A SUSPECTED ASTROVIRUS-ASSOCIATED FOODBORNE OUTBREAK AMONG CHILDREN AND STAFF ATTENDING A GROUP OF CHILDCARE CENTRES IN GAUTENG PROVINCE, NOVEMBER 2018

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Executive summary

In November 2018 a suspected outbreak of gastroenteritis, reported from multiple branches of a childcare and education facility (crèche) chain in Gauteng Province, was investigated. The outbreak affected children attending the crèches as well as adult employees. A standardised questionnaire was used to collect food history from the adult cases at selected crèche branches. A single caterer pre-prepared meals and delivered to all crèches. Food retention samples collected from the caterer were tested for pathogens commonly associated with foodborne disease, and stool specimens collected from adults and children at several crèches were tested for selected enteric viruses, bacteria and parasites. A total of 279 cases was identified. Where date of birth was available, 87% (235/270) of case-patients were children and 13% (35/270) were adults. The median age among children was 3 years (range 8 months – 5 years) and the median age among adults was 30 years (range 19-62 years). Illness in children preceded that in adults, suggesting secondary infection in adults. No pathogens were detected in the food samples; however, these samples were collected well after the outbreak was reported, and no food retention samples dating to the start of the outbreak were available for testing. Human astroviruses (HAstVs) were detected in 54% (7/13) of the stool specimens. Nucleic acid sequence analysis and phylogenetic tree comparisons showed that the HAstVs identified were highly related (99%) to each other and to another HAstV type 8 strain that was detected in South Africa in 1998. The HAstV strains were detected in case-patients (six adults and one child) from two crèches. These data strongly suggest that catered food from a single supplier was the source of the HAstV outbreak, perpetuated by person-to-person transmission.

Introduction

Human astroviruses (HAstVs) are transmitted by the faecal-oral route, either directly from person-to-person or indirectly via contaminated food and water, and have been implicated in large foodborne gastroenteritis outbreaks.[1] In 1991, HAstV-contaminated school lunches resulted in an outbreak of acute gastroenteritis that involved 10 primary and four junior high schools in Katano City, Japan. More than 4 700 people, including pupils and adults, were affected. The primary source of the HAstV outbreak was contaminated food from a common supplier.[2] The ability of the virus to spread from person-to-person is well described and has been demonstrated by human volunteer studies.[3]

Astrovirus (AstV) infection has an incubation period of 1-4 days and typically presents as watery diarrhoea that resembles a mild form of rotavirus gastroenteritis. Astrovirus diarrhoea is usually seen in young children aged 6 months to 2 years and may be associated with anorexia, fever, vomiting and abdominal pain. Although AstV diarrhoea does not normally result in significant dehydration or hospitalisation, persons with poor nutritional status, immunodeficiency, mixed infections, or underlying gastrointestinal disease are at risk for developing complications.[4] In individuals with immunosuppression (other than HIV), AstVs have been associated with non-diarrhoeal symptoms such as coeliac disease and neurotropic conditions.[5]

Human AstVs are classified into eight classic strains, HAstV-1 to HAstV-8, and recombinant and novel strains have also been identified.[4-5] Classic HAstVs are globally distributed and are the third most common cause of viral gastroenteritis, following rotavirus and norovirus.[6]

There is limited data on the epidemiology of HAstVs in South Africa (SA). Most investigations have reported the rates of HAstVs detection for specific geographical areas.[7-11] HAstVs were first detected in SA in 1979, when the star-shaped virions were viewed by electron microscopy in stool specimens collected from a six-month-old baby.[12] Since then, several small studies have investigated the presence of HAstVs in SA, reporting detection of HAstVs in human stool specimens and environmental water samples.[7-9, 11, 13-15] In 1997, HAstVs were detected in 37% of stool specimens collected in a diarrhoeal disease outbreak in a child care centre in Tshwane, Gauteng Province.[16]

A study carried out between 2009 and 2014 reported HAstVs in 7% of stool specimens collected from hospitalised patients under the age of five years.[17] The study was conducted in selected areas in SA and included sites from Mpumalanga, Gauteng and KwaZulu-Natal provinces. However, since these pathogens are typically associated with milder infections, community and outpatient-focused studies are required to determine the broader epidemiology of HAstVs.

Outbreak background

The Outbreak Response Unit of the National Institute for Communicable Diseases (ORU-NICD) received a notification of a suspected outbreak of gastroenteritis on 6 November 2018, reportedly affecting multiple branches of a childcare and education facility (crèche) chain in Gauteng Province. There are ten branches in total: four in the City of Johannesburg, four in the City of Tshwane, one on the West Rand and one in Ekurhuleni District (Figure 1). The cases included children attending the crèches as well as adult employees. All crèches received food from the same caterer daily. The caterer provided pre-prepared lunch and snacks to the crèches, while each crèche prepared their own breakfast (which included porridge made from either Mabele, oats or mielie-meal). All crèches have a similar menu, rotated on a two-weekly basis.

Food supply was identified as the only common epidemiological link between the crèches. The investigating team hypothesised that contaminated food from the caterer was the likely vehicle of infection. An epidemiological investigation was thus conducted with the aim of determining the magnitude of the outbreak, identifying the source of the outbreak, and providing recommendations to prevent occurrence of similar outbreaks.

Methods

Study setting

The outbreak affected both children and adults at the ten crèches. The crèches are distributed across Gauteng Province (Figure 1) and enrol children aged six weeks to five years. The children are categorised by age into separate classes, namely: baby (3 weeks – 12 months), young toddler (1–2 years) and 2 to 5 year olds.

