Published in final edited form as:

J Infect Dis. 2020 April 07; 221(9): 1499–1505. doi:10.1093/infdis/jiz621.

Infectious etiologies of intussusception among children <2 years old in 4 Asian countries

Eleanor Burnett[1,*], **Furqan Kabir**[2,*], **Nguyen Van Trang**[3], **Ajit Rayamajhi**[4,5], **Syed M Satter**[6], **Jie Liu**[7,*], **Mohammad Tahir Yousafzai**[2], **Dang Duc Anh**[3], **Anupama Thapa Basnet**[5], **Meerjady S. Flora**[8], **Eric Houpt**[7], **Saqib Hamid Qazi**[2], **Tran Minh Canh**[9], **Anjana Karki Rayamajhi**[4], **Bablu K Saha**[10], **Nasir Saleem Saddal**[11], **Sehrish Muneer**[2], **Pham Hoang Hung**[12], **Towhidul Islam**[13], **Syed Asad Ali**[2], **Jacqueline E. Tate**[1], **Catherine Yen**[1], **Umesh D. Parashar**[1] **Asian Intussusception Surveillance Network**

[1]Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, USA

[2]Dept. of Pediatrics and Child Health, Aga Khan University Karachi, Pakistan

[3]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

[4]Janak Medical and Research Center, Balaju, Kathmandu, Nepal

[5]Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal

[6]icddr,b, Dhaka, Bangladesh

[7]Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, USA

[8]Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, Bangladesh

[9]Hai Phong Children's Hospital, Hai Phong, Vietnam

[10]Rangpur Medical College Hospital, Rangpur, Bangladesh

[11]Dept. of Surgery, National Institute of Child Health Karachi, Pakistan

[12]Central Hue Hospital, Hue, Vietnam

[13]Sher E Bangla Medical College Hospital, Barisal, Bangladesh

[14]Rajshahi Medical College Hospital, Rajshahi, Bangladesh

[15]Jalalabad Ragib-Rabeya Medical College and Hospital, Sylhet, Bangladesh

[16]National Public Health Laboratory, Teku, Kathmandu, Nepal

Correspondence and reprints: Eleanor Burnett, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, MS A-34, Atlanta, GA 30329, wwg7@cdc.gov, 404 718 6761.

*Equal contributions

Asian Intussusception Surveillance Network: Md Nowshad Ali[14], Mohammad T Azad[15], Fatima Aziz[2], Muhammad Buksh[2], Runa Jha[16], Adil Kalam[2], Zahirul K Khan[17], Hoang Mai Linh[12], Huma Saleem[2], Suzanne Stroup[7], Ho Huu Thien[12]

Conflicts of interest: The authors indicate that they have no conflicts of interest relevant to this article to disclose.

[17]Jahurul Islam Medical College and Hospital, Bajitpur, Bangladesh

Abstract

Background—The etiology of intussusception, the leading cause of bowel obstruction in infants, is unknown in most cases. Adenovirus has been associated with intussusception, and a slightly increased risk of intussusception with rotavirus vaccination has been found in several countries. We conducted a case-control study among children <2 years old in Bangladesh, Nepal, Pakistan, and Vietnam to evaluate infectious etiologies of intussusception before rotavirus vaccine introduction.

Methods—From 2015-2017, we enrolled one-to-one matched intussusception cases and hospital controls; 249 pairs are included. Stool specimens were tested for 37 infectious agents using TaqMan Array technology. We used conditional logistic regression to estimate the odds ratio and 95% confidence interval (CI) of each pathogen associated with intussusception in a pooled analysis and in quantitative sub-analyses.

Results—Adenovirus (OR: 2.67, 95%CI: 1.75, 4.36) and human herpes virus 6 (OR: 3.50, 95%CI: 1.15, 10.63) were detected more frequently in cases than controls. Adenovirus C detection <20 quantification cycles was associated with intussusception (OR: 18.59, 95%CI: 2.45, 140.89). Wild-type rotavirus was not associated with intussusception (OR: 1.07, 95%CI: 0.52, 2.22).

Conclusions—In this comprehensive evaluation, adenovirus and HHV-6 were associated with intussusception. Future research is needed to better understand mechanisms leading to intussusception, particularly after rotavirus vaccination.

Summary:

Using state-of-the-art molecular testing for >30 gastrointestinal pathogens, adenovirus and human herpes virus 6 were associated with intussusception among children <2 years old before rotavirus vaccine introduction in Bangladesh, Nepal, Pakistan and Vietnam, while wild type rotavirus was not.

Keywords

adenovirus; rotavirus; intussusception; intestinal obstruction; viral pathogens

Introduction

Intussusception, an invagination of the intestine, is the leading cause of bowel obstruction in infants [1] and can lead to vascular compromise, necrosis of the intestine, and death when not reduced by enema or during surgery [2–4] . Some intussusception cases may resolve spontaneously [2–4]. Worldwide, an estimated 74 intussusceptions per 100,000 infants occur annually [5]. However, the incidence of intussusception varies by country: rates have been documented as high as 300 per 100,000 infants in Korea and Vietnam and as low as nine per 100,000 infants in Bangladesh [5]. Other differences between populations have also been noted in the age distribution, clinical management, and outcomes of intussusception in infants and young children [5–7].

Some cases of naturally occurring intussusception are caused by anatomical lead points; however, in most cases the cause is unknown. Because some studies have reported a seasonality to intussusception cases, several viral pathogens have been considered as possible etiologies [5]. Adenovirus, in particular type C, has been consistently associated with intussusception in infants and young children [8–15]. Enterovirus, norovirus and human herpesvirus 6 (HHV-6) have been shown to have statistically significant relationships with intussusception in some studies. However, findings for these viruses are sparse [9, 11, 14, 15]. No causative association has been found between intussusception and certain other viruses including astrovirus, sapovirus, and echovirus [8–11, 13–17].

The relationship between wild-type rotavirus and intussusception is also of interest because of a slightly increased risk of intussusception detected following vaccination with three different rotavirus vaccines based on different rotavirus strains (Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium), RotaTeq (Merck & Co., West Point, PA, USA), and RotaShield (Wyeth, Collegeville, PA, USA)) [18–24]. Wild-type rotavirus was not associated with intussusception in evaluations from six countries before the introduction of rotavirus vaccines; two additional studies conducted in pre-vaccine introduction settings reported a protective effect [8, 10, 13, 15–17]. One study found an association with rotavirus; however, the study was conducted after rotavirus vaccine introduction and the researchers were unable to distinguish between wild-type and vaccine rotavirus strains [9]. The mechanism for the increased risk of intussusception associated with rotavirus vaccines is unknown and this relationship has only been found in high- and middle-income settings to date [18–24]. Post-licensure safety evaluations are ongoing for additional rotavirus vaccines including Rotasiil (Serum Institute of India Pvt. Ltd., Pune, India), ROTAVAC (Bharat Biotech International Ltd., Hyderabad, India), Rotavin (PolyVac, Hanoi, Vietnam), and Lanzhou lamb rotavirus vaccine (Lanzhou Institute of Biological Products, Lanzhou, China) [25–27].

From 2014-2017, the Asian Intussusception Surveillance Network conducted active surveillance in a network of sentinel hospitals in four Asian countries (Bangladesh, Nepal, Pakistan, and Vietnam) to describe the epidemiology of intussusception [6]. An improved understanding of infectious pathogens associated with intussusception will help guide post-vaccine implementation safety monitoring. Pakistan implemented rotavirus vaccination in 2017 and the other three countries are planning to introduce rotavirus vaccine over the next few years. In this paper, we present the findings of a matched case-control study to evaluate potential infectious etiologies from stool specimens of intussusception cases using a custom developed TaqMan Array card in these four Asian countries before rotavirus vaccine introduction.

Methods

Enrollment

The Asian Intussusception Surveillance Network's case surveillance methods have previously been described [6]; intussusception cases enrolled in the surveillance platform were eligible for inclusion in this evaluation. Children <2 years old hospitalized from 2015-2017 at any of the 15 surveillance hospitals for their first episode of intussusception

meeting the Brighton Collaboration level 1 criteria for diagnostic certainty and who provided a stool specimen were included as cases in this evaluation [4]. Controls were children <2 years old admitted as inpatients to the same hospitals for non-infectious medical or surgical conditions which were unrelated to intussusception or other gastrointestinal conditions requiring bowel surgery. Children enrolled as controls also provided a stool specimen. Informed consent was obtained from the caregivers and a standardized questionnaire was administered. Additional information was obtained from clinical staff and the child's medical record. All data were entered into an Epi Info database.

We individually matched one control to each case for date of birth (+/−62 days), date of hospital admission (+/−31 days), and residence in the same, or similar, district. Cases and controls enrolled in Bangladesh, Nepal, and Pakistan were matched by study staff at the time of enrollment. In Vietnam, cases and controls were enrolled in parallel and matched using statistical software before the analysis [28]. We estimated that 140 case-patients and 140 controls would be necessary to demonstrate a 10% difference in pathogen prevalence between case-patients and controls with a power of 80% [8].

Laboratory methods

Stool samples were collected from cases and controls within 48 hours of enrollment and stored at −70°C. All specimens were transported to and tested at Infectious Disease Research Laboratory (IDRL) of Aga Khan University, Karachi, Pakistan using TaqMan Array technology [29]. Briefly, total nucleic acid was extracted from 200 milligram of stool samples with QIAamp Fast Stool DNA Mini kit (Qiagen) using a modified protocol previously described [29]. 20 μl of the 200 μl eluate was mixed with 50 μl AgPath One Step RT-PCR buffer (Thermo Fisher), 4 μl AgPath Enzyme mix, 26 μl nuclease free water, then loaded onto the TaqMan Array Card. Each microfluidic TaqMan Array card (TAC) has 384 wells and can test eight specimens for up to 90 targets using real time RT-PCR under the cycling conditions of 45°C for 20 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 1min. The cards are modular and customizable; two versions of the TAC were used for testing in this study and the previously validated pathogens included on either card are listed in supplementary Table 1 [30]. The pathogens included on either version of the card had a suggested or hypothesized association with intussusception in the published literature. Data validity was determined according to the TAC quality control scheme, based on external controls spiked into each sample (MS2 for RNA targets and Phocine herpesvirus for DNA targets), extraction blanks incorporated into each batch of extraction, and software flags (Supplementary Figure 1). The positive results were valid only when the corresponding extraction blank was negative for the relevant targets; the negative results were valid only when the external controls were positive on a given sample; data in the reaction wells flagged with manufacturer defined BADROX (bad passive reference signal) combined with NOISE (noise higher than others in card) or SPIKE (noise spikes) or both were determined invalid. The invalid results were excluded from analysis.

Analytic methods

Descriptive analyses are presented as percentages and medians with interquartile ranges (IQR) for the demographic characteristics of cases and as percentages of cases and controls

for prevalence of pathogens in the stool specimens. We used conditional logistic regression to estimate the odds ratio and 95% confidence interval (CI) of intussusception and each pathogen, controlling for country. As a secondary analysis, we considered potential associations separately for Vietnam and for Bangladesh, Nepal and Pakistan as a group due to differences in the descriptive epidemiology of intussusception cases in Vietnam [5, 6]. All analyses were performed using SAS v9.4.

In the primary analysis, we used quantification cycle (Cq) 35 as the analytical cutpoint for all pathogens, where a Cq-value <35 was considered positive. Cq values indicate the abundance of the target nucleic acid in the sample, with lower values indicating more nucleic acid. As a secondary analysis of adenovirus type C and pan adenovirus to better understand the differences between Vietnam and Bangladesh, Nepal, and Pakistan, we divided positive specimens into 4 categories based on Cq value: <20, 20-24, 25-29, and 30-34. A specimen was considered positive for adenovirus non- type C if it was positive for pan adenovirus and negative for adenovirus type C.

This activity was approved by the institutional review boards at icddr,b in Dhaka, Bangladesh, Nepal Health Research Council in Kathmandu, Nepal, Aga Khan University in Karachi, Pakistan, the National Institute of Hygiene and Epidemiology in Hanoi, Vietnam, and the Centers for Disease Control and Prevention in Atlanta, USA.

Results

In total, 803 specimens were collected and tested, of which there were 311 uniquely matched case and control pairs. In total, 249 matched pairs are included in the analysis. Because the cases and controls from Vietnam were matched retrospectively, we were unable to match all of the cases. Of the matched pairs, 22 were from Bangladesh, 60 were from Nepal, 61 were from Pakistan, and 106 were from Vietnam. The remaining specimen pairs were excluded because the control did not meet the inclusion definition (n=46) or the number of days between the case and control birth or admission dates was greater than 2 and 1 months, respectively (n=16). The median age of cases was 9 months (IQR: 6-13) and 62% were male. Among controls, 69% were male. The majority of cases (86%) experienced at least one common symptom of intussusception; fever, vomiting, diarrhea, constipation, and bloody stools were considered common symptoms. More than half of the intussusceptions (54%) were reduced by enema and 46% were reduced during surgery; six (2%) died.

The median number of pathogens detected among cases and controls was 2 (cases: IQR: 1-3; controls: IQR:1-4). The most commonly detected pathogens among all 498 case and control specimens were pan adenovirus (n=195, 39%), enteroaggregative *E. coli* (n=195, 39%), adenovirus type C (n=176, 35%), enterovirus (n=171, 34%), and cytomegalovirus (n=83, 17%). One case child was positive for the Rotarix vaccine strain; none of the children were positive for RotaTeq or ROTAVAC vaccine strains.

Across all four countries, intussusception cases were nearly 3 times more likely to have adenovirus detected than controls (OR: 2.67, 95%CI: 1.75, 4.36) (Table 1). Using four Cq value categories for positive specimens, this relationship was statistically significant when

adenovirus was detected in intussusception cases at <20 Cq (OR: 22.65, 95%CI: 4.79- 106.96), 20-24 Cq (OR: 10.87; 95%CI: 3.67- 32.26) and 25-29 Cq (OR: 2.89; 95%CI: 1.31- 6.41) but not at 30-34 Cq. Adenovirus type C was not associated with intussusception (OR: 1.24, 95%CI: 0.82- 1.86) with a binary Cq cut point. However, using the four Cq value categories for positive specimens, adenovirus C was statistically significantly associated with intussusception when detected at <20 Cq (OR: 16.60, 95%CI: 2.20, 125.50) and 20-24 Cq (OR: 8.60, 95%CI: 1.08, 68.16). There was no association between intussusception and adenovirus type C detected at 25-29 and 30-34 Cq. Using a binary Cq categories, non-C species of adenovirus were also statistically significantly associated with intussusception (OR: 1.90, 95%CI: 1.11, 3.27) in all countries.

Similar to the overall results, there was a statistically significant association between adenovirus and intussusception in the analysis including cases enrolled in Bangladesh, Nepal, and Pakistan (OR: 3.27, 95%CI: 1.67, 6.43) and Vietnam alone (OR: 2.36, 95%CI: 1.26, 4.40). No association between adenovirus type C and intussusception was detected using binary Cq categories (Bangladesh, Nepal, and Pakistan: OR: 1.77, 95%CI: 0.97- 3.20; Vietnam: OR: 0.88, 95%CI: 0.50- 1.56) (Table 2). When stratifying by the Cq value categories, adenovirus C detection of Cq value <20 was statistically significantly associated with intussusception in Bangladesh, Nepal, and Pakistan (OR: 9.99, 95%CI: 1.20, 83.11) but not in Vietnam (undefined). Non-C species of adenovirus were statistically significantly associated with intussusception in Vietnam (OR: 8.50, 95%CI: 1.96, 36.79) but not in Bangladesh, Nepal, and Pakistan (OR: 1.17, 95%CI: 0.62, 2.19).

Intussusception cases were about 3 times more likely to have HHV-6 detected than controls in the analysis of all 4 countries (OR: 3.50, 95%CI: 1.15, 10.63) (Table 1), in Bangladesh, Nepal, and Pakistan (OR: 3.67, 95%CI: 1.02, 13.14), and Vietnam (OR: 3.00, 95%CI: 0.32, 28.84) (Table 2), though the odds ratio in the Vietnam only analysis was not statistically significant. Of cases with HHV-6 detected, 43% (n=6) were co-infected with adenovirus. Norovirus GII was significantly protective against intussusception in all four countries (OR: 0.50, 95%CI: 0.28, 0.90) and in Vietnam (OR: 0.30, 95%CI: 0.12, 0.75). *Campylobacter jejuni/C. coli* was also protective against intussusception (OR: 0.39, 95%CI: 0.16- 0.93) in all four countries and the analysis with Bangladesh, Nepal, and Pakistan (OR: 0.23, 95%CI: 0.07- 0.81). In Bangladesh, Nepal, and Pakistan, *Campylobacter* pan (OR: 0.38, 95%CI: 0.15, 0.96) and *C. difficile* (OR: 0.38, 95%CI: 0.15, 0.96) were protective against intussusception, although this was not found in the four-country pooled analysis or the analysis of Vietnam alone. There was no statistically significant association detected between intussusception and enterovirus (OR: 1.00, 95%CI: 0.67, 1.49), wild-type rotavirus (OR: 1.07, 95%CI: 0.52, 2.22), or any of the other pathogens tested. Similar results were observed when limiting the analysis to children <1 year old, within which age range rotavirus vaccine was given (supplementary Table 2).

Discussion

This comprehensive etiologic assessment of intussusception used state-of-the-art molecular diagnostics to detect a broad range of pathogens and has the largest number of matched case-control pairs to date by pooling data from four countries under a common protocol. Our

finding that pan adenovirus is associated with intussusception is consistent with findings from previous evaluations, although the magnitude of association is somewhat less than earlier case-control studies in the overall results [8–11, 15]. When categorized by Cq value, the highest viral load category had a similar magnitude of association as earlier studies. We also found an association with intussusception when there was a high viral load of adenovirus type C and the point estimates from this sub analysis by Cq value were comparable to point estimates for adenovirus type C in other studies [8, 9]. This suggests that acuity of infection may be an important factor in this relationship. Although the relationship between intussusception and adenovirus has been consistently documented, a large number of case and control stool specimens in our study were positive for adenovirus indicating not all adenovirus infections lead to intussusception meeting the Brighton level 1 criteria.

Like earlier evaluations in countries that had not introduced rotavirus vaccine [8, 10, 15–17], we found no association between wild-type rotavirus and intussusception in our pooled primary analysis with data from all four countries or our sub analyses. One might expect wild-type rotavirus to have a causative relationship with intussusception given that three rotavirus vaccines based on different rotavirus strains (rhesus-human reassortant, bovine-human reassortant, and human) have been linked to intussusception. The lack of an association may suggest that the high titer of virus in vaccine or the oral administration route might have a particular link. Alternatively, these studies lack statistical power to detect a possible low risk with wild-type rotavirus. There are other hypotheses about the cause of intussusception following rotavirus vaccination but as our study was conducted in settings without routine use of rotavirus vaccines, we are unable to comment on these hypotheses.

We found a similar magnitude association between intussusception and HHV-6 in the pooled four country analysis; Bangladesh, Nepal, and Pakistan; and Vietnam alone. Previous studies had reported no association with HHV-6 alone but a causative association when the child was co-infected with adenovirus [11, 14]. The absolute number of specimens positive for HHV-6 was very small, therefore a limited percentage of all intussusceptions are likely due to HHV-6. In this evaluation, we found a statistically significant protective effect with norovirus group II. In the published literature, there was one significant, protective result for norovirus and one null result [9, 13]. We did not find a causative relationship between any of the bacterial pathogens and intussusception.

This study has several limitations. First, our cases and controls were enrolled from a limited number of sentinel sites; these findings may not be generalizable widely within these four countries, regionally, or globally. Furthermore, this study was not powered at an individual country level but rather for a pooled primary analysis. Enrolling appropriate controls proved challenging and thus our final population was a small subset of the over 1,400 cases enrolled during the 2 year surveillance period [6]. Second, we collected stool specimens after hospital admission and may not have captured infections from prior to the hospitalization that were no longer detectible in stool. Also, we only collected and tested stool specimens, unlike some earlier studies that included throat swabs in addition to stool specimens [14, 15]. Specimens from alternate anatomical sites may have provided additional information about viruses associated with intussusception, especially as adenovirus is typically an illness with

respiratory symptoms. Finally, the assay we used for adenovirus type C is more sensitive than the assay for pan adenovirus, which complicates the interpretation of the analysis of adenovirus non-C. Nonetheless, we think these findings are informative.

In conclusion, this matched case-control study found that adenovirus and HHV-6 are associated with intussusception, however these infections do not account for all of the cases of intussusception in our study population. Wild-type rotavirus was not associated with intussusception. Future research is needed to better understand the mechanisms that lead to intussusception, particularly after rotavirus vaccination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Acute intussusception in infants and children: Incidence, clinical presentation and management: a global perspective. Geneva: World Health Organization Vaccines and Biologicals, 2002.
2. Kornecki A, Daneman A, Navarro O, Connolly B, Manson D, Alton DJ. Spontaneous reduction of intussusception: clinical spectrum, management and outcome. Pediatr Radiol 2000; 30:58–63. [PubMed: 10663512]
3. Munden MM, Bruzzi JF, Coley BD, Munden RF. Sonography of pediatric small-bowel intussusception: differentiating surgical from nonsurgical cases. AJR Am J Roentgenol 2007; 188:275–9. [PubMed: 17179377]
4. Bines JE, Kohl KS, Forster J, et al. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine 2004; 22:569–74. [PubMed: 14741146]
5. Jiang J, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood intussusception: a literature review. PLoS One 2013; 8:e68482. [PubMed: 23894308]
6. Burnett E, Van Trang N, Rayamajhi A, et al. Preparing for safety monitoring after rotavirus vaccine introduction - Assessment of baseline epidemiology of intussusception among children <2years of age in four Asian countries. Vaccine 2018; 36:7593–8. [PubMed: 30414781]
7. Clark AD, Hasso-Agopsowicz M, Kraus MW, et al. Update on the global epidemiology of intussusception: a systematic review of incidence rates, age distributions and case-fatality ratios among children aged <5 years, before the introduction of rotavirus vaccination. Int J Epidemiol 2019.
8. Bines JE, Liem NT, Justice FA, et al. Risk factors for intussusception in infants in Vietnam and Australia: adenovirus implicated, but not rotavirus. J Pediatr 2006; 149:452–60. [PubMed: 17011313]
9. Minney-Smith CA, Levy A, Hodge M, et al. Intussusception is associated with the detection of adenovirus C, enterovirus B and rotavirus in a rotavirus vaccinated population. J Clin Virol 2014; 61:579–84. [PubMed: 25464971]
10. Mansour AM, El Koutby M, El Barbary MM, et al. Enteric viral infections as potential risk factors for intussusception. J Infect Dev Ctries 2013; 7:28–35. [PubMed: 23324817]
11. Lappalainen S, Ylitalo S, Arola A, Halkosalo A, Rasanen S, Vesikari T. Simultaneous presence of human herpesvirus 6 and adenovirus infections in intestinal intussusception of young children. Acta Paediatr 2012; 101:663–70. [PubMed: 22296119]
12. Okimoto S, Hyodo S, Yamamoto M, Nakamura K, Kobayashi M. Association of viral isolates from stool samples with intussusception in children. Int J Infect Dis 2011; 15:e641–5. [PubMed: 21757385]

13. Jang J, Lee YJ, Kim JS, et al. Epidemiological Correlation between Fecal Adenovirus Subgroups and Pediatric Intussusception in Korea. J Korean Med Sci 2017; 32:1647–56. [PubMed: 28875609]
14. Hsu HY, Kao CL, Huang LM, et al. Viral etiology of intussusception in Taiwanese childhood. Pediatr Infect Dis J 1998; 17:893–8. [PubMed: 9802631]
15. Ukarapol N, Khamrin P, Khorana J, Singhavejsakul J, Damrongmanee A, Maneekarn N. Adenovirus infection: A potential risk for developing intussusception in pediatric patients. J Med Virol 2016; 88:1930–5. [PubMed: 27097123]
16. Velazquez FR, Luna G, Cedillo R, Torres J, Munoz O. Natural rotavirus infection is not associated to intussusception in Mexican children. Pediatr Infect Dis J 2004; 23:S173–8. [PubMed: 15502698]
17. Bahl R, Saxena M, Bhandari N, et al. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among indian children. J Infect Dis 2009; 200 Suppl 1:S277–81. [PubMed: 19817609]
18. Tate JE, Mwenda JM, Armah G, et al. Evaluation of Intussusception after Monovalent Rotavirus Vaccination in Africa. N Engl J Med 2018; 378:1521–8. [PubMed: 29669224]
19. Buttery JP, Danchin MH, Lee KJ, et al. Intussusception following rotavirus vaccine administration: post-marketing surveillance in the National Immunization Program in Australia. Vaccine 2011; 29:3061–6. [PubMed: 21316503]
20. Yih WK, Lieu TA, Kulldorff M, et al. Intussusception risk after rotavirus vaccination in U.S. infants. N Engl J Med 2014; 370:503–12. [PubMed: 24422676]
21. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. N Engl J Med 2011; 364:2283–92. [PubMed: 21675888]
22. Weintraub ES, Baggs J, Duffy J, et al. Risk of intussusception after monovalent rotavirus vaccination. N Engl J Med 2014; 370:513–9. [PubMed: 24422678]
23. Kramarz P, France EK, Destefano F, et al. Population-based study of rotavirus vaccination and intussusception. Pediatr Infect Dis J 2001; 20:410–6. [PubMed: 11332666]
24. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001; 344:564–72. [PubMed: 11207352]
25. Luan le T, Trang NV, Phuong NM, et al. Development and characterization of candidate rotavirus vaccine strains derived from children with diarrhoea in Vietnam. Vaccine 2009; 27 Suppl 5:F130–8. [PubMed: 19931712]
26. Burnett E, Tate JE, Kirkwood CD, et al. Estimated impact of rotavirus vaccine on hospitalizations and deaths from rotavirus diarrhea among children <5 in Asia. Expert Rev Vaccines 2018:1–8.
27. Kirkwood CD, Ma LF, Carey ME, Steele AD. The rotavirus vaccine development pipeline. Vaccine 2017.
28. Mounib EL, Satchi T. Automating the Selection of Contols in Case-Control Studies. SAS Users Group International Conference, 2000.
29. Liu J, Kabir F, Manneh J, et al. Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhoea: a multicentre study. Lancet Infect Dis 2014; 14:716–24. [PubMed: 25022434]
30. Liu J, Gratz J, Amour C, et al. Optimization of Quantitative PCR Methods for Enteropathogen Detection. PLoS One 2016; 11:e0158199. [PubMed: 27336160]

Table 1.

Association between pathogens included on TaqMan Array cards and intussusception among children <2 years old in 4 Asian countries.

	Cases		Controls			
	N=249	**%**	**N=249**	**%**	**OR[1]**	**95%CI**
Adenovirus pan	119	48	76	31	**2.67**	**1.75- 4.36**
<20 Cq-value[2]	29	12	7	3	**22.65**	**4.79- 106.96**
20-24 Cq-value[2]	31	13	6	2	**10.87**	**3.67- 32.26**
25-29 Cq-value[2]	23	9	16	6	**2.89**	**1.31- 6.41**
30-34 Cq-value[2]	36	15	47	19	1.25	0.71-2.22
Type C	93	39	83	35	1.24	0.82- 1.86
<20 Cq-value[2]	20	8	3	1	**16.60**	**2.20- 125.50**
20-24 Cq-value[2]	10	4	2	1	**8.60**	**1.08- 68.16**
25-29 Cq-value[2]	13	5	21	9	0.71	0.30- 1.66
30-34 Cq-value[2]	50	21	57	24	0.93	0.56- 1.53
Non-Type C	43	18	25	10	**1.90**	**1.11- 3.27**
Type F[3]	7	7	4	4	2.00	0.50- 8.00
Ancylostoma	0	0	0	0	-	-
Ascaris	1	0	0	0	-	-
Astrovirus	11	4	16	7	0.69	0.30- 1.62
C. difficile	36	15	42	17	0.90	0.54- 1.51
Campylobacter pan	18	7	27	11	0.64	0.34- 1.24
C. jejuni/C. coli	8	3	20	8	**0.39**	**0.16- 0.93**
Cytomegalovirus	41	17	42	17	1.00	0.62- 1.62
Cryptosporidium	11	4	10	4	1.14	0.41- 3.15
Enteroaggregative *E. coli* (EAEC)	101	41	94	38	1.20	0.79- 1.81
Enteroinvasive *E. coli* (EIEC)	8	3	5	2	1.60	0.52- 4.89
Atypical enteropathogenic *E. coli* (aEPEC)	37	15	38	15	1.10	0.67- 1.82
Typical enteropathogenic *E. coli* (tEPEC)	11	5	22	9	0.52	0.25- 1.09
Enterotoxigenic *E. coli* (ETEC)	29	12	24	10	1.29	0.69- 2.44
E. coli O157	4	2	1	0	4.00	0.45- 35.79
Shiga-toxin producing *E. coli* (STEC)	3	1	2	1	1.50	0.25- 8.98
E. histolytica	0	0	0	0	-	-
Epstien Barr virus	2	1	2	1	1.00	0.14- 7.10
Enterovirus	86	35	85	35	1.00	0.67- 1.49
Giardia	8	3	12	5	0.64	0.25- 1.64
H. pylori	1	0	1	0	1.00	0.06- 15.00
Human herpesvirus 6	14	6	4	2	**3.50**	**1.15- 10.63**
Human herpesvirus 7	1	0	0	0	-	-

	Cases		Controls			
	N=249	**%**	**N=249**	**%**	**OR[1]**	**95%CI**
Necator	0	0	0	0	-	-
Norovirus group I	5	2	8	3	0.63	0.20- 1.91
Norovirus group II	19	8	36	15	**0.50**	**0.28- 0.90**
Rotarix specific NSP2	1	0	0	0	-	-
RotaTeq specific VP6[3]	0	0	0	0	-	-
RotaVac specific G9[3]	0	0	0	0	-	-
Rotavirus	18	7	17	7	1.07	0.52- 2.22
<20 Cq-value[2]	3	1	0	0	-	-
20-24 Cq-value[2]	4	2	8	3	0.47	0.14- 1.61
25-29 Cq-value[2]	5	2	2	1	2.50	0.49- 12.89
30-34 Cq-value[2]	6	2	7	3	0.73	0.22- 2.46
Salmonella	4	2	9	4	0.44	0.14- 1.44
Sapovirus	16	6	24	10	0.62	0.31- 1.24
Strongyloides	0	0	0	0	-	-
Trichuris	1	0	0	0	-	-
Y. enterocolitica	8	3	2	1	4.00	0.85- 18.84

[1] Odds ratio

[2] Quantification cycle

[3] Only 1 version of the card therefore 191/498 samples were tested

Table 2.

Association between pathogens and intussusception among children <2 years old by country group.

	Bangladesh, Nepal, and Pakistan						Vietnam					
	Cases		Controls				Cases		Controls			
	N=143	%	N=143	%	OR[1]	95%CI	N=106	%	N=106	%	OR[1]	95%CI
Adenovirus pan	65	46	41	29	**3.27**	**1.67- 6.43**	54	51	35	33	**2.36**	**1.26- 4.40**
<20 Cq-value[2]	18	13	6	4	**13.62**	**2.77- 67.08**	11	10	1	1	-	-
20-24 Cq-value[2]	12	9	4	3	**7.02**	**1.66- 29.69**	19	18	2	2	**23.05**	**2.94- 180.99**
25-29 Cq-value[2]	12	9	7	5	**3.99**	**1.21- 13.10**	11	10	9	8	2.29	0.76- 6.87
30-34 Cq-value[2]	23	16	24	17	1.83	0.83- 4.01	13	12	23	22	0.78	0.33- 1.86
Type C	52	38	39	27	1.77	0.97- 3.20	41	40	44	46	0.88	0.50- 1.56
<20 Cq-value[2]	8	6	1	1	**9.99**	**1.20- 83.11**	12	12	2	2	-	-
20-24 Cq-value[2]	3	2	0	0	-	-	7	7	2	2	5.15	0.61-43.41
25-29 Cq-value[2]	10	7	9	6	1.71	0.51- 5.66	3	3	12	13	0.22	0.05-1.04
30-34 Cq-value[2]	31	22	29	21	1.40	0.70- 2.79	19	18	28	29	0.53	0.25- 1.15
Non-Type C	24	17	22	15	1.17	0.62- 2.19	19	18	3	3	**8.50**	**1.96- 36.79**
Type F[3]	5	23	3	14	2.00	0.37- 10.92	2	3	1	1	2.00	0.18- 22.06
Ascaris	1	1	0	0	-	-	0	0	0	0	-	-
Astrovirus	10	7	13	9	0.80	0.32- 2.03	1	1	3	3	0.33	0.04- 3.21
C. difficile	6	4	19	13	**0.38**	**0.15- 0.96**	30	28	23	22	1.50	0.76- 2.95
Campylobacter pan	9	6	20	14	**0.38**	**0.15- 0.96**	9	8	7	7	1.33	0.46- 3.84
C. jejuni/C. coli	4	3	15	10	**0.23**	**0.07- 0.81**	4	4	5	5	0.80	0.22- 2.98
Cytomegalovirus	27	19	25	17	1.16	0.63- 2.14	14	13	17	16	0.79	0.36- 1.73
Cryptosporidium	10	7	9	6	1.17	0.39- 3.47	1	1	1	1	1.00	0.06- 15.99
Enteroaggregative *E. coli* (EAEC)	78	55	71	50	1.33	0.79- 2.26	23	22	23	22	1.00	0.51- 1.96
Enteroinvasive *E. coli* (EIEC)	7	5	5	4	1.40	0.44- 4.41	1	0	0	0	-	-
Atypical enteropathogenic *E. coli* (aEPEC)	21	15	20	14	1.29	0.64- 2.59	16	15	18	17	0.93	0.45- 1.93
Typical enteropathogenic *E. coli* (tEPEC)	8	6	16	11	0.53	0.23- 1.26	3	3	6	6	0.50	0.13- 2.00

	Bangladesh, Nepal, and Pakistan						Vietnam					
	Cases		Controls				Cases		Controls			
	N=143	%	N=143	%	OR[1]	95%CI	N=106	%	N=106	%	OR[1]	95%CI
Enterotoxigenic *E. coli* (ETEC)	15	11	16	11	0.92	0.40- 2.08	14	13	8	8	2.20	0.76- 6.33
E. coli O157	0	0	1	0	-	-	4	4	0	0	-	-
Shiga-toxin producing *E. coli* (STEC)	0	0	2	1	-	-	3	3	0	0	-	-
Epstien Barr virus	2	1	0	0	-	-	0	0	2	2	-	-
Enterovirus	56	39	52	36	1.15	0.69- 1.92	30	29	33	32	0.81	0.43- 1.53
Giardia	7	5	12	8	0.55	0.20- 1.48	1	1	0	0	-	-
H. pylori	1	1	0	0	-	-	0	0	1	1	-	-
Human herpesvirus 6	11	8	3	2	**3.67**	**1.02- 13.14**	3	3	1	1	3.00	0.32- 28.84
Human herpesvirus 7	0	0	0	0	-	-	1	1	0	0	-	-
Norovirus group I	5	4	8	6	0.63	0.20- 1.91	0	0	0	0	-	-
Norovirus group II	12	8	15	11	0.79	0.36- 1.73	7	7	21	20	**0.30**	**0.12- 0.75**
Rotarix specific NSP2	0	0	0	0	-	-	1	1	0	0	-	-
Rotavirus	13	9	15	11	0.85	0.38- 1.89	5	5	2	2	4.00	0.45-35.79
Salmonella	1	1	6	4	0.17	0.02- 1.38	3	3	3	3	1.00	0.20- 4.96
Sapovirus	11	8	18	13	0.56	0.25- 1.27	5	5	6	6	0.80	0.22- 2.98
Trichuris	1	0	0	0	-	-	0	0	0	0	-	-
Y. enterocolitica	7	5	2	1	3.50	0.73- 16.85	1	1	0	0	-	-

[1]Odds ratio

[2]Quantification cycle

[3]Only 1 version of the card

J Infect Dis. Author manuscript; available in PMC 2020 July 20.