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Original Article

Successful containment of horizontal enterovirus infection in a neonatal unit in Singapore through diagnosis by polymerase chain reaction (PCR) and direct sequence analysis

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ABSTRACT

Background: Enterovirus (EV) outbreaks often coincide with seasonal peaks in the community. However, they may also sporadically occur in neonatal units. Identification of EV infection in neonates can be challenging, as they tend to present with mild or nonspecific symptoms. This study reports an EV outbreak in the Neonatal Unit at KK Women's and Children's Hospital, Singapore.
Methods: This is a single-center, retrospective cohort study of neonates who had positive results for EV during the outbreak. Demographic characteristics, clinical presentations and outcomes were analyzed. Control measures used to limit the spread of infection are reported.
Results: A total of 7 cases of EV infection were identified. Their median birth weight and gestational age were 1240 g (750 –2890 g) and 28 weeks (26–35 weeks), respectively. Symptoms occurred at a median age of 48 days (9–103 days). All cases presented initially with recurrent apnea and 4 needed assisted ventilator support with CPAP (2) and mechanical ventilation (2). Serious complications occurred in 3 infants (2 with necrotizing enterocolitis and 1 with meningitis) and none died. EV was detected from rectal swabs (n = 6), CSF (n = 2) and nasopharyngeal swabs (n = 2). Viral subtyping uniformly revealed echovirus 25. Surveillance of all exposed infants by nasopharyngeal swabs was implemented, along with strict contact precautions and cohorting measures.
Conclusions: Premature infants with EV are more prone to serious complications, which can lead to significant morbidity. Thus, early recognition of symptoms, rapid diagnosis and prompt implementation of infection control measures are key to prevent further spread of infection.
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Introduction

Identification of enterovirus (EV) infection in neonates can be challenging, as they tend to present with mild or non-specific respiratory and gastrointestinal symptoms due to their underlying immature immune system. This predisposes them to serious complications such as meningitis, necrotizing enterocolitis (NEC) and septicemia, with resulting significant long-term morbidities and even death. The genus *Enterovirus* belongs to the family *Picornaviridae* and consists of small positive-sense, single-stranded ribonucleic acid (RNA) viruses. The genus consists of 15 species, namely *Enterovirus A–L* and *Rhinovirus A–C*, as distinguished by genetic and antigenic differences [1]. *Enterovirus A–D* and *Rhinovirus A–C* infect humans and cause various neurologic, respiratory, cardiovascular and other manifestations [2]. EV infections are transmitted through the fecal-oral route, by respiratory

Abbreviations: NICU, Neonatal intensive care unit; SCN, Special care nursery; EV, Enterovirus; PCR, polymerase chain reaction; RNA, Ribonucleic acid; CPAP, continuous positive airway pressure; CSF, Cerebrospinal fluid; NEC, Necrotizing enterocolitis.

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droplets and by fomites and can follow endemic and epidemic patterns of circulation.

Prevalence rates of confirmed EV infections in newborns admitted to hospital for presumed sepsis have been reported to be 3–14% [3,4]. EV infections can be acquired by neonates vertically in utero, at the time of delivery or postnatally [5]. In the community, the diagnosis of EV infection should be considered in a sick or febrile infant, and the disease often occurs in seasonal peaks [5]. In a protected environment such as a neonatal intensive care unit (NICU), horizontal transmission can occur, and this may lead to nosocomial outbreaks [4]. Such outbreaks can rapidly spread if infected cases are not recognized and containment measures are not implemented early on. We report an outbreak of EV infections which occurred in the NICU and special care nursery (SCN) of a tertiary-level hospital, KK Women's and Children's Hospital, Singapore. We analyzed the demographic characteristics, clinical details, and laboratory data of the affected infants, as well as described the infection control measures implemented to contain and mitigate the EV outbreak in the unit.

