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Influenza Virus Detection in the Stool of Children with Acute Gastroenteritis

Jianling Xie, MD, MPH¹, Xiao-Li Pang, PhD^{2,3}, Gillian A.M. Tarr, PhD⁴, Yuan Mu, PhD^{3,5}, Ran Zhuo, PhD³, Linda Chui, PhD^{2,3}, Bonita E. Lee, MD, MPH^{6,7}, Otto G. Vanderkooi, MD⁸, Phillip I. Tarr, MD⁹, Samina Ali, MDCM^{6,7}, Shannon E. MacDonald, RN PhD^{10,4}, Stephen B. Freedman, MDCM, MSc^{11,12,13}

¹Section of Pediatric Emergency Medicine, Department of Pediatric, Alberta Children Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta

²Provincial Laboratory for Public Health, Alberta Public Laboratories, Edmonton, Alberta, Canada

³Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

⁴Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

⁵Department of Clinical Laboratory, Children's Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

⁶Department of Pediatrics, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta, Canada

⁷Women & Children's Health Research Institute (WCHRI), University of Alberta, Edmonton, Alberta, Canada

Corresponding author: Dr. Stephen B. Freedman, Sections of Pediatric Emergency Medicine and Gastroenterology, Department of Pediatrics, Alberta Children's Hospital and Research Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. T3B 6A8, Stephen.freedman@ahs.ca.
CRediT authorship contribution statement

Jianling Xie MD, MPH: Methodology, Formal analysis, Writing - Original Draft, Visualization **Xiao-Li Pang PhD:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition

Gillian A.M. Tarr PhD: Methodology, Formal analysis, Writing - Review & Editing, Visualization

Yuan Mu PhD: Methodology, Validation, Investigation, Data Curation, Writing - Review & Editing

Ran Zhuo PhD: Methodology, Validation, Investigation, Data Curation, Writing - Review & Editing

Linda Chui PhD: Resources, Writing - Review & Editing, Funding acquisition

Bonita E. Lee MD, MPH: Conceptualization, Writing - Review & Editing, Visualization, Funding acquisition

Otto G. Vanderkooi MD: Conceptualization, Writing - Review & Editing

Phillip I. Tarr MD: Conceptualization, Writing - Review & Editing, Visualization

Samina Ali, MDCM: Conceptualization, Writing - Review & Editing, Visualization

Shannon E. MacDonald RN PhD: Conceptualization, Writing - Review & Editing

Stephen B. Freedman MDCM, MSc: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition

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⁸Department of Pediatrics, Microbiology, Immunology and Infectious Diseases, Pathology & Laboratory Medicine and Community Health Sciences and the Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

⁹Department of Pediatrics, Division of Gastroenterology, & Nutrition, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA

¹⁰Faculty of Nursing, University of Alberta, Edmonton, Alberta, Canada

¹¹Section of Pediatric Emergency Medicine, Department of Pediatrics, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

¹²Section of Pediatric Gastroenterology, Department of Pediatrics, Alberta Children's Hospital, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

¹³Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

Abstract

Objectives: To determine if the clinical characteristics of children with gastroenteritis and influenza identified in their stool differ from those whose stool was influenza-negative.

Methods: Children <18-years with gastroenteritis whose stool tested negative for enteropathogen were tested for influenza in stool. The clinical features between influenza-positive and influenza-negative gastroenteritis cases were compared. Stools from controls without infection were also tested for influenza.

Results: Among the 440 gastroenteritis cases, those who were influenza test-positive were older [median age 4.0 (IQR: 2.3, 5.5) vs. 1.5 (IQR: 0.5, 4.0) years; $P=0.008$], more likely to present in fall or winter (92.3% vs. 48.0%; $P=0.001$), be febrile (84.6% vs. 30.6%; $P<0.001$), have respiratory symptoms (91.7% vs. 44.8%; $P=0.002$), have dehydration [median Clinical Dehydration Scale score: 4 (IQR: 1.5, 4.5) vs. 2 (IQR: 0, 3); $P=0.034$], and have higher Modified Vesikari Scale scores [median: 13 (IQR: 10.5, 14.0) vs. 10 (IQR: 9.0, 13.0); $P=0.035$], than those who tested negative. Thirteen gastroenteritis cases (13/440; 3.0%) including one child without respiratory symptoms vs. one control (1/250; 0.4%) were influenza stool positive.

