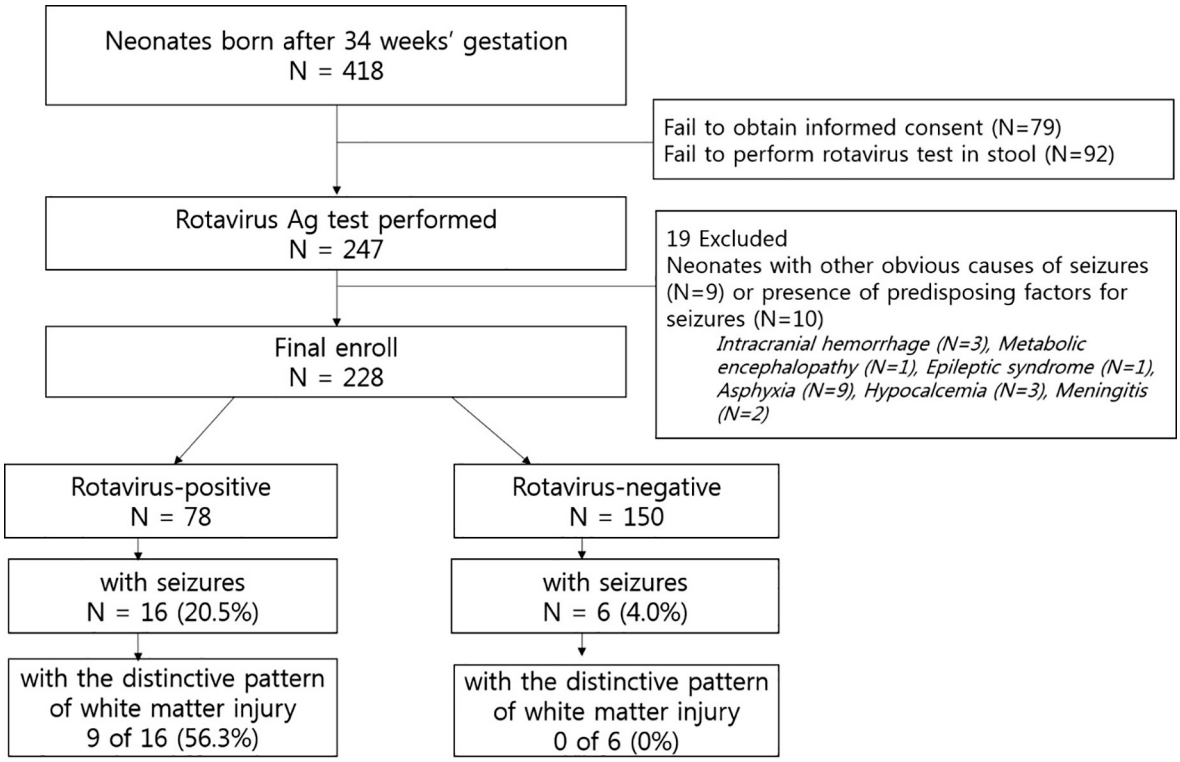

Fig. 1. Study population.

stratified analysis according to probiotic administration immediately after birth showed that rotavirus infection was a significant risk factor only in patients without probiotic medication (OR, 4.83; 95%CI, 1.36–17.10, $p = 0.01$) (Table 3).

3.4. Clinical characteristics for the WMI pattern in neonatal seizures

The clinical features of 9 patients with WMI pattern on DWI were summarized (Table 4). The mean gestational age and birth weight were 39.1 ± 1.9 weeks and 3.4 ± 0.6 kg, respectively. No abnormal findings were found on metabolic screening. All patients were treated with antiepileptic drugs. Multifocal-clonic seizures were the most common seizures. CSF, serum, and stool specimens were analyzed for rotavirus, parechovirus, and enterovirus using PCR in all nine patients. Rotavirus was detected in the stool specimens of all patients, but not in the CSF or serum. Only one patient was detected parechovirus in the stool and serum, while the others were not detected parechovirus nor enteroviruses in any specimen. DWI findings in all 9 patients were nearly identical (Fig. 2). On comparing between patients with and without the WMI pattern during neonatal seizures (Table 5), rotavirus infection (100% vs. 53.8%, $p = 0.004$) and seizure onset between day 4 and 6 of life (66.7% vs. 15.4%, $p = 0.02$) were characteristic of neonatal seizures with WMI pattern. No probiotics were administered immediately after birth in the subgroup with WMI pattern (0% vs. 23.1%, $p = 0.24$).