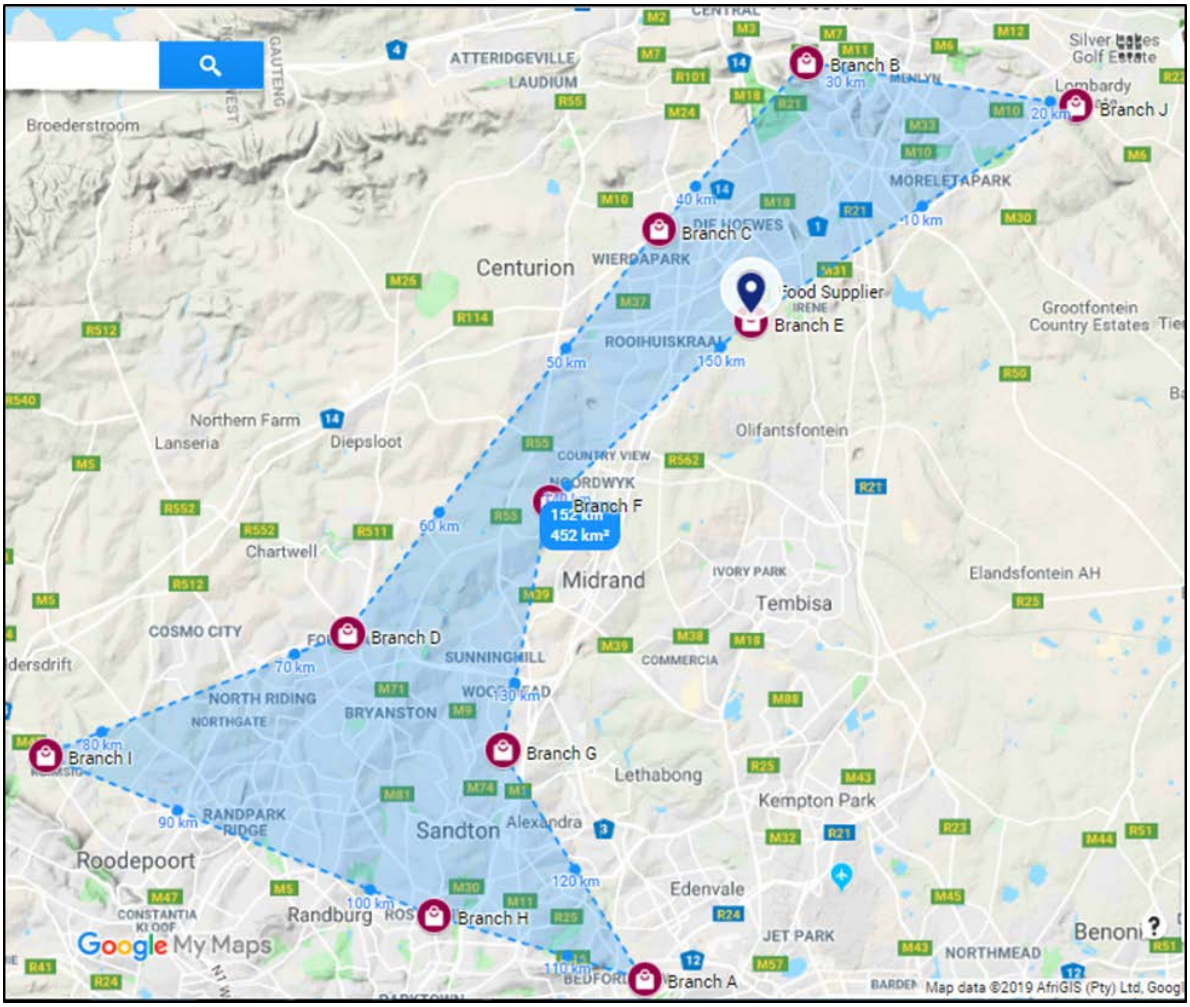

Figure 1: Distribution of crèche branches, human astrovirus (HAstV) outbreak, Gauteng Province, South Africa, November 2018.

Study design

A descriptive study was conducted amongst children and adults from all ten branches, and a case-control study was conducted amongst adults at four of the ten branches. A case was defined as a person of any age who presented with diarrhoea or vomiting with/without fever between 17 October and 23 November 2018. A control was defined as any adult crèche employee working between 17 October and 23 November who did not develop symptoms of gastroenteritis.

Data collection

Epidemiological investigation

A line list including cases (children and adults) from all branches was compiled. The investigating team visited four of the ten branches and completed a detailed hard-copy semi-standardised questionnaire with consenting adults for cases and controls. The team also visited the caterer and

completed questionnaires with food handlers working at the facility. Data collected through the questionnaire included demographic, clinical and food history.

Laboratory investigations

Clinical specimens

Stools specimens and rectal swabs were collected between 13 and 29 November 2018 from four crèches and from food handlers at the caterer's premises. These specimens were sent to the Centre for Enteric Diseases (CED) for enteric pathogens testing.

In total, 13 specimens were received at CED. Nucleic acid extracts from these specimens were screened using one-step multiplex real-time kits (Fast Track Diagnostics; Luxembourg) for the presence of selected enteric viruses (FTlyo Viral gastroenteritis), bacteria (FTlyo Bacterial gastroenteritis) and parasites (FTlyo Stool parasites).

The HAstVs detected were further characterised by reverse transcriptase-polymerase chain reaction (RT-PCR) and nucleotide sequence analysis. Amplicons were sequenced and analysed on an ABI 3500 Genetic Analyