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ORIGINAL ARTICLE

Pseudo-outbreak of rotavirus infection in a neonatal intensive care unit

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Background: A rotavirus outbreak in a neonatal intensive care unit (NICU) may have catastrophic consequences for young infants receiving critical care. From May 13, 2011 to July 11, 2011, a significant increase in stool samples testing positive for rotavirus antigens in the NICU of a university affiliated hospital was observed. Due to lack of clinical presentations suggestive of rotavirus infection in the patients and the rarity of rotavirus infection in the NICU in the past, a pseudo-outbreak was suspected.

Methods: Infection control measures were reinforced initially. To investigate the outbreak, a prospective laboratory-based active surveillance of all infants in the NICU was conducted right after the cluster was identified. Repeated testing using a modified enzyme immunoassay (EIA) kit, rotavirus RNA polyacrylamide gel electrophoresis (PAGE), reverse transcription polymerase chain reaction (RT-PCR), and retrospective chart review methods were used to confirm the pseudo-outbreak.

Results: Seven infants in the NICU, with or without gastrointestinal symptoms, tested positive for the rotavirus antigen using the old version of an EIA kit, which indicated a possible outbreak. Active surveillance with repeated tests for recollected stool samples using a modified EIA kit showed negative results in all 24 infants in the NICU. Seven stored stool samples

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from four infants, which previously tested positive for the rotavirus antigen, tested negative for rotavirus using the modified EIA kit, PAGE, and RT-PCR. Chart reviews showed no clinical difference between index cases and controls. False positivity might arise from unsatisfactory specificity of the old EIA kit. After the introduction of the modified EIA kit, no rotavirus was detected in the NICU for at least 7 months.
Conclusion: This cluster of patients who tested positive for the rotavirus antigen in stools was confirmed to be a pseudo-outbreak. Interpretation of the old EIA for rotavirus in an NICU setting should be done with caution until the mechanism of the false-positive reaction is elucidated.

Introduction

Rotavirus is one of the leading causes of gastroenteritis in young children and infants, including nosocomial infections in neonates.[1–3] Young infants are especially susceptible to rotavirus infections. In one of our previous studies, 40% of nosocomial rotavirus infections occurred in children younger than 6 months of age.[4] Although neonatal rotavirus infections can be mild or asymptomatic,[5–7] life-threatening events such as necrotizing enterocolitis and secondary bacteremia may develop as well.[8–10] Diagnosis of rotavirus infection in neonates or infants is relatively difficult, as atypical clinical manifestations, such as bradycardia or apnea,[11] in addition to the gastrointestinal symptoms, may be present. Hence, a rapid and effective laboratory test is necessary for diagnosis. In the 1970s and early 1980s, the electron microscope served as an important tool for diagnosis of rotavirus infections,[12] however, most hospitals now use the immunoassay methods. Despite advancements in diagnostic tools, limitations still exist and sometimes cause unexpected events such as pseudo-outbreaks when false-positives occur in clusters.

Herein, we describe a pseudo-outbreak of a rotavirus infection in the neonatal intensive care unit (NICU) at National Taiwan University Hospital (NTUH). Rotavirus outbreaks in the NICU have been rare in the past. However, stool samples from seven infants tested positive for rotavirus within 2 months, from May 13, 2011 to July 11, 2011. Several infection control measures and investigations were implemented. After further testing, this situation was determined to be a pseudo-outbreak.

Methods

Hospital setting and study population

NTUH is a 2500-bed, university-affiliated teaching hospital and medical center in Northern Taiwan. The NICU contains 25 beds including one isolation room, and is located in the children's hospital at NTUH. The NICU provides both primary and tertiary care for severely sick neonates and young infants. Most of the patients at NTUH were born locally, but some were transferred from other hospitals or nursery settings. Prospective, hospital-wide nosocomial infection surveillance has been conducted since 1981. Standard precautions are strictly executed by healthcare personnel in the NICU as a daily practice.