3.5. Genotypes of rotavirus

Rotavirus genotypes were analyzed in 45 of 78 neonates from the rotavirus-positive group (GenBank accession numbers: VP4, KY 419229–4192279; VP7, KY 419280–4192330). Overall, the most common strains were G1P[8] (33.3%), G9P[8] (26.6%), G9P[4] (20.0%), and G3P[8] (15.5%) (see supplementary Table 1). Of these, G9P[8] was the most common strain in neonates with seizures (5 of 10 tested) and in those who were out-born (9 of 24 tested). G9P[8] ($n = 4$), G1P[8] ($n = 1$), and G3P[8] ($n = 1$) were detected in neonates with the WMI pattern. Interestingly, G9P[8] was also commonly detected in neonates without seizures (7 of 35) and in those who were in-born (3 of 21). G4P[6] was detected in only one patient without seizures.

4. Discussion

In this prospective observational study, we firstly showed that rotavirus is an independent risk factor of neonatal seizures (4.46-fold higher risk), but probiotic administration at birth might decrease the risk of

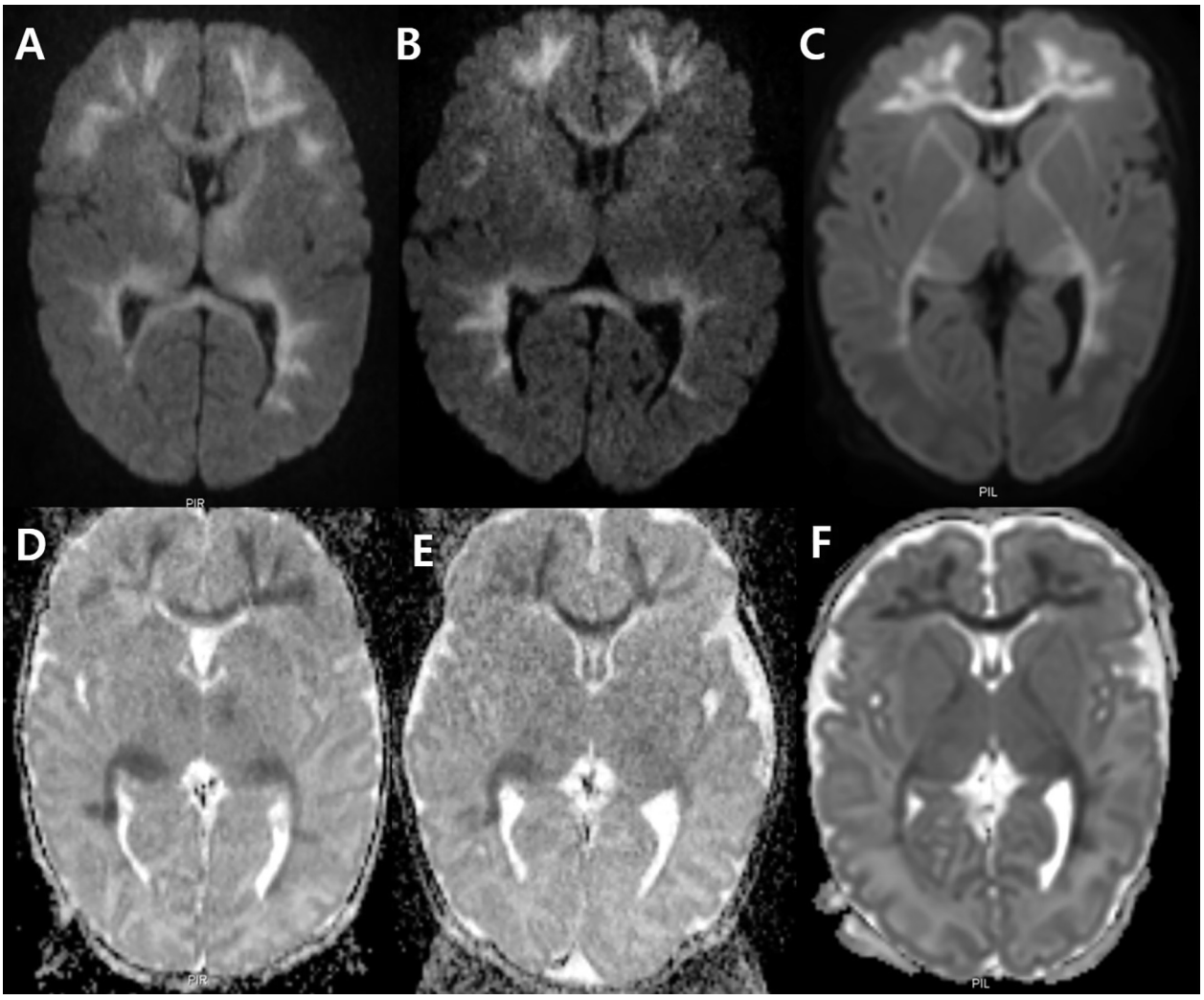

Fig. 2. Diffusion-weighted imaging findings in three neonates within 5 days of seizure onset Patients 1 (A, D, images on the day of the seizures, 10 days of age), 2 (B, E, images on the day of the seizures, 5 days of age), and 7 (C, F, images on the second day of the seizures, 6 days of age). Extensive and symmetrical areas of restricted diffusion were noted in specific regions including periventricular white matter and deep white mater (A, B, C). On apparent diffusion coefficient maps, the corresponding regions revealed low signal intensity (D, E, F).

rotavirus-associated seizure. The clinical diagnosis of neonatal seizures is not easy because many manifestations are entirely subclinical [13]. In perinatal asphyxia, which is the most common cause of neonatal seizures, electrographic-only seizures are common [14]. However, we did not routinely perform EEG monitoring in neonates with these conditions. To avoid unintended selection bias, we excluded all neonates with known risk factors for seizures (e.g., perinatal asphyxia) regardless of clinical seizure occurrence. Thus, our study was specifically designed to investigate the association between rotavirus infection and otherwise-unexplained neonatal seizures.