Conclusions: Fever, respiratory symptoms, more severe illness, and older age were more common in children with gastroenteritis with influenza detected in stool, compared to those tested negative

Keywords

influenza; enteropathogens; gastroenteritis; pediatrics; reverse transcriptase-quantitative polymerase chain reaction

INTRODUCTION

Influenza is one of the most important infectious pathogens affecting nearly 10 percent in children <18 years of age annually (1) with outbreaks occurring predominantly during the winter months in temperate climates. Manifestations vary with age and in young children,

infection is often associated with vomiting and diarrhea (2, 3). The presence of enteric symptoms in children is highlighted in a Centers for Disease Control and Prevention (CDC) report which states that “Among young children with influenza, nausea, vomiting or diarrhea may also occur” (4).

Although influenza virus has been detected in the stools of adult patients with uncomplicated diarrhea in the absence of respiratory symptoms (5), as it does not replicate in the human gastrointestinal tract, it is generally not considered to be an enteropathogen. Thus, pathogen detection in stool in the presence of gastrointestinal symptoms can lead to diagnostic and therapeutic decision dilemmas. As children play a crucial role in the spread of influenza (6), and the importance of influenza epidemic control as an international public health priority (7), clarity regarding the role influenza plays in children with gastroenteritis symptoms is needed to inform prevention and control programs. As such, we sought to investigate whether children with vomiting and/or diarrhea with influenza-positive stool present with different or more severe clinical features from those with influenza-negative stool. We also explore if the presence of influenza virus in the gastrointestinal tract is associated with vomiting and diarrhea in children.

METHODS

Study Design

We performed a secondary analysis employing data from the Alberta Provincial Pediatric Enteric Infection TEam (APPETITE) study (8). Between August 1, 2015 and February 28, 2018, children with vomiting and/or diarrhea (cases) and those without gastrointestinal and respiratory symptoms (controls) were consecutively enrolled in the province of Alberta, Canada. Cases were aged <18 years, had ≥3 episodes of vomiting and/or diarrhea in a 24-hour period and <7 days of symptoms. They were recruited in two urban, pediatric tertiary-care emergency departments (ED) [Alberta Children’s Hospital (Calgary), Stollery Children’s Hospital (Edmonton), Alberta, Canada], and through Health Link (Alberta Health Services), a provincewide telephone advice line. Controls were consecutively enrolled from the same EDs and from a public health clinic located in Calgary, if they were <18 years of age and had no vomiting, diarrhea, fever, rhinorrhea, or cough during the preceding 7 days. To focus on the impact of influenza as the sole pathogen, children in both study cohorts were excluded if they had any enteropathogen detected in their stool specimens.

The study protocol was approved by the ethics committees of the University of Alberta (Pro00050790) and the University of Calgary (REB14-1122). Consent was obtained from the caregivers of eligible children along with assent, when appropriate.

Definitions

Respiratory symptoms were an increase in rhinorrhea or cough from participant’s baseline state. Fever was defined as a rectal temperature or adjusted rectal temperature of ≥38.0°C (9). Dehydration was quantified using the Clinical Dehydration Scale (CDS) score, with scores of 0, 1-4, and 5-8 indicating none, mild to moderate, and severe dehydration,

respectively (10). The Modified Vesikari Scale (MVS) score, which ranges from 0 to 20, with higher scores indicating more severe disease, was used to assess gastroenteritis illness severity (11). Seasons were categorized as winter (January–March), spring (April–June), summer (July–September) and fall (October–December). Influenza virus was deemed present in stool if real-time reverse transcription (RT) polymerase chain reaction (PCR) detected the M or the NS 1 gene of influenza A or B, respectively. Viral load was defined by the RT-PCR cycle threshold (C_T) values (12).

Objectives

The primary objective was to determine, among gastroenteritis cases, if children with influenza-positive stool samples had clinical features that differed from test-negative children. The secondary objective was to compare the likelihood of influenza virus detection in stool submitted by gastroenteritis cases without respiratory symptoms vs. those provided by controls. We also sought to evaluate if the influenza viral load and the strains detected differed between influenza-positive cases and controls.

Outcome Measures

The primary outcome measure focused on clinical features including the presence of fever, respiratory symptoms, and CDS and MVS scores. Secondary outcome measures included stool influenza virus detection, influenza viral load, and strains identified.

Specimen Acquisition

One stool sample was collected in sterile containers (V302-F; Starplex Scientific Inc., ON, Canada) from each ED participant. If stool was not provided prior to discharge, specimens were collected by caregivers at home. Health Link participants received specimen collection kits by courier and caregivers collected specimens as per ED protocols. All specimens collected at home were placed in coolers with ice packs and returned to the laboratory by courier within 12 hours of collection.

Sample Testing

Stool specimens were analyzed by laboratorians blinded to case/control status and clinical symptoms.

Enteropathogen Testing: Stool specimens were tested using the Luminex xTAG Gastrointestinal Pathogen Panel (GPP; Luminex Molecular Diagnostics, ON, Canada) (13), and an in-house real time PCR gastroenteritis virus panel (GVP) that tested for adenovirus (generic), astrovirus, norovirus, rotavirus and sapovirus (14). The GPP is a multi-analyte, bead-based, molecular diagnostic assay that detects adenovirus 40/41, *Campylobacter* spp., *Clostridioides difficile*, *Cryptosporidium*, *Entamoeba histolytica*, *Escherichia coli* O157, enterotoxigenic *E. coli*, *Giardia*, norovirus GI and GII, rotavirus, *Salmonella* spp., Shiga toxin-producing *E. coli*, *Shigella* spp., *Vibrio cholerae*, and *Yersinia enterocolitica*. All specimens underwent routine enteric bacteria culture as per the Alberta Public Health Laboratory's protocol (Supplementary 1), after which stool specimens and accompanying total nuclei acid (NA) extractions were stored at -80°C .

Stool specimen NA extracts of without an identified pathogen on the aforementioned testing platforms were tested for influenza virus A and B. We tested all eligible stool from gastroenteritis cases. For every two cases, one control, matched for month and year, was randomly identified for testing using the random sampling procedure in SPSS 25.0 (IBM Corp.) which led to the exclusion (i.e. non-testing) of 520 controls.

Influenza Testing: The one step RT-qPCR (15) was used to detect universal influenza virus A and B in stools. Influenza A virus specimens underwent H-subtyping using an identical RT-qPCR protocol as employed for influenza A virus testing except for the primers and probes (16) (Supplementary 2). RT-qPCR testing was performed in a closed tube system using the ABI 7500 fast real-time PCR system (Applied Biosystems) with a 10 µl reaction volume containing 2.5 µl TaqMan Fast Virus 1-Step Master Mix (Thermofisher, ON, Canada), 0.8 µM of each primer, 0.2 µM of probe and 5 µl of NA. PCR reactions included initial incubation at 50°C for 5 minutes and then 95°C for 20 seconds followed by 45× amplification cycles at 95°C for 3 seconds and 60°C for 30 seconds. Amplification data were collected and analyzed with Sequence Detection Software version 2.0 (Applied Biosystems). The C_T cutoff for a positive result was set at 38 for both influenza A/B and H typing.

Statistical Analysis

For the primary objective, clinical characteristics of gastroenteritis cases were summarized with frequencies and percentages along with 95% confidence interval (CI), or medians along with interquartile ranges (IQR), as appropriate. Between-group (i.e. influenza positive vs. negative) comparisons were performed using Chi-square and Mann-Whitney U tests for categorical and continuous data, respectively. To control for false discovery, we corrected P values using the Benjamini-Hochberg method within sets of tests. For the secondary objectives, we compared influenza detection in stool between controls and cases, we first included all cases, then performed a sensitivity analysis including only cases without respiratory symptoms (i.e. gastrointestinal symptoms only) to reduce potential bias introduced by the absence of respiratory symptoms in controls. Influenza detection rate and strains were summarized as frequencies and percentages and C_T values as median, IQR and range.

Two-tailed P values of <0.05 were considered statistically significant. Analyses were conducted using SPSS 25.0 (IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.).

RESULTS

Specimens from a total of 440 cases with gastroenteritis and 250 controls were selected for influenza virus testing (Figure 1). Cases and the controls were well matched by age, sex, and season and year of enrollment (Table 1).

Primary Outcome

Thirteen (2.7%, 95% CI: 95% CI: 1.6%, 5.0%) gastroenteritis cases tested positive for influenza A or B. Children whose stool was influenza test-positive were older [median age

4.0 (IQR: 2.3, 5.5) vs. 1.5 (IQR: 0.5, 4.0) years; $P=0.008$] and more likely to present for care during the fall or winter [92.3% (95%CI: 64.0%, 99.8%) vs. 48.0% (95%CI: 43.2%, 52.9%); $P=0.001$], compared to cases who were influenza test-negative. Influenza-positive children were more likely to have fever [84.6% (95%CI: 54.6%, 98.1%) vs. 30.6% (95%CI: 26.3%, 35.3%); $P=0.001$], respiratory symptoms [91.7% (95%CI: 61.5%, 99.8%) vs. 44.8% (95%CI: 39.8%, 49.9%); $P=0.002$], dehydration [CDS median: 4.0 (IQR: 1.5, 4.5) vs. 2.0 (IQR: 0, 3); $P=0.034$], and higher MVS scores assessed at enrollment [median: 11.0 (IQR: 9.5, 12.0) vs. 9 (IQR: 7.0, 11.0); $P=0.015$] and total illness MVS scores [median: 13.0 (IQR: 10.5, 14.0) vs. 10 (IQR: 9.0, 13.0); $P=0.044$], compared to those who were influenza test-negative. Half (6/13, 46.2%; 95%CI: 20.4%, 73.9%) of children whose stool were influenza-positive had diarrhea with a median of 7 (IQR: 3, 15) episodes in the 24-hours preceding enrollment (Table 2).

Secondary Outcomes

Among all gastroenteritis cases, 13 (13/440; 3.0%; 95% CI: 1.6%, 5.0%) had stool positive for influenza A or B vs. one control (1/250; 0.4%; 95%CI: 0%, 2.2%) had influenza A (H1 negative and H3 negative) virus detected in stool; absolute difference: 2.6% (95%CI: 0.03%, 4.6%; $P=0.02$). Among the gastroenteritis cases without respiratory symptoms (214/440; 48.6%), influenza A H3 was detected in only one child (1/214; 0.5%; 95% CI: 0.02%, 3.0%); absolute difference 0.07% (95%CI: -2.1%, 2.6%; $P>0.99$). For strain identified, influenza A (H1 negative and H3 negative), influenza A H3 and Influenza B was detected in 4 (4/440, 0.9%; 95% CI: 0.3%, 2.5%), 5 (5/440, 1.1%; 95%CI: 0.4%, 2.8%), and 4 (4/440, 0.9%; 95% CI: 0.3%, 2.5%) gastroenteritis cases, respectively. During the study period the predominant circulating strain in Alberta shifted from A H1N1 (2015-2016) to H3N2 (2016-2018); Figure 2. The median C_T value of influenza A for the nine gastroenteritis cases was 31.8 (IQR: 29.6, 34.8; range: 28.3, 35.6), and for the one control it was 35.1. The median C_T value of influenza B was 32.6 (IQR: 31.1, 35.3; range: 30.9, 35.9).

DISCUSSION

Our case-control study describes the clinical features of children with stool influenza-positive, enteropathogen-negative acute gastroenteritis. Extensive microbial testing excluded participants infected with other enteropathogens to eliminate concerns regarding pathogen co-detection and disease attribution. Using this approach, children with gastroenteritis in whom influenza was detected were older and more likely to present during the fall or winter. Their clinical symptoms were more severe, as manifested by greater degree of dehydration and higher MVS scores. We detected influenza more frequently in the stool of symptomatic gastroenteritis, most of whom also had respiratory symptoms, compared to asymptomatic controls.