Cases and definitions

Data from stool samples tested with enzyme immunoassay (EIA) for rotavirus between January 2007 and February 2012 were extracted from the virology database at NTUH. Monthly distributions of positive and negative samples were further analyzed. The monthly positive rate for rotavirus is defined as the number of positive rotavirus tests from stool specimens divided by the number of all specimens submitted in the corresponding month. The mean monthly positive rate in the past 5 years was used as the baseline rotavirus positive rate. The epidemic threshold is defined as the baseline rotavirus positive rate plus two standard deviations. Outbreak is defined as when the rotavirus positive rate exceeds the epidemic threshold for 2 consecutive months. Pseudo-outbreak is a condition when an apparent cluster of infection is subsequently shown to be artifactual. It may be caused by inadequate processing or contamination of specimens, laboratory errors, or changes in surveillance techniques.[13]

The infants who tested positive during the outbreak period were defined as index cases, while all the remaining patients in the NICU were defined as the controls.

Infection control measures

The infection control measures conducted during the outbreak period included reinforcement of existing hygiene measures, implementation of additional infection control measures, and execution of active surveillance.

The initial infection control reinforcement emphasized the importance and the execution of hand hygiene, as previously described.[14] Besides, every infant who tested positive for the rotavirus was under strict contact isolation and cohort care. Visitors and healthcare workers in the NICU were firmly required to follow standard precautions.

Stringent infection control methods targeting rotavirus transmissions were implemented. In addition to hand washing, visitors were required to wear a mask and gown before entering the NICU. Environmental and terminal disinfections were performed thoroughly, especially in the

beds, the incubators, and the surrounding area where the index infants stayed. Medical devices, such as the probes of the sonogram machine, were disinfected with 0.06% sodium hypochlorite after every use. Additional measures were applied to feeding procedures in order to prevent fecal-oral transmission. Feeding bottles and orogastric tubes were well-sterilized, pacifiers were rinsed with hot water, new feeding syringes were replaced before every feeding, and nursing sets were individualized. Nurses were requested to discard used diapers and excrement in a proper way. Infection committee personnel visited the NICU periodically to evaluate the execution of the aforementioned infection control measures.

Active surveillance was aimed at prospectively identifying rotavirus infections. It started right after the identification of the cluster of cases. This included laboratory screening of all young infants staying in the NICU during the defined outbreak period, using the recollected stool samples. Additional tests were performed on particular stored stool samples. The methods used are described below.

Rotavirus detection and confirmation

The stool specimens were tested by EIA for the qualitative determination of the rotavirus antigen (RIDASCREEN Rotavirus, R-Biopharm AG, Darmstadt, Germany) by staff in the virology section of the central laboratory at NTUH, which met the requirements for internal and external quality control of the Proficiency Test and was accredited by the College of American Pathologists (CAP). This CAP accreditation is renewed every 2 years. The test uses monoclonal antibodies against VP6, a protein of the inner capsid of the rotavirus. This commercial kit was reported to exhibit sensitivity of 100%, specificity of 99.73%, a positive predictive value of 93.74%, and a negative predictive value of 100%.[15] The reagents were stored at a temperature of 4°C as required. The temperature of the refrigerator was continuously monitored. The shelf-life, storage conditions (including the temperature and humidity), and the expiration date of the kit were checked every month by the laboratory personnel. All technicians were well trained.

The stool specimens were suspended to approximately 10% in sample dilution buffer and centrifuged at 2500 $\times$ g for 5 minutes. The remaining suspensions were stored at -20°C after initial testing. Two drops (100 μL) of conjugate were added into the microwell with the fecal suspension and mixed. Next, the mixture was incubated at room temperature (20—25°C) for 60 minutes, and then washed five times with a washing buffer. Afterwards, two drops of substrate were added, mixed, and then incubated at room temperature (20—25°C) in the dark for 15 minutes. Then, one drop of stop reagent was added and mixed. Finally, the optical density was measured photometrically at 450 nm.