Similar to previous reports [5,7], our study demonstrated that rotavirus infection and seizures around day 5 of life were characteristics of late-preterm and healthy-term newborns with the WMI pattern. The incubation period of rotavirus is 2 to 3 days [15], and the interval from diarrhea to seizures in children with rotavirus infection is 1–3 days [16]. Thus, in newborns with the WMI pattern, rotavirus infection likely occurs just after birth. A healthy gut microbiome is important to protect the host against rotavirus infection [17]. Because human gut is sterile at birth [18], the gut might be relatively sterile at the time of rotavirus infection in patients demonstrating the WMI pattern. Together with a particular developmental window in the central nervous system (CNS), rotavirus infection before maturation of the gut microenvironment may be important. As previously reported, rotavirus was not detected in the serum or CSF of patients with the WMI pattern [5,6]. WMI mechanism may involve something other than direct viral invasion of the CNS.

Table 1
Baseline Characteristics of Rotavirus-Positive and –Negative Neonates.

Variable Mean (±SD) or *n* (%)	Rotavirus-positive (*n* = 78)	Rotavirus-negative (*n* = 150)	Total (*n* = 228)	*p*[a]
Sex, male	47 (60.3)	74 (49.3)	121 (53.1)	0.12
Gestational age, weeks	37.7 (±2.1)	36.9 (±1.9)	37.2 (±2.0)	0.003
Late-preterm (34–36 weeks)	27 (34.6)	73 (49.7)	100 (44.4)	.03
Term (37 weeks)	51 (65.4)	74 (50.3)	125 (55.6)	
Birth weight, kg	2.9 (±0.6)	2.7 (±0.5)	2.8 (±0.6)	0.001
Age at rotavirus test, days	6.2 (±4.1)	5.4 (±4.1)	5.7 (±4.1)	0.15
1-min Apgar score[b]	7.7 (±1.5)	7.3 (±1.5)	7.4 (±1.5)	0.07
5-min Apgar score	9.2 (±1.0)	8.9 (±1.0)	8.9 (±1.1)	0.05
Meconium aspiration syndrome	1 (1.3)	4 (2.7)	5 (2.2)	0.66
Obstetric complications	13 (16.7)	26 (17.3)	39 (17.1)	1.00
Nulliparity of mother	43 (55.1)	80 (53.3)	123 (53.9)	0.89
Out born	47 (60.3)	73 (48.7)	120 (52.6)	0.12
Probiotics at birth[c]	32 (41.0)	100 (66.7)	132 (57.9)	<.001
Hospital stay, days	11.3 (6.2)	12.0 (8.8)	11.7 (8.0)	0.57
At least one symptom	47 (60.3)	36 (24.0)	83 (36.4)	<.001
Fever	12 (15.4)	11 (7.3)	23 (10.1)	0.07
Vomiting	6 (7.7)	9 (6.0)	15 (6.6)	0.78
Diarrhea	18 (23.1)	3 (2.0)	21 (9.2)	<.001
Seizures	16 (20.5)	6 (4.0)	22 (9.6)	<.001
Apnea/bradycardia	9 (11.7)	11 (7.3)	20 (8.8)	0.32

Abbreviations: SD, standard deviation.
[a] *p*-values obtained using the χ^2 or *t*-test.
[b] Apgar score not obtained in 32 patients.
[c] Probiotics administration within 24 h of birth.