The clinical features of stool influenza-positive gastroenteritis cases in our study were in keeping with the classic features associated with influenza infection, notably, fever and respiratory symptoms (2, 17). That our influenza-positive gastroenteritis cases were older than those who were influenza-negative may reflect the fact that school-age children are more likely to be in close proximity to other infected children, thereby facilitating influenza

transmission (18). This is in line with published data that children aged 5 to 17 years have the highest incidence of influenza (19). As our study was conducted while the nasal live attenuated influenza vaccine was being administered in Alberta, it is possible that, as has been demonstrated with the oral rotavirus vaccines (20), that stool influenza-positive case represent vaccine strain excretion by the children. However, 12 of the 13 influenza-positive children were enrolled between December and March, which does not correspond to our local influenza vaccination time period. Moreover, overall population vaccination rates in Alberta during the study period ranged from a low of 27% to a high of 30%.(21)

Gastrointestinal symptoms are frequently present in individuals with acute respiratory infections.(22, 23) Enteric symptoms have been reported in 10% to 30% of influenza-infected children (2, 24, 25). Several explanations have been proposed for the observed gastrointestinal symptoms seen during acute respiratory infections. Notably, acute respiratory and gastrointestinal viruses both tend to peak in terms of frequency during the winter, which may cause a spurious association. Second, acute diarrhea in particular may result from the administration of antibiotics used to treat respiratory pathogens.(26) Lastly, gastrointestinal symptoms may be a manifestation of direct or indirect respiratory viral effects such as lung-derived CD4+ cell-induced dysbiosis which leads to intestinal injury. (27)

Prevalence of influenza virus in stool ranges from 2.9% to 71% in children with classic respiratory symptom predominant influenza (28–31). That influenza virus can induce the exposure of cellular receptors involve in intestinal inflammation could explain the diarrhea seen in some patients with influenza infection (32). In our study, a significantly greater number of cases with gastroenteritis relative to controls were influenza positive. This may be explained by the presence of respiratory symptoms in the gastroenteritis group and their exclusion, by definition, in the controls. When we excluded cases with respiratory symptoms to make the case and control groups more comparable, the influenza detection rate in stool did not differ between groups. To better understand the etiologic role of influenza in gastroenteritis, a control group recruited with respiratory symptoms would be necessary.

Even though none of our participants had influenza A/H1N1 detected in their stool samples, which has been associated with gastrointestinal symptoms in children,(33, 34) the influenza-positive cases had a median of 7 diarrheal episodes in the 24 hours preceding stool collection. These patients represent a significant potential source of influenza virus shedding and transmission through aerosol and droplet nuclei shed in respiratory secretions, and contact with stool. Future studies should examine influenza virus transmission via feces and vomitus.

Prior reports provided conflicting results when pathogen loads were correlated with clinical severity. In children with influenza H1N1, the median initial stool specimen C_T values does not appear to differ by baseline symptom duration (30). Similarly, the fecal detection of influenza H1N1 influenza virus does not correlate with the presence of gastrointestinal symptoms (35). Conversely, viral load concentrations appear to decrease as the time interval from symptom onset increases (36). Although, in our study influenza C_T values were lower among cases than the one control, given the small number of positive children in our study,

these results should be considered inconclusive. It should also be noted that viral cultures were not performed and the viral RNA detected may represent fragmented, free influenza nucleic acid, which may not be infectious. Thus, our data does not provide evidence of intestinal tropism and active replication in the gastrointestinal tract which is characteristic of enteropathogens.

Our findings raise the provocative question of whether future expanded syndromic enteric panels should include influenza as a target. The influenza detection rate of 3% in this sub-cohort was higher than the detection rate (i.e. 1%) of some enteropathogens included in our expanded testing algorithm, such as *Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia enterocolitica*, Enterotoxigenic *E. coli*, Shiga-toxin producing *E. coli*, *Vibrio cholera*, *Cryptosporidium*, *Entamoeba*, and *Giardia* (37). On the other hand, children with respiratory symptoms which was found in >90% of our gastroenteritis cases with influenza should have a nasopharyngeal swab taken for respiratory virus testing.

Our study has several limitations which should be considered. We did not collect nasopharyngeal swabs, and thus we could not incorporate the detection of nasopharyngeal influenza into our analysis. We also did not have a control group with classical influenza symptoms (i.e. predominant respiratory symptoms in the absence of vomiting and diarrhea), which could have served as a comparison to our gastroenteritis cases. Future studies incorporating these design features are needed to assess causality. Lastly, the small number of cases in whom influenza was detected limited the power of our study and ability to draw firm conclusions.

Nonetheless, we found that symptomatic gastroenteritis cases with influenza-positive stool specimens, were older, more likely to have fever, prominent respiratory symptoms, and more severe illness when compared to children with influenza-negative stool specimens. These results have infection control implications, particularly in the hospital setting. Ideally, the presence of respiratory symptoms should be captured by clinicians to enable appropriate diagnostic testing and infection precautions. Clinicians managing children for whom an etiology of gastroenteritis cannot be identified, should consider the possibility of influenza infection in children with respiratory symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

APPETITE	Alberta Provincial Pediatric EnTeric Infection TEam
CDC	Centers for Disease Control and Prevention
CDS	Clinical Dehydration Scale
CI	Confidence Interval
CT	Cycle Threshold
IQR	Interquartile Range
MVS	Modified Vesikari Scale
RT-qPCR	Reverse Transcriptase-quantitative Polymerase Chain Reaction

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Highlights

- 3% of children with acute gastroenteritis had influenza virus detected in stool
- Influenza virus positive children are older and present during the fall and winter
- Influenza positive children are more likely to be febrile and respiratory symptoms
- Influenza positive children tend to have more severe episodes of gastroenteritis

Summary of the main point:

Influenza virus may be detected in the stool of children with vomiting and diarrhea. Among children with vomiting and diarrhea, stool influenza-positive children are older and more likely to be febrile, have respiratory symptoms and more severe illness.

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Figure 1.
Study Flow Diagram

Figure 2.

Distribution of confirmed influenza strains in the province of Alberta (i.e. non-study participants) during the study period in comparison to strains detected in the stool of study participants (i.e. acute gastroenteritis cases and controls). In 2015-2016, the number of influenza positive subjects was 5311, 6, and 0 in the province of Alberta, study acute gastroenteritis cases, and study controls, respectively; in 2016-2017, the numbers were 4494, 1, and 1 respectively; in 2017-2018, the numbers were 9069, 6, and 0, respectively.

Table 1.

Characteristic of the gastroenteritis cases and controls without gastroenteritis.

	Symptomatic Gastroenteritis Cases N=440	Asymptomatic Controls N=250
Age (year)	1.56 (0.6, 4.05)	1.50 (0.5, 3.45)
Sex (male)	234 (53.2)	145 (58.0)
Season Enrolled		
January-March	88 (20.0)	57 (22.8)
April-June	82 (18.6)	45 (18.0)
July-September	141 (32.0)	75 (30.0)
October-December	129 (29.3)	73 (29.2)
Year Enrolled		
2015	59 (13.4)	29 (11.6)
2016	169 (38.4)	91 (36.4)
2017	191 (43.4)	111 (44.4)
2018	21 (4.8)	19 (7.6)
Vomiting	347 (78.9)	0 *
Diarrhea	241 (54.8)	0 *
Fever^b	139/431 (32.3)	0 *
Upper Respiratory Symptoms^c	184/398 (46.2)	0 *
Stool Influenza Virus Positive^d	13 (3.0)	1 (0.4)

Data presented as medians (interquartile range) for continuous variables, and frequencies (proportions) for categorical variables.

^aChi-square test and Mann-Whitney U test were used for between group comparisons for categorical and continuous variables, respectively.^bFever was defined as a documented temperature of ≥38.0°C rectal temperature or adjusted rectal temperature. Temperatures were adjusted for the location of measurement: 1.1°C was added to axillary temperatures and 0.6°C was added to oral temperatures.(9)^cData missing for 42 participants as collection initiated in September 2015.^dInfluenza virus A H3 was detected in 9 cases and 1 in control; influenza virus B was detected in 4 cases.

* By definition asymptomatic controls had no clinical symptoms of infection.

Table 2.

Clinical characteristic comparison between symptomatic gastroenteritis cases with influenza-positive and influenza-negative stool tests.