Regarding quality control, positive and negative control samples were used each time the test was carried out. The extinction (optical density) for the negative control was $<$ 0.2 at 450 nm and the measured value for the positive control was $>$ 0.8 at 450 nm. If the value was $>$ 0.2 for the negative control, insufficient washing was likely, and the washing steps were corrected. If the values differed from the required values, or if the substrate was turbid or had turned blue before it was added to the wells, then it was possible that the reagents had expired and would not be used. If the stipulated values were not met, the following points would be checked before repeating the test: expiry date of the reagents used, functionality of the equipment being used (e.g., calibration), correct test procedure, contamination or leaks of the kit components, and abnormal appearance (e.g., turned blue) of the substrate solution.[16]

Before July 27, 2011, the test was examined using the old protocol (denoted as old EIA kit) where only one conjugate was used. Starting from July 27, 2011, the old EIA method was replaced by a new modified protocol (denoted as modified EIA kit). The two kits differ in the steps of adding the conjugate(s). The old EIA kit added peroxidase-conjugated mouse monoclonal antirotavirus antibodies together with the sample. Whereas the modified EIA kit added conjugates in two steps, first adding biotinylated monoclonal antirotavirus antibodies together with the sample, and after a washing step, adding streptavidin-conjugated peroxidase. This change may be related to the improvement of specificity.[16] The modified EIA kit applied during the active surveillance was performed by the independent laboratory at the Center for Infection Control, NTUH.

Molecular detection of the rotavirus was performed in particular stool samples by the laboratory of the Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University. Rotavirus RNA was extracted with phenol-chloroform and further purified with guanidine thiocyanate-silicon dioxide.[17] Polyacrylamide gel electrophoresis (PAGE) of extracted rotavirus RNA product was performed using reference strain DS-1 and Wa as positive controls. The rotavirus VP7, VP4, VP6, and NSP4 genes were amplified by reverse transcription polymerase chain reaction (RT-PCR) using DS-1 as a positive control. The primers were described previously.[17—20]

Clinical information and statistical analysis

Medical charts were reviewed retrospectively after the outbreak. Demographic, clinical, and laboratory data were collected. Data from the index and control cases were compared using the Fisher's exact test for categorical variables and the Mann-Whitney *U* test for continuous variables. A p value of $<$ 0.05 was considered significant. All statistical operations were two-tailed and performed with SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Fig. 1A shows the number of stool samples which tested positive for rotavirus at the NICU from January 2007 to February 2012. Rotavirus infection in the NICU from 2007 to the first half of 2010 was rare and occurred sporadically. Cases increased in the second half of 2010 and peaked from February to July of 2011. Meanwhile, the number of stool samples which tested positive for rotavirus in other units of NTUH (excluding those in the NICU) showed characteristics of seasonality (Figure 1B). The number peaked around February to May in most years.

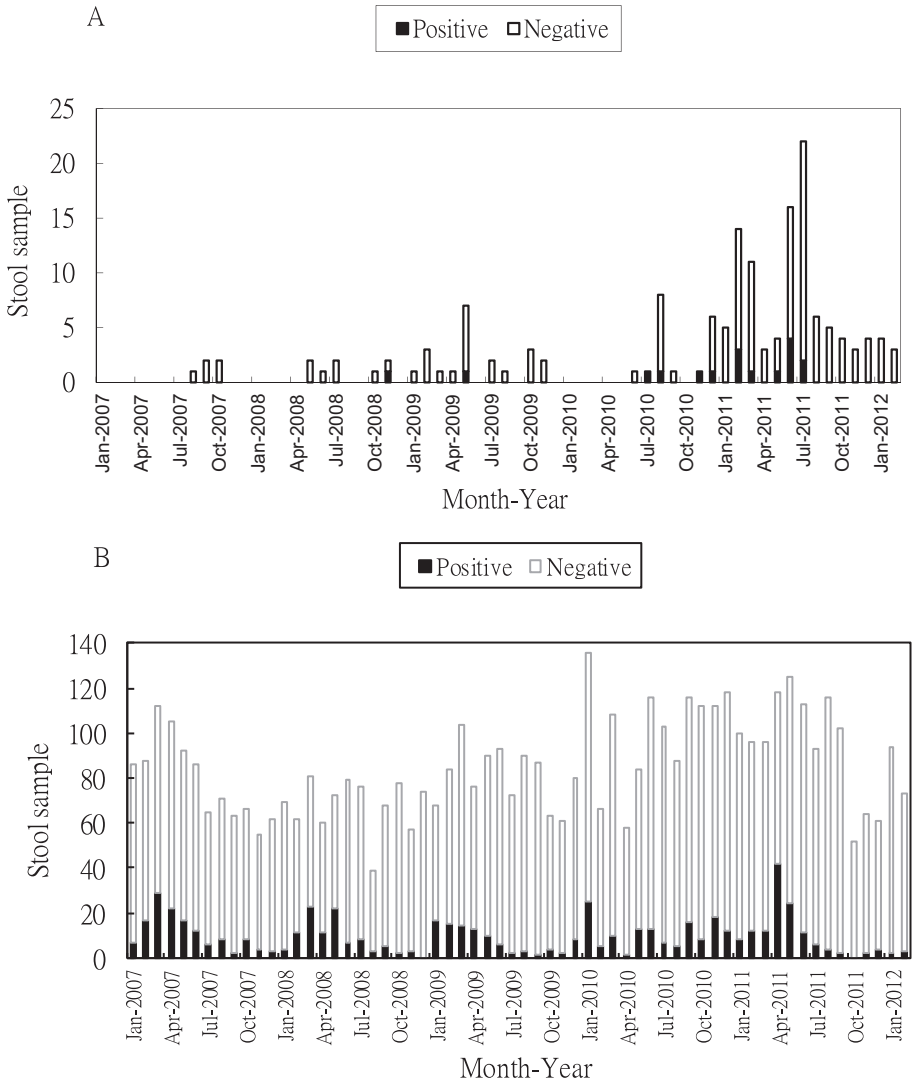

Figure 1. Monthly distribution of stool samples tested for rotavirus in the A. neonatal intensive care unit (NICU) and B. at National Taiwan University Hospital (NTUH) excluding the NICU from January 2007 to February 2012.

After rotavirus infection was identified in three cases in February and one in March 2011, the NICU staff were required to reinforce contact precautions and hand hygiene practices. In April, there was no rotavirus detected in the NICU. However, from May 13, 2011 to July 11, 2011, seven infants in the NICU tested positive for rotavirus in their stool samples. These cases had gastrointestinal symptoms such as blood tinged stool, abdominal distension or feeding intolerance. Due to the high alertness of the clinicians, checkup of stool samples from closely neighbored patients who had no symptoms or only nonspecific symptoms were done. Surprisingly, the tests revealed positive results in a portion of patients. Repeated tests were then performed for stool samples from the seven patients which gave 21 (47%) positive results in 45 samples (Table 1). Rotavirus EIA was performed frequently in the NICU from May to July of 2011. A total of 56 stool samples were tested within this period, comprising 33% of the total 172 tests performed in the NICU from year 2007 to 2011. During this period of time, the positive rates of detection in May and June achieved 25%, respectively, which exceed the epidemic threshold of the corresponding months (21% in May and 22% in June) and indicated a potential outbreak.

The unexpected increase of rotavirus infections in the NICU attracted special attention from the infection control committee of NTUH. Infection control measures were re-emphasized and an outbreak investigation was carried out.

First of all, active surveillance was conducted. Stool samples from all 24 patients in the NICU, including the seven index cases, were screened for rotavirus from July 14, 2011 to July 19, 2011. These tests were examined by an independent laboratory supervised by the infection control committee using the modified EIA kit, instead of the old EIA kit used previously. All 24 screening tests revealed negative results. Additional studies were performed on the seven stored stool samples from four infants which previously tested positive using the old EIA kit. On July 26, 2011 these stored stool samples were rechecked using the modified EIA kit and all were negative. For further confirmation, the same seven stored stool samples were further tested by

Table 1 Laboratory tests performed and their results for index cases

Index case	Age at the time of detection (d)	Sex (M/F)	GA at birth (wk)	Symptoms	Relative date [a]	Positive old EIA/number tested	Positive modified EIA/number tested [b]	Positive RT-PCR/ number performed [c]
1	53	M	25	Bloody stool	Day 1	4/4	0/1	NA
2	20	F	27	Abdominal distension, gastroesophageal reflux	Day 21	9/14	0/1	0/4
3	62	M	29	Abdominal distension	Day 22	2/11	0/1	NA
4	30	F	24	Abdominal distension	Day 46	1/4	0/1	0/1
5	5	M	29	Ileus, abdominal distension	Day 46	2/5	0/1	0/1
6	13	M	26	Abdominal distension	Day 53	2/4	0/1	0/1
7	18	M	31	Abdominal distension, feeding intolerance	Day 60	1/3	0/1	NA
Total						21/45	0/7	0/7

[a] Relative date of the first positive EIA report for each index case.
[b] The tests were performed on recollected stool by an independent laboratory from the infection control committee.
[c] RT-PCR was performed on previous old EIA-positive stored stool samples by an independent laboratory at the Department of Laboratory Medicine, National Taiwan University.
Cases are listed in a sequence of the date of the first positive stool rotavirus enzyme immunoassay (EIA) during the pseudo-outbreak period (May 13, 2011—July 11, 2011).
EIA = enzyme immunoassay; GA = gestational age; NA = not applicable; RT-PCR = reverse transcription polymerase chain reaction.

RNA-PAGE and RT-PCR. Analysis of PAGE did not show any similar bands on the gel compared with the positive controls, indicating no detectable rotavirus genomic RNA from these stool samples. RT-PCR aimed at detecting VP7, VP4, VP6 and NSP4 genes were all negative (Table 1).

The central laboratory then changed the stool detection method from using the old EIA kit to the modified EIA kit after July 27, 2011. Since then, there have still been some detectable rotavirus infections in other units at NTUH but not in the NICU.

Table 2 shows the demographics and clinical manifestations of the seven index cases and 17 controls. Comparisons of the laboratory data were also made between the index cases and controls (Table 3). There were no statistically significant differences in the demography except significantly younger postmenstrual age, lower birth weight, and lower body weight in the index case group. The clinical features between these two groups, including the common clinical manifestations of rotavirus infection, and possible protective and risk factors, were not significantly different. In the laboratory data, two groups showed no difference in hemogram, liver enzymes, renal function, electrolytes, C-reactive protein, and stool examinations. The only difference was hemoglobin, which was lower in the index group. Three index cases and 14 controls were taking premature or regular infant formulas. Some cases were taking the following oral medication while their stools tested positive for rotavirus: metoclopramide (8 cases), theophylline (6 cases), ferric hydroxide (5 cases), furosemide (1 case), kaolin/pectin (1 case), and sildenafil citrate (1 case).

Discussion

Using the modified EIA kit for the fresh and stored stool samples, RT-PCR for the stored stool samples, and the clinical data analysis, we concluded that the increased rotavirus infections in the NICU was a pseudo-outbreak. Pseudo-outbreaks often become apparent when inconsistency exists between clinical, epidemiological, and laboratory findings. Mitigation requires collaboration between clinical, laboratory, and public health personnel.[13] This event was first suspected as a pseudo-outbreak mainly due to two reasons. First, most patients did not have typical clinical symptoms and signs, which are presumed to be present in young children. Second, a cluster of rotavirus infections has rarely been detected in the NICU in the past.

Comparison of rotavirus infections in the NICU and other units over 5 years (Fig. 1A and B) showed that rotavirus infections in the NICU were unique in that they were mainly sporadic without any seasonality. Our study of rotavirus infections in neonatal intermediate ICUs in the past was consistent with this finding.[2] This is explained by the fact that the NICU is a relatively isolated environment with stricter infection control practices. Most patients in the NICU were neonates or small infants who were born prematurely in the hospital. They experienced very limited community exposure, if any. In true NICU outbreaks, nosocomial transmission plays a major role. In units other than the NICU, the trend is similar to other temperate countries where rotavirus infection is common in the winter and early spring.[21] Our study results suggest the cluster of positive cases at the NICU was attributed to decreased specificity of the EIA kit and frequent stool testing in a given, short period of time. Decreased specificity directly increases the number of false-positive results, decreases the positive predictive value of the test, and increases the perceived incidence of infection.[22] Repeated testing increases the number of false-positive results, with false-positive results greatly outnumbering true-positive results.

After realizing the increase of positive rotavirus results was actually a pseudo-outbreak, the central laboratory of the hospital introduced a newer modified EIA kit. It applies

Table 2 Comparison of demographics and clinical features between index cases and controls [a]

	Total (n = 24)	Index (n = 7)	Control (n = 17)	p
Demographics				
Postnatal age (d)	36.2 ± 40.1 (0～161)	28.7 ± 21.2 (5～62)	39.2 ± 46 (0～161)	0.727
Postmenstrual age (wk)	36 1/7 ± 6 (28 3/7～53 4/7)	32 ± 3 4/7 (28 3/7～38 1/7)	37 6/7 ± 6 (30 4/7～53 4/7)	0.013
Gestational age (wk)	31 ± 5 4/7 (22 6/7～41 2/7)	27 6/7 ± 2 4/7 (24 1/7～31 6/7)	32 2/7 ± 6 (22 6/7～41 2/7)	0.152
Birth weight (g)	1513 ± 1009 (496～3800)	701 ± 178 (496～910)	1847 ± 1022 (528～3800)	0.008
Vaginal delivery	5 (21%)	2 (29%)	3 (18%)	0.608
Male patients	17 (71%)	5 (71%)	12 (71%)	1.000
Weight (g)	1861 ± 1128 (430～4530)	778 ± 254 (430～1072)	2307 ± 1001 (856～4530)	0.000
Hospitalization period (d)	35 ± 40 (1～162)	30 ± 21 (6～63)	37 ± 46 (1～162)	0.494
Underlying disease	17 (71%)	6 (86%)	11 (65%)	0.625
Clinical features				
Unstable vital signs	4 (16.7%)	1 (14%)	3 (18%)	1.000
Emesis	2 (8.3%)	0 (0%)	2 (12%)	1.000
Feeding intolerance	3 (12.5%)	1 (14%)	2 (12%)	1.000
Abdominal distension	12 (50%)	6 (86%)	6 (35%)	0.069
Bloody stool	1 (4.2%)	1 (14%)	0 (0%)	0.292
Defecation frequency (/d)	3.1 ± 2.7 (0～10)	2.3 ± 2.1 (0～6)	3.5 ± 2.9 (0～10)	0.300
Stool amount (g/kg/d)	6.2 ± 5.1 (0～20.6)	6.7 ± 6 (0～17.8)	6.1 ± 4.9 (0～20.6)	0.799
Abdominal surgery	2 (8%)	0 (0%)	2 (12%)	1.000
NG tube/OG tube prior 7 days	23 (96%)	7 (100%)	16 (94%)	1.000
NPO status	7 (29%)	4 (57%)	3 (18%)	1.134
Breast milk feeding	12 (50%)	3 (43%)	9 (53%)	1.000
Feeding amount (mL/kg/d)	54 ± 65 (0～179)	35 ± 61 (0～150)	61 ± 67 (0～179)	0.164
Oral drug use	8 (33%)	2 (29%)	6 (35%)	1.000
Parenteral nutrition	16 (67%)	7 (100%)	9 (53%)	0.054
Parenteral antibiotic	10 (42%)	3 (43%)	7 (41%)	1.000