Table 2
Crude Odds Ratio (OR) of potential risk factors associated with seizures.

VariableMean (±SD) or *n* (%)	With seizures (*n* = 22)	Without seizures (*n* = 206)	OR (95% CI)	*p*[a]
Sex, male	11 (50.0)	110 (53.4)	0.87 (0.36–2.10)	0.87
Gestational age, weeks	38.5 (±1.5)	37.0 (±2.0)	1.47 (1.15–1.88)	0.002
Late-preterm (34–36 weeks)	3 (13.6)	99 (48.1)	0.17 (0.04–0.59)	0.005
Term (>37 weeks)	19 (86.4)	107 (51.9)	Reference	
Body weight, kg	3.2 (±0.5)	2.7 (±0.6)	4.56 (1.96–10.61)	<.001
Meconium aspiration syndrome	1 (4.5)	4 (1.9)	2.40 (0.25–22.51)	0.44
Fever	1 (4.5)	22 (10.7)	0.39 (0.05–3.10)	0.38
Diarrhea	2 (9.1)	19 (9.2)	0.98 (0.21–4.53)	0.98
Probiotics at birth[b]	3 (13.6)	129 (62.6)	0.09 (0.02–0.32)	<.001
Out born	21 (95.5)	99 (48.1)	22.69 (2.99–171.89)	0.003
Nulliparity of mother	9 (40.9)	114 (55.3)	0.55 (0.23–1.37)	0.20
Maternal obstetric conditions	3 (13.0)	36 (17.5)	0.74 (0.20–2.65)	0.65
Cesarean section	13 (56.5)	127 (62.3)	0.72 (0.30–1.76)	0.48
Rotavirus infection	16 (72.7)	62 (30.1)	6.19 (2.31–16.57)	<0.001

Abbreviations: SD, standard deviation; CI, confidence interval.
[a] *p*-values obtained from univariate logistic regression analysis.
[b] Probiotics administration within 24 h of birth.

Table 3
Adjusted Odds Ratio (OR) for risk of seizures associated with rotavirus-infection.

Sample	Adjusted OR (95%CI)	*p*
Overall[a], *n* = 228	4.46 (1.49–13.35)	0.007
Probiotics medication at birth[b], n = 132	2.44 (0.18–32.15)	0.49
Without probiotics medication at birth[c], *n* = 96	4.83 (1.36–17.10)	0.01

Abbreviations: CI, confidence interval.
[a] Adjusted for gestational age, birth weight, out-born birth, probiotic medication at birth.
[b] Adjusted for gestational age, birth weight, out-born birth, and restricted to patients with probiotics medications at birth.
[c] Adjusted for gestational age, birth weight, out-born birth, and restricted to patients without probiotics medications at birth.

Table 4
Clinical characteristics of neonates with the WMI pattern on diffusion-weighted imaging.

Pt. No.	GA (wks)	BW (kg)	sex	RV in stool	Ad. month	age of seizure onset (d)	AS (1/5)	Underlying condition	DM	nulliparity	CSF WBC (/mm^3)	other Sx	Sz type	AEDs	detection of other viruses
1	38	2.2	m	+	Feb	10	8/9	No	c/s	No	2	No	FC	Pb	No
2	40	3.8	m	+	Mar	5	9/10	No	c/s	yes	4	diarrhea	MC	MDZ, Pb	No
3	39	3.1	f	+	Mar	5	8/9	No	c/s	No	1	No	MC	Pb	No
4	40	4.2	m	+	May	7	9/10	No	v	yes	8	diarrhea	FC	Pb	No
5	36	2.9	m	+	June	4	8/9	No	c/s	No	1	No	MC	MDZ, Pb	No
6	40	3.3	f	+	Oct	4	9/10	No	v	No	1	No	S	MDZ	No
7	37	3.5	f	+	Oct	5	9/10	No	v	No	0	No	MC	Pb	No
8	39	3.5	f	+	Dec	6	9/10	No	c/s	No	1	No	MC	MDZ, Pb	No
9	39	3.4	m	+	Dec	2	8/10	No	v	No	39	fever	S, MC	MDZ, Pb, PT	Parechovirus in serum, stool

Pt. No., patient number; GA, gestational age; wks, weeks; d, day; RV, rotavirus; Ad, admission; DM, delivery mode; CSF, cerebrospinal fluid; WBC, white blood cell; Sx, symptom; Sz, seizure; AEDs, antiepileptic drugs; m, male; f, female; AS, Apgar score; c/s, Cesarean section; v, vaginal delivery; FC, focal clonic; MC, multifocal clonic; S, subtle; Pb, phenobarbital; MDZ, midazolam; PT, phenytoin.

In the present study, we demonstrated that probiotic administration immediately after birth was associated with 10-fold decreased risk of seizures (OR, 0.09; $p <$ .001). When a stratified analysis was performed, rotavirus infection remained a risk factor only in patients without probiotic administration (OR, 4.83; $p = .01$). The probiotics taken by patients were almost entirely *Saccharomyces boulardii* and only one took *Lactobacillus casei*. Both probiotics have been reported to prevent and treat various gastrointestinal diseases including rotavirus gastroenteritis [19,20]. In particular, *S. boulardii* prevented rotavirus-induced chloride secretion and oxidative stress in enterocytes via the inhibition of NSP4 [21]. NSP4 is a non-structural protein that functions as a viral enterotoxin, and has previously been hypothesized to be involved in rotavirus-associated neurological injury [22,23]. *S. boulardii* also has been shown to confer beneficial effects in various infections by its immunomodulatory effects [24]. Inflammation outside the CNS could induce white matter injury, particularly in the developing brain [25]. Therefore, the probiotics might reduce the risk of rotavirus-associated neonatal seizures via NSP4 inhibition or through modulation of the inflammatory signals from the gut.

Another interesting finding in our study was that almost all rotavirus-associated seizures (93.8%) and all seizures with the WMI pattern (100%) occurred in the out-born patients, while rotavirus seemed to be equally common in both groups (in-born, 28.7%; out-born, 39.2%). The present finding was consistent with our previous observations. We have encountered clusters of neonates with the WMI pattern (about 34 neonates, including 9 of the present study and previously reported 18 patients [5]) for a decade; however, all affected neonates were transferred from postpartum care centers or local nurseries and were infected with rotavirus. Similar findings have never been observed among inborn births in our NICU for the past decade, suggesting a clear link between outborn birth and rotavirus-associated WMI. Our NICU is the sole tertiary referral center in the western Gyeongnam province of Korea, and one of the few centers in this region with a policy of routine probiotic administration (*S. boulardii*) following birth, which could explain these interesting findings. In the present study, no significant difference was observed in genotype distribution between the groups (in-born vs. out-born, and seizures vs. without seizures). In a recent Korean study [6], G4P[6] was the predominant genotype in neonates with the WMI pattern. However, our study showed that G9P[8] was most common in neonates with and without seizures, while G4P[6] was only detected in one neonate without seizures. These findings are suggest that a particular pathogenic genotype may not involve the mechanism of WMI, but our sample was too small to draw definitive conclusions. Indeed, further analysis of genotypes are needed

Table 5
Association of WMI pattern on Diffusion-Weighted Imaging in Neonates With Seizures.

Variable Mean (±SD) or *n* (%)	With WMI pattern ($n = 9$)	Without WMI pattern ($n = 13$)	p[a]
Sex, male	6 (66.7)	5 (38.5)	0.38
Gestational age, weeks	39.1 (±1.3)	37.4 (±3.2)	0.15
Term (>37 weeks)	8 (88.9)	11 (84.6)	1.00
Body weight, kg	3.4 (±0.6)	3.2 (±0.5)	0.40
1-min Apgar score	8.7 (±0.5)	7.7 (±2.6)	0.58
5-min Apgar score	9.7 (±0.5)	8.7 (±2.5)	0.49
Seizure onset between day 4 and 6 of life	6 (66.7)	2 (15.4)	0.02
Out-born birth	9 (1 0 0)	18 (90.0)	1.00
Cesarean section	5 (55.6)	7 (53.8)	1.0
Nulliparity of mother	2 (22.2)	7 (53.8)	0.20
Maternal obstetric conditions	0 (0)	2 (15.4)	0.49
Rotavirus Ag positive in stool	9 (100.0)	7 (53.8)	0.004
Probiotics at birth[b]	0 (0)	3 (23.1)	0.24

Abbreviations: SD, standard deviation.
[a] p-value obtained using the χ^2 or Mann-Whitney U test.
[b] Probiotics administration within 24 h of birth.

because G9P[8] is a common global genotype but rarely detected in neonates [26].