	Influenza A or B Positive N=13	Influenza A and B Negative N=427	P-Value ^a
Age (year)			0.008
<1.0 year, n (%)	4.0 (2.3, 5.5)	1.5 (0.5, 4.0)	
1.0 to 3.0 years, n (%)	1 (7.7)	172 (40.3)	
>3.0 years, n (%)	3 (23.1)	117 (27.4)	
	9 (69.2)	138 (32.3)	
Sex (male)	10 (76.9)	224 (52.5)	0.096
Season of Illness			0.003
Winter (January–March)	7 (53.8)	81 (19.0)	
Spring (April–June)	1 (7.7)	81 (19.0)	
Summer (July–September)	0 (0)	141 (33.0)	
Fall (October–December)	5 (38.5)	124 (29.0)	
Year of Presentation			0.089
2015	0 (0)	59 (13.8)	
2016	7 (53.8)	162 (37.9)	
2017	4 (30.8)	187 (43.8)	
2018	2 (15.4)	19 (4.4)	
Travel Outside of C.A. or U.S. in Past 12 Months	2 (15.4)	60 (14.1)	>0.99
Family Member With Similar GI Symptoms	2 (15.4)	60 (14.1)	>0.99
Vomiting (any)	11 (84.6)	336 (78.7)	0.746
Maximum Number of Vomiting Episodes/24-hrs^b	5 (3, 8)	7 (4, 8)	0.264
Maximum Number Vomiting Episodes Preceding 24-Hours Before enrollment^b	5 (3, 8)	4 (3, 7)	0.994
Vomiting Duration at Enrollment (hrs)	25.8 (13.0, 62.0)	15.5 (3.3, 46.9)	0.240
Diarrhea (any)	6 (46.2)	235 (55.0)	0.579

	Influenza A or B Positive N=13	Influenza A and B Negative N=427	P-Value ^a
Maximum Number Diarrhea Episodes/24-hrs ^c	7 (3, 15)	6 (3, 10)	0.632
Maximum Number Diarrhea Episodes in Preceding 24-hrs Before Enrollment ^c	7 (3, 12)	5 (3, 7)	0.401
Diarrhea Duration at Enrollment (hrs)	0 (0, 51.9)	5.5 (0, 62.4)	0.665
Abdominal Pain (any)	12/12 (100)	333/381 (87.4)	0.375
Fever ^d	11/13 (84.6)	128/418 (30.6)	0.0001
Highest Temperature (adjusted rectal in Celsius)	40.0 (38.9, 41.5)	39.1 (38.4, 40.0)	0.068
Respiratory Symptoms (any) ^e	11/12 (91.7)	173/386 (44.8)	0.002
CDS Score (10)	4 (1.5, 4.5)	2 (0, 3)	0.034
Intravenous Fluid Administration	3/12(23.1)	72/410(17.6)	0.710
Subsequent Emergency Department Visit	1/13 (7.7)	39/408 (9.6)	>0.99
Hospitalization	2/12 (15.4)	48/411 (11.7)	>0.99
MVS Score at Enrollment (11)	11 (9.5, 12)	9 (7, 11)	0.015
MVS Score Following the ED Visit	6 (2, 9)	5 (3, 8)	0.882
MVS Score, Entire Illness	13 (10.5, 14)	10 (9, 13)	0.044
Interval from Gastrointestinal Symptom Onset to Stool Collection (days)	4.0 (2.0, 5.0)	3.0 (1.0, 4.0)	0.098

Data presented as medians (interquartile range) for continuous variables, and frequencies (proportions) for categorical MVS. Modified Vesikari Scale; ED, Emergency Department; GI, gastrointestinal; CDS, Clinical Dehydration Scale..

^a Chi-square test and Mann-Whitney U test were used for between group comparisons for categorical and continuous variables, respectively. P value 0.044 was considered significant after correction via Benjamini-Hochberg procedure for multiple comparisons (n=26).

^b Among those who had vomiting.

^c Among those who had diarrhea.

Fever was defined as a documented temperature of $\geq 38.0^{\circ}\text{C}$ rectal temperature or adjusted rectal temperature. Temperatures were adjusted for the location of measurement: 1.1°C was added to axillary temperatures and 0.6°C was added to oral temperatures.⁹⁾

Data missing for 42 participants as collection initiated in September 2015