[a] Please see text for the definitions of index cases and controls.
NG = Nasogastric; NPO = Nil per os; OG = Orogastric.

two antibody conjugates in the procedures to increase the specificity of the test. The true reason why performances of these two kits differed remained unclear, because we did not test the same stool specimens using the same method (e.g., old EIA kit) by two different laboratories at the same time. However, by using modified EIA as well as molecular and clinical analysis, we have clearly demonstrated the initial results from the central lab were not good.

After this event, rotavirus stool testing was still ongoing in clinically suspicious cases, but arbitrary tests were being discouraged and restricted. There was no further detection of rotavirus in the NICU for at least 7 months after July 2011. Continuous surveillance is implemented to monitor the performance of the new modified EIA method as well as rotavirus infections in the NICU and other units at NTUH.

There was a time interval between the first positive stool samples using the old EIA test and the negative confirmation using the modified EIA test on recollected fresh stool samples. One may question the duration of viral shedding in stool after infection. Previous studies have shown that rotavirus shedding in neonates is longer when compared to children and may extend up to 75 days postinfection.[7,23] In our cases, the time interval mentioned was shorter, with a mean of 28 days and a median of 21 days. In addition, we also used RT-PCR, a sensitive method which was able to detect low copy numbers of rotavirus RNA. The performance of the modified EIA test, especially false-negative results, was not of major concern, as true rotavirus infections were still detectable in other settings at NTUH using the modified EIA.

Quality control procedures are needed to ensure that different laboratories are performing the commercial assay at high proficiency.[24] The virology section of the central laboratory at NTUH fulfilled the requirements for internal and external quality control of the proficiency test by the CAP. The section processed all samples sent from all settings at NTUH but false-positive results were only found in the NICU samples. We believe that flaws in infection control and laboratory processes were less likely to be the cause of positivity in rotavirus detection.

False-positivity of immunoassay detection methods for stool samples has been described previously.[25-27] Faden et al[28] reported an adenovirus gastroenteritis pseudo-outbreak in an NICU. The cause of false-positive results

Table 3 Comparison of laboratory data between index cases and controls [a]

	Number of samples (*n*)	Total	Index	Control	*p*
Hemoglobin (g/dL)	22	13.2 ± 2.7 (9∼18.8)	11 ± 1.7 (9∼13.5)	14.1 ± 2.6 (9.7∼18.8)	0.016
Platelets (K/μL)	22	263.7 ± 125.7 (38∼575)	211 ± 103.3 (38∼335)	283.4 ± 130.4 (61∼575)	0.284
WBC (K/μL)	22	11.1 ± 4.1 (3∼18.4)	11.3 ± 4.8 (3∼17)	11.1 ± 4 (5∼18.4)	0.658
AST (U/L)	12	59.8 ± 56.7 (12∼192)	53.5 ± 71.8 (12∼161)	63 ± 53 (29∼192)	0.124
ALT (U/L)	13	18.2 ± 11.5 (4∼42)	16.8 ± 17 (6∼42)	18.8 ± 9.5 (4∼32)	0.486
Blood urine nitrogen (mg/dL)	14	9.3 ± 4.6 (2∼16.7)	12.1 ± 4.2 (6.9∼16.7)	8.2 ± 4.5 (2∼15)	0.157
Serum creatinine (mg/dL)	16	0.6 ± 0.2 (0.4∼1.2)	0.6 ± 0.2 (0.4∼0.8)	0.7 ± 0.3 (0.4∼1.2)	0.820
Sodium (mmol/L)	18	135.9 ± 3.7 (127∼143)	134.2 ± 6.2 (127∼143)	136.6 ± 2.3 (133∼140)	0.297
Potassium (mmol/L)	17	4.6 ± 0.7 (3.6∼5.6)	4.2 ± 0.6 (3.6∼5.1)	4.7 ± 0.6 (3.7∼5.6)	0.153
C-reactive protein (mg/dL)	19	0.9 ± 1.7 (0∼5.7)	0.3 ± 0.5 (0∼1.2)	1.1 ± 1.9 (0∼5.7)	0.255
Stool occult blood	10	7 (70%)	4 (80%)	3 (60%)	1.000
Stool pus cell	5	1 (20%)	1 (33%)	0 (0%)	1.000
Positive stool culture	3	0 (0%)	0 (0%)	0 (0%)	