Why, then, is this pattern of rotavirus-associated WMI almost exclusively seen in Korea? The most likely explanation relates to traditional approaches to postpartum care in Korea, referred to as *Sanhujori*, in combination with emerging trends associated with this culture. Traditionally, *Sanhujori* services were provided at home by the mother's extended family, with newborn infants isolated from other children or visitors for at least 21 days after birth. However, with the decline of the traditional extended family system in Korea, many of these services are now provided by private postpartum care facilities (*Sanhujori* centers) [27]. Today, one-third of mothers currently use postnatal care facilities [28], in which newborns are usually separated from their mother and housed together in a communal nursery room for a week or more. Not surprisingly, time spent at these facilities has been shown to be an important risk factor for neonatal infections [28]. As asymptomatic infected newborns born outside the hospital have been identified as the main source of rotavirus infection in NICU environment [29], infection rate of this virus in Korean NICUs ranging from 13.1% to 31.4% [29–31]. This problem is particularly pronounced in Gyeongnam Province, where our institution is located, in which only 26% of newborns receive rooming-in care (the baby and mother shared a single room) in postpartum care facilities [32]. Given that all studies of rotavirus-associated WMI in newborns performed to date have been performed at NICUs in Gyeongnam Province [5–7].

On the other hand, the recent introduction of two rotavirus vaccines in Korea has led to significant reductions in the overall incidence of rotavirus gastroenteritis, but these benefits did not extend to young infants (<6 months of age, particularly <2 months of age), in whom modest increases in infections were reported [33]. Rotavirus vaccines are not indicated for newborns, and therefore cannot be used to protect newborns from rotavirus infections. These findings also suggest that vaccine introduction may reduce maternally-transferred immunity in young infants (due to the reduction of natural booster effects according to overall infection decrease). Rotavirus antibodies have been shown to protect against seizures in rotavirus gastroenteritis [23]. Taken together, this series of overlapping reasons (the recent trend of postnatal care and changes of maternally transferred immunity) likely explains why WMI is almost exclusively seen in Korea.

However, rotavirus-associated WMI might not be a local phenomenon limited to Korea. Verboon-Maciolek et al. [34] first reported this issue of rotavirus infection in eight neonates from The Netherlands, Switzerland, Norway, and Australia during a period of six years. All eight patients had rotavirus-related symptoms such as diarrhea, vomiting, or fever [34]. In contrast, we reported on nine patients with WMI at our single center in two years, but only three of them showed rotavirus-related symptoms. Thus, if only patients with gastrointestinal symptoms or fever were tested for the presence of rotavirus infection, we could not reveal the relationship between infection and WMI pattern. In addition, the rotavirus infection rates in NICUs seem to be higher than expected even in developed western countries. A study in the US [8] documented a rotavirus infection prevalence of 18.4% in their NICU. The rotavirus infection prevalence reported in our study is similar to that reported at the NICU of a Greek tertiary hospital (about 30%) [35], but is considerably lower than that reported in studies conducted in India (43.9%–78%) [36–37]. A recent *meta*-analysis demonstrated that rotavirus is the most common pathogen of viral origin for nosocomial infections in NICUs [38]. Thus, rotavirus infection is still an important problem in the NICUs in

some countries. We do not know why patients with rotavirus-associated WMI pattern were rarely accompanied by the gastroenteritis symptoms. However, these findings suggest that neurological complications of rotavirus in neonates may be more common than we thought [39], and this phenomenon is not limited to Korea alone.

Our study has some limitations. First, patients without stool specimens or parental consent were excluded; second, the study was conducted in a single center with high rotavirus infection rate; third, only 14 out of 22 patients with seizures had CSF viral studies. We could not estimate breast-milk effects, as supplement types and frequency of breast-milk feeding varied with time. However, we consider it unlikely that breast-milk feeding affected our results significantly as most inborn neonates were fed formula at least for the first 2–3 days after birth, followed by a combination of formula and breast milk for the first week of life. Additionally, MRI was not performed in rotavirus-positive neonates without neonatal seizures, which may have skewed our findings, as those without clinical seizures may have also shown WMI, though less severe. Although we defined one patient co-infected with parechovirus (patient no. 9 in Table 4) to have rotavirus-associated WMI, parechovirus may have played a leading role in the pattern of injury rather than rotavirus because the patient had fever and CSF pleocytosis. Outborn birth may simply be a confounding factor in the association between neonatal seizures and rotavirus, casting doubt on the apparent protective association between probiotics and seizures. Thus, the present study might be insufficient to confirm any preventive effects of probiotics on neonatal seizures associated with rotavirus infection. Further studies involving NICUs without routine probiotics medication will be necessary to test this hypothesis.

Despite its limitations, our study might suggest that the risk of neonatal seizures is increased in rotavirus-positive newborns, and that administration of probiotics immediately after birth likely reduces the risk. From a public health perspective, our findings throw an important message in a policy of nursery care facilities for healthy-term newborns; strict preventive measures should be enforced in newborn nurseries, and complimentarily, it is advisable to take probiotics immediately after birth, particularly in areas where the rate of neonatal rotavirus infection is high but maternally-transferred immunity is likely to be reduced.

5. Funding and all other required statements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (2015-0578). This manuscript has been presented in the 14th Asian and Oceanian Congress of Child Neurology.

6. Competing interests

Nothing to report.

Acknowledgment

The biospecimens and data used for this study were provided by the Gyeongsang National University Hospital, a member of the Korea Biobank Network. We thank So-Hee Kim, a coordinating nurse, who received the written consent forms and collected the clinical data.

Contributors' Statements.

Dr Yeom conceptualized and designed the study, carried out initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Dr Park designed and coordinated the data collection, carried out initial analyses, and contributed to the writing of the final manuscript. She is designated as co-first author, because she contributed equally to this work.

Drs YS Kim, Seo, ES Park, Lim, Woo, Youn, and Choi carried out the initial analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Dr RB Kim supervised data collection, carried out statistical analyses, and approved the final manuscript as submitted.

Dr Han coordinated data collection, carried out the initial analyses, and approved the final manuscript as submitted.

Drs Chung and CH Park conceptualized and designed the study, supervised data collection, carried out the initial analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted. They contributed equally to this work and should be considered co-corresponding authors.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.braindev.2018.07.001.

References

[1] Johansen K, Hedlund KO, Zweygberg-Wirgart B, Bennet R. Complications attributable to rotavirus-induced diarrhoea in a Swedish paediatric population: report from an 11-year surveillance. Scand J Infect Dis 2008;40:958–64.

[2] Kang B, Kim DH, Hong YJ, Son BK, Kim DW, Kwon YS. Comparison between febrile and afebrile seizures associated with mild rotavirus gastroenteritis. Seizure 2013;22:560–4.