Subjects and methods

KK Women's and Children's Hospital (KKH) is the largest tertiary-level referral perinatal center in Singapore with an annual delivery rate of approximately 12,000 babies. Its neonatal services comprise four newborn nurseries, two level II SCN and one level III NICU. Neonates who were tested positive for EV from 5th June 2014 to 8th July 2014 in the NICU or SCN were included. For babies diagnosed with EV infection, the hospital's electronic medical database and laboratory database was reviewed for the affected patients. Basic demographic, clinical and laboratory data were collected. This included gender, birth weight, gestational age at birth, time of symptom onset, patients' ward location, clinical presentation, respiratory support required, household contact with patient who presented with respiratory tract infection, laboratory tests performed and final diagnosis of the respective EV manifestation. Data were analyzed in a simple descriptive manner, except that median and range was calculated for gestational age, birth weight and time of symptom onset. An epidemic curve was also created to assess temporal relationships between cases. Information on outbreak control interventions were extracted from outbreak control meeting minutes.

EV was diagnosed by real-time reverse polymerase chain reaction (rRT PCR) based on a protocol by Corless et al. [6] with modified primers and probes, as follows: forward primer EV-f, 5′–CCCCTGAATGCGGCTAATC, reverse primer, EV-r 5′-ATTGTCACCATAAGCAGCCA and probe, EV-p FAM 5′-CGGAACCGACTACTTTGGGTGWCCGT-3′ TAMRA. Positive specimens were genotyped at the National Public Health Laboratory, as part of Singapore's national surveillance program, using primers targeting the EV VP1 gene according to a published protocol [7]. After initial sequencing results showed a match with Echovirus 25, Echovirus-specific primers (ECHO_E25_3685R, 5′-GACGTCAATATAGACACATGCCG; ECHO_E25_4575 F, 5′-TTCAAGTCCMAATGYCGTATTGA; ECHO_E25_7620R, 5′-CGCACCGAAYGCGGARAATTT-3′) were used in combination with two pan-EV primers (pan-EV-F2, 5′–CCCTGAATGCGGCTAATCC; 222R, 5′-CICCIGGIGGIAYRWACAT) to amplify and sequence larger fragments from the VP1–4 genes directly from clinical specimens.

A total of 56 "contact" infants, defined as infants exposed to confirmed cases, were also screened with EV PCR from 6/6/2014 to 2/7/2014 and all results returned negative. All medical staff were required to wear personal protective equipment (PPE) and maintain strict hand hygiene while handling EV- positive and "contact" patients. Virucidal label-hand rubs were introduced and environment disinfection of cubicles was strictly implemented.

This study was approved by the institutional review board of the hospital, with a waiver of informed consent, due to the retrospective nature of the study as well as the de-identification of subjects reported.

Results

Description of outbreak

Upon detection of a confirmed case of EV infection, medical staff vigilance and close surveillance confirmed the detection of an EV cluster.

Seven cases of EV infection were identified and isolated during the outbreak. It is part of our departmental policy to isolate positive cases from the rest of the patient cohort in order to reduce the risk of further spread. This was an observed increase in EV incidence for 2014, as there were 7 patients who tested positive during this episode and only 1 case was seen in November.

The index case was identified in SCN Pink (Fig. 1) on 3rd June 2014 The patient was a premature infant of 31 week and 1 day gestation with a birth weight of 1738 g, born well without extensive resuscitation and hospitalized due to prematurity, awaiting target weight and gestation to be discharged. He presented with recurrent episodes of apnea and tachypnea on day 17 of life (corrected age of 33 weeks and 4 days) which required admission to NICU for continuous positive airway pressure (CPAP) ventilation. Full sepsis workup including blood investigations for full blood group (FBC), C- reactive protein (CRP), lumbar puncture for cerebrospinal fluid (CSF) cell count, culture and viral PCRs, as well as nasopharyngeal aspirate for common respiratory virus antigens (immunofluorescent test for adenovirus, influenza A, B, parainfluenza, respiratory syncytial virus, metapneumovirus), was conducted. All tests were negative, except for CSF, which showed a positive polymerase chain reaction (PCR) result for EV, leading to a diagnosis of EV meningitis.

The second affected patient was also hospitalized in SCN pink, and the child presented with both respiratory and gastrointestinal symptoms 1 day after the clinical presentation of the index case. He subsequently developed Stage 2B necrotizing enterocolitis (NEC). On the basis of heightened clinical vigilance, CSF and a rectal swab specimen were examined by PCR for EV. He required invasive mechanical ventilation for 3 days, was kept nil by mouth and treated with empiric antibiotics during this episode. Subsequently, 5 patients from other wards (SCN Blue and MRSA ward) were identified to have EV infections via rectal and nasopharyngeal swabs from multiplex PCR.

It is observed that all affected patients were premature infants who were clinically stable and breathing room air prior to becoming infected. The median birth weight of affected neonates was 1240 g (range, 750–2890 g); the onset of symptoms occurred at a median postnatal age of 48 days (9 days to 103 days), with a corresponding median post-menstrual age of 28 + 5 weeks (26 + 5 weeks to 35 + 5 weeks). Characteristics of cases are shown in Table 1.

All cases initially presented with episodes of apnea and blood oxygen desaturation, and 4 patients were admitted to level III NICU for respiratory support. Two infants needed nasal-prong CPAP. The other 2 infants deteriorated, needing mechanical ventilation, and were diagnosed with NEC stage IIb, with prominent gaseous distension of bowel loops and pneumatosis intestinalis reported from radiographic findings. Three other patients remained under close observation at the isolated area of the level II SCN. EV was detected from rectal swabs (6 patients), CSF (2 patients) and nasopharyngeal swabs (2 patients). One patient had positive EV PCR results from both CSF and a rectal swab. Treatment for the affected infants

Fig. 1. Special care nursery pink and blue, with isolation areas for enterovirus-infected and methicillin-resistant *Staphylococcus aureus* (MRSA)-infected or colonized patients' area in front of both SCNs.

was mainly supportive, and all infants recovered well from the outbreak.

All infants were also investigated with bacterial cultures and received antibiotics, but none of the blood cultures yielded positive results. They were also tested for common respiratory viruses using direct immunofluorescence tests, due to their presentations with respiratory symptoms, which all returned negative. No death was seen during this outbreak which lasted for 15 days. We also screened a total of 56 "contact" infants, but their results were negative. Upon discharge of "contact" or EV-positive infants, parents were also given pamphlets and advised to look out for possible symptoms.

There were no apparent common factors identified for the initial cases, as they were cared for in different cubicles in the SCN at the time of presumed acquisition. The positive results coincided with a time of deteriorating clinical status in each of the infants. However, it was noticed that the virus had spread from SCN Pink to SCN Blue and to the MRSA ward throughout the initial period, despite the spatial location of different areas (see floor plan, Fig. 1), in addition to the fact that those infants were cared by different teams of doctors and nurses. Cross-infection was speculated to have occurred during night shifts when the same on-call team doctors would take care of the NICU and two SCN babies, in addition to the rotating schedule of the nursing staff covering those areas. The epidemic curve of the outbreak is shown in Fig. 2.

Virus subtyping

Virus subtyping was performed on all 7 cases by the National Public Health Laboratory, Singapore. All 7 strains were identified as echovirus 25, which supports the notion of cross-infection within the cohort.

Outbreak control measures

All EV-positive infants from NICU and SCN were isolated in a separate, designated isolation area (yellow shaded area in the floor plan, Fig. 1). Both SCN Pink and SCN Blue were used to house all the "contact" infants who had had exposure to the EV positive cases, and these areas were closed to new admissions during the period of the outbreak. In the NICU, 6 other "contact" infants were transferred to separate isolation cubicles. The cubicles where the positive cases were located underwent thorough environmental cleaning before opening to new admissions (NICU floor plan, Fig. 3).

Surveillance of all exposed infants by nasopharyngeal swabs and PCR testing for EV were performed, along with strict contact precautions and cohorting measures. Personal protective equipment was initiated for both EV-positive and "contact" infants. All medical personnel and allied health staff were required to strictly adhere to hand hygiene according to the 5 moments of hand hygiene, wearing of gowns, gloves and apron when handling EV-positive and "con-

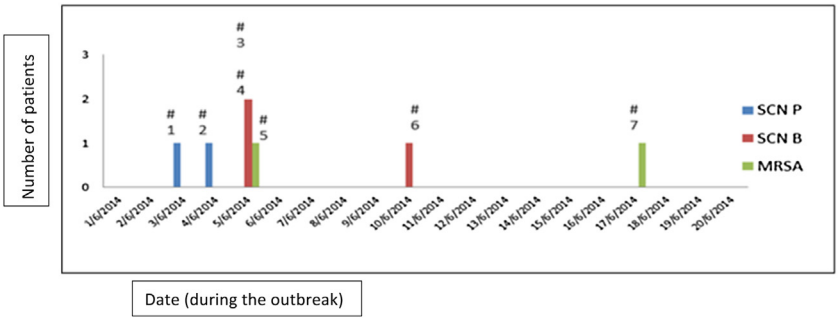

Fig. 2. Epidemic curve of the enterovirus outbreak in Neonatal department, Kandang Kerbau Women's and Children's Hospital, Singapore.

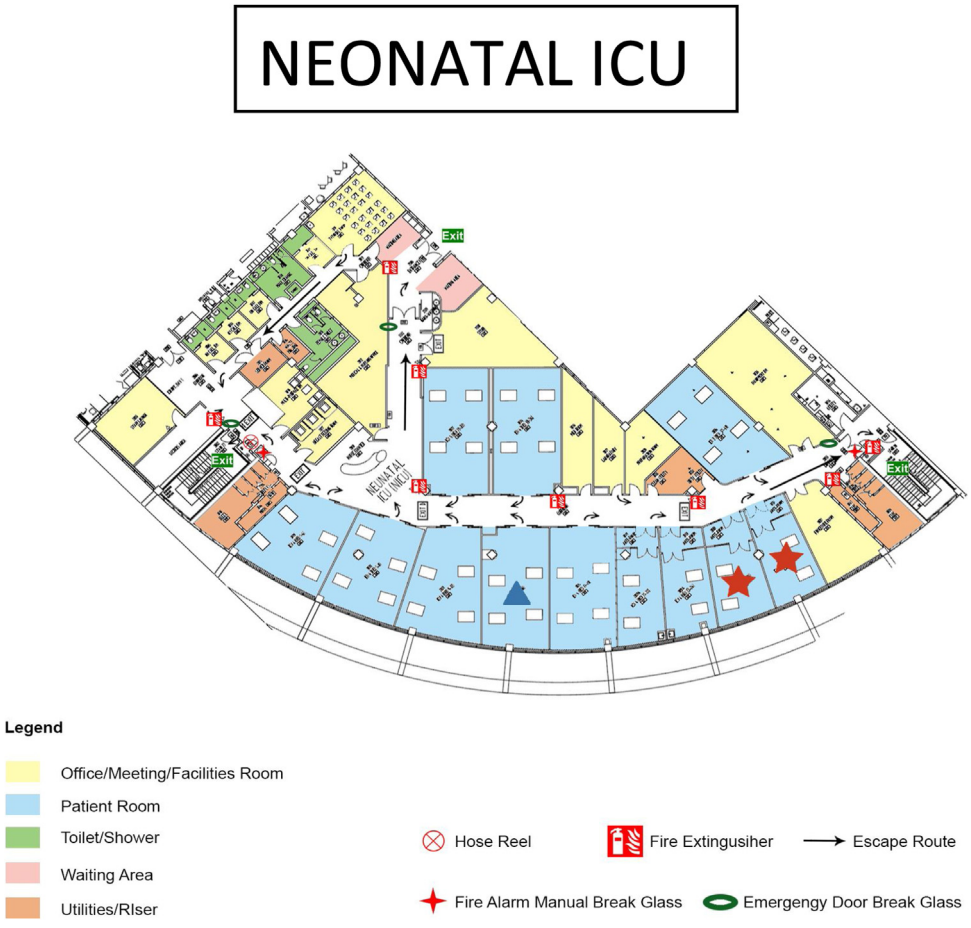

Fig. 3. NICU cubicles where ★ represent isolation cubicles for enterovirus contact infants and ▲ cubicle hosting positive cases.

tact" infants. Caregivers and visitors were also advised to adhere to strict hand hygiene before and after patient contact.