[a] Please see text for the definitions of index cases and controls.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; WBC = white blood cell.

was difficult to determine. However, the presence of large amounts of protein A of *Staphylococcus aureus* in the specimen has never been reported as a possible cause.[29] Hence, it is reasonable to suspect undetermined interfering substances in the stool in our cases as a possible cause of false positivity. Most of our patients were fed with breast milk or infant formula and some oral medications were used. Occult blood was detected in the stool of some cases. Among the seven index cases, three received stool cultures for *Salmonella*, *Shigella*, *Campylobacter*, and *Clostridium difficile*. All yielded negative results.

False-positive results might have biased the clinicians' clinical judgment. The index cases were frequently regarded as symptomatic solely due to abdominal distension. Index cases had significantly younger postmenstrual ages and lower weights than the controls. They were more eventful than controls, which misled clinicians to suspect rotavirus infection and further perform EIA on these patients. The lower hemoglobin in index cases also can be explained by the fact that they were at a higher risk for anemia of prematurity and may have more frequent blood samplings, due to unstable clinical conditions. Comparison of index cases and controls in the current study did not show any difference in clinical symptoms between them. These results further support that the index cases were not truly infected by rotavirus.

The pseudo-outbreak carried certain negative impacts. Several studies have shown that patients who are placed in contact precautions are less likely to receive complete vital signs recording and examination.[30–32] In addition, they are at increased risk if they cohort with those truly infected by rotavirus. Although this did not really happen in our case, we still took the precautions before all the cohorted patients proved to be false-positive. During a pseudo-outbreak, patients would probably receive excessive examination and treatment.[13] Unnecessary infection prevention measures increase the burden upon healthcare providers and utilization of hospital administrative resources and are not cost-beneficial. It also results in a lack of confidence in the credibility of the laboratory.[13] Psychologically, this pseudo-outbreak also created worry and anxiety among parents. More or less, this has weakened the rapport between the parents and healthcare providers and may further affect the image of the hospital.

There are several limitations in this study. First, the retrospective chart review confers an imperfect follow up and collection of data. Although the performance of the modified EIA could be challenged, theoretically it would show higher specificity using two antibody conjugates instead of one during the test procedures. Finally, we did not identify the etiology in this pseudo-outbreak, although unknown content in the stool was highly suspected to be the cause of the false-positivity.

In conclusion, we described a pseudo-outbreak of a rotavirus infection in an NICU at a tertiary-care hospital. Comparison between index cases and controls, modified EIA, PAGE, and RT-PCR methods confirmed the false-positivity of the EIA formerly used. Detection of rotavirus in the NICU is a serious occurrence that requires immediate and thorough investigation. The methods used to detect rotavirus must be reliable. Any test with an unsatisfactory specificity should not be used in the NICU. Despite an EIA kit with higher specificity being substituted, interpretation of all positive results should be done with caution until the mechanism of the false-positive reaction is elucidated.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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