[3] Hung JJ, Wen HY, Yen MH, Chen HW, Yan DC, Lin SJ, et al. Rotavirus gastroenteritis associated with afebrile convulsion in children: clinical analysis of 40 cases. Chang Gung Med J 2003;26:654–9.
[4] Abe T, Kobayashi M, Araki K, Kodama H, Fujita Y, Shinozaki T, et al. Infantile convulsions with mild gastroenteritis. Brain Dev 2000;22:301–6.
[5] Yeom JS, Kim YS, Seo JH, Park JS, Park ES, Lim JY, et al. Distinctive pattern of white matter injury in neonates with rotavirus infection. Neurology 2015;84:21–7.
[6] Oh KW, Moon CH, Lee KY. Association of rotavirus with seizures accompanied by cerebral white matter injury in neonates. J Child Neurol 2015;30:1433–9.
[7] Lee KY, Oh KW, Weon YC, Choi SH. Neonatal seizures accompanied by diffuse cerebral white matter lesions on diffusion-weighted imaging are associated with rotavirus infection. Eur J Paediatr Neurol 2014;18(5):624–31.
[8] Sharma R, Hudak ML, Premachandra BR, Stevens G, Monteiro CB, Bradshaw JA, et al. Clinical manifestations of rotavirus infection in the neonatal intensive care unit. Pediatr Infect Dis J 2002;21:1099–105.
[9] Cornblath M, Ichord R. Hypoglycemia in the neonate. Semin Perinatol 2000;24:136–49.
[10] Tarulli A. Seizures and epilepsy. In: Neurology: A Clinician's Approach. New York, NY: Cambrige University Press; 2011. p. 160–77.
[11] Wong DS, Poskitt KJ, Chau V, Miller SP, Roland E, Hill A, et al. Brain injury patterns in hypoglycemia in neonatal encephalopathy. AJNR Am J Neuroradiol 2013;34:1456–61.
[12] Han TH, Kim CH, Chung JY, Park SH, Hwang ES. Genetic characterization of rotavirus in children in South Korea from 2007 to 2009. Arch Virol 2010;155:1663–73.
[13] Clancy RR, Legido A, Lewis D. Occult neonatal seizures. Epilepsia 1988;29:256–61.
[14] Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, Wang A, Cook N, Donnelly M, et al. Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. J Child Neurol 2011;26:724–8.
[15] Kapikian AZ, Wyatt RG, Levine MM, Black RE, Greenberg HB, Flores J, et al. Studies in volunteers with human rotaviruses. Dev Biol Stand 1983;53:209–18.
[16] Hongou K, Konishi T, Yagi S, Araki K, Miyawaki T. Rotavirus encephalitis mimicking afebrile benign convulsions in infants. Pediatr Neurol 1998;18:354–7.
[17] Shornikova AV, Casas IA, Mykkanen H, Salo E, Vesikari T. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatr Infect Dis J 1997;16:1103–7.
[18] Morelli L. Postnatal development of intestinal microflora as influenced by infant nutrition. J Nutr 2008;138:1791S–5S.
[19] Das S, Gupta PK, Das RR. Efficacy and safety of *Saccharomyces boulardii* in acute rotavirus diarrhea: double blind randomized controlled trial from a developing country. J Trop Pediatr 2016;62:464–70.
[20] Kelesidis T, Pothoulakis C. Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders. Therap Adv Gastroenterol 2012;5:111–25.
[21] Buccigrossi V, Laudiero G, Russo C, Miele E, Sofia M, Monini M, et al. Chloride secretion induced by rotavirus is oxidative stress-dependent and inhibited by *Saccharomyces boulardii* in human enterocytes. PLoS One 2014;9 e99830.
[22] Goldwater PN, Rowland K, Thesinger M, Abbott K, Grieve A, Palombo EA, et al. Rotavirus encephalopathy: pathogenesis reviewed. J Paediatr Child Health 2001;37:206–9.
[23] Yeom JS, Kim YS, Jun JS, Do HJ, Park JS, Seo JH, et al. NSP4 antibody levels in rotavirus gastroenteritis patients with seizures. Eur J Paediatr Neurol 2016;21:367–73.
[24] Stier H, Bischoff SC. Influence of Saccharomyces boulardii CNCM I-745 on the gut-associated immune system. Clin Exp Gastroenterol 2016;13:269–79.
[25] Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol 2015;11:192–208.
[26] Than VT, Kang H, Lim I, Kim W. Molecular characterization of serotype G9 rotaviruses circulating in South Korea between 2005 and 2010. J Med Virol 2013;85:171–8.
[27] Hwang NM, Lee IY, Park YT, Seo DW. A study on the management of community postpartum service facilities in Korea. Korea Institute for Health and Social Affairs (KIHASA), Ministry of Health and Welfare; 2001. May, Report No. 2001-28.
[28] Yoon HS, Shin YJ, Ki M. Risk factors for neonatal infections in full-term babies in South Korea. Yonsei Med J 2008;49(4):530–6.
[29] Kim CR, Oh JW, Yum MK, Lee JH, Kang JO. Rotavirus infection in neonates at a university hospital in Korea. Infect Control Hosp Epidemiol 2009;30:893–5.
[30] Shim JO, Son DW, Shim SY, Ryoo E, Kim W, Jung YC. Clinical characteristics and genotypes of rotaviruses in a neonatal intensive care unit. Pediatr Neonatol 2012;53:18–23.
[31] Moon HH, Kim DS, Park SH, Kim HM. Clinical characteristics and genotype of rotavirus infection in newborn infants. Korean J Perinatol 2012;23:266–72.
[32] Kim YM, Kim E-Y. Maternal and hospital factors impacting the utilization of rooming-in care in South Korea: secondary analysis of national health data. J Korean Acad Nurs 2011;41:593–602.
[33] Park DK, Chung JY. The changes in the outbreak of rotavirus gastroenteritis in children after introduction of rotavirus vaccines: a retrospective study at a tertiary hospital. Korean J Pediatr Infect Dis 2014;21:167–73.
[34] Verboon-Maciolek MA, Truttmann AC, Groenendaal F, Skranes J, Døllner H, Hunt RW, et al. Development of cystic periventricular leukomalacia in newborn infants after rotavirus infection. J Pediatr 2012;160:165–8.
[35] Koukou D, Chatzichristou P, Trimis G, Siahanidou T, Skiathitou A-V, Koutouzis EI, et al. Rotavirus gastroenteritis in a neonatal unit of a Greek Tertiary Hospital: clinical characteristics and genotypes. PLoS One 2015;10 e0133891.
[36] Ramani S, Sowmyanarayanan TV, Gladstone BP, Bhowmick K, Asirvatham JR, Jana AK, et al. Rotavirus infection in the neonatal nurseries of a tertiary care hospital in India. Pediatr Infect Dis J 2008;27:719–23.
[37] Cicirello HG, Das BK, Gupta A, Bhan MK, Gentsch JR, Kumar R, et al. High prevalence of rotavirus infection among neonates born at hospitals in Delhi, India: predisposition of newborns for infection with unusual rotavirus. Pediatr Infect Dis J 1994;13:720–4.
[38] Civardi E, Tzialla C, Baldanti F, Strocchio L, Manzoni P, Stronati M, et al. Viral outbreaks in neonatal intensive care units: what we do not know. Am J Infect Control 2013;41:854–6.
[39] de Vries LS, Bearden D. Neurologic complications of rotavirus in neonates: More common than we thought? Neurology 2015;84:13–4.