On 9 June 2014, the infection control unit advised to change from regular alcohol-based hand rub to a hand rub with virucidal label claims (Desderman Pure Gel, Schülke, Norderstedt, Germany). Environmental disinfection of cubicles housing EV-positive and "contact" patients as well as isolation rooms was performed with sodium dichloroisocyanurate (Biospot, Hydrachem, London, UK) at a concentration of 1000 ppm. Subsequent to these measures, there was a decline in the spread of infection, and only two further cases occurred (cases 6 and 7) 5 and 12 days later, respectively. We monitored the development of EV infection until the last case was discharged home about 1 month later.

Discussion

The most common presenting symptoms of EV infection in neonates include fever, poor feeding, lethargy, respiratory distress and irritability. Studies have reported that at least half of the infants during the course of EV infection developed respiratory distress, including grunting, tachypnea, apnea, nasal flaring or cough, and nasal discharge [8]. A smaller proportion of infants (about 20%) develop gastrointestinal symptoms consisting of abdominal distension, bloody stools, or even NEC [9]. Premature infants are vulnerable to even more serious manifestation of disease, likely due to their immature immune system, specifically their low B cell and T cell functions and low immunoglobulin levels [10]. In our cohort

Table 1
Baseline demographics, clinical presentation and course of illness in affected neonates during the enterovirus outbreak.

Case	Gestational age at birth	Birth weight	Age of onset (Day of life/post menstrual age)	Clinical presentation	Diagnosis	Investigations and tests	Source of specimen	Level of care	Respiratory status prior EV infection	Respiratory support during illness
#1	31+1 weeks	1738 g	Day 17 OL/ 33w+4d	Apneas and desaturations	Enterovirus meningitis	Enterovirus PCR	CSF	III	Room Air	CPAP x 3 days
#2	28+5 weeks	1240 g	Day 48 OL/ 35w+4d	Desaturations, tachycardia, tachypnoea and abdominal distension	Enterovirus stage IIb NEC	Enterovirus PCR	Rectal swab and CSF	III	Room Air	IPPV x 3 days
#3	30 weeks	1100 g	Day 60 OL/ 38w+4d	Apneas and desaturations	Enterovirus stage IIb NEC	Enterovirus PCR Abdominal Xray: dilated bowel loops	Rectal swab	III	Room Air	IPPV x 3 days
#4	28+5 weeks	1215 g	Day 100 OL/ 43 w	Apneas and desaturations	Enterovirus infection	Enterovirus PCR	Stool	II	Room Air	Nasal cannula O2
#5	35+5 weeks	1950 g	Day 9 OL/ 37w	Apneas and desaturations	Enterovirus infection	Enterovirus PCR	NPA and Rectal swab	III	Room Air	CPAP
#6	26+5 weeks	750 g	Day 103 OL/ 41w+3d	Apneas and desaturations	Enterovirus infection	Enterovirus PCR	NPA and Stool	II	Room Air	Room air
#7	33+4 weeks	2890 g	Day 42 OL/ 39w+4d	Mild, self-limiting transient desaturations	Enterovirus infection	Enterovirus PCR	Rectal swab	II	Room Air	Room air

(OL = of life, w = weeks, NEC – necrotizing enterocolitis, DNA- deoxyribonucleic acid, PCR- polymerase chain reaction, NPA- nasopharyngeal aspirate, IPPV- intermittent positive pressure ventilation, CPAP- continuous positive airway pressure).

of affected premature infants, they presented with a wide spectrum of symptoms, ranging from mild respiratory distress to NEC needing mechanical ventilation and inotropic support. As symptoms may be variable in this population, and preventing further spread of infections is important, it is necessary to obtain a detailed neonatal history pertaining to the onset of symptoms and history of contact or exposure, such as visiting family members or staff. Careful measures like these can potentially prevent an occurrence of an outbreak in the hospital or even community.

EV outbreaks in hospitals often coincide with seasonal peaks of hand, foot, and mouth disease in the community [11]. The EV outbreak in our neonatal department resulted in in 7 cases and lasted 15 days. Six of 7 EV-positive patients were diagnosed by PCR from rectal swabs. Viral subtyping confirmed echovirus-25 in all cases, as opposed to the common circulating coxsackie viruses 16 and 6. Traditionally, EV detection is based on viral isolation in cell culture, and once a virus is isolated, further use of antisera or immunofluorescence staining allows identification of the infecting serotype [12]. Samples from the upper respiratory tract (throat swabs or nasopharyngeal aspirate), gastrointestinal tract (stool or rectal swabs) and cerebrospinal fluid have the highest isolation yield in comparison to blood and urine specimens [13,14]. The use of reverse transcriptase polymerase chain reaction (RT-PCR) has largely replaced the older methods of cell culture as it provides for shorter turnover times of 24 h and has both high sensitivity and specificity, up to 100% and 97%, respectively [15,16].

During the outbreak, all samples with positive results for EV were sent to the Singapore National Public Health Laboratory for viral subtyping. Identification of virus types was performed by direct sequence analysis. Echovirus-25 was found in all 7 samples, and this type had not been reported in national surveillance prior to the KKH outbreak. Thus, there is little information concerning its virulence based on local cases. One report describes conjunctivitis, pneumonia and gastrointestinal disease associated with NEC in echovirus-22 infection [17]. Another report describes symptoms of echovirus-18 infection as acute exanthematous disease [18]. One article reported echovirus-25 infections in an infant home; this was associated with maculopapular rashes, diarrhea, respiratory tract illness and aseptic meningitis [19]. As opposed to other reports that described gastroenteritis related to echovirus type-22 and acute exanthematous disease related to echovirus-18 [17,18], we noticed a tendency of echovirus-25 infected patients to develop respiratory distress and to require either assisted or mechanical ventilation.

Immediate isolation and separation in cohorts were put in place after the first case was detected on 5 June 2014. All EV-positive infants were isolated in a designated isolation room with additional contact precautions such as gloves, masks and gowns. A specific "isolation" team of doctors and nurses were formed to take care of the infected patients with the aim to contain the spread of viruses. The alcohol-based hand rub was also temporarily changed from a standard one to a virucidal one that included EV in its label claims.

All cases were detected in different areas and taken care of by different medical and nursing teams before isolation. Based on the detection of the same subtype of EV, it appears possible that there might have been cross-infection during night shifts or nursing changeovers. Thus, it appeared more likely that the spread of this EV strain occurred within the unit rather than the strain having been transmitted from the community to the babies on separate occasions. Thus, awareness of strict hand hygiene protocols and early detection was most important for infection control. The results of ad-hoc investigations and epidemiological studies guided the infection control policies and procedures that were implemented. All infants in the exposed cohort were screened with nasopharyngeal swabs and observed for any symptoms, with advice given to parents for observation even after discharge. No new admissions were allowed into the cohort areas during this period of outbreak. With

the active involvement and combined effort of multi-disciplinary teams, the outbreak lasted for 15 days with no new cases diagnosed after that.

Conclusions

EV infections can present with a spectrum of different clinical symptoms in neonates. Premature infants are more prone to serious complications such as NEC and meningitis when infected, which can lead to significant morbidities. Early recognition of symptoms, rapid diagnosis and prompt implementation of infection control measures are important to prevent the further spread of infection.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

None declared.

Ethical approval

This study was approved by the local institutional review board and a waiver of informed consent was granted due to the retrospective nature of the study as well as de-identication of patients in the report.

CRediT authorship contribution statement

Yee Yin Tan: Methodology, Data curation, Investigation, Writing - original draft, Writing - review & editing. **Bin Huey Quek:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Koh Cheng Thoon:** Conceptualization, Data curation, Investigation, Methodology, Writing - review & editing. **Matthias Maiwald:** Conceptualization, Data curation, Investigation, Methodology, Writing - review & editing. **Chee Fu Yung:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Victor Samuel Rajadurai:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Juin Yee Kong:** Conceptualization, Methodology, Data curation, Investigation, Writing - review & editing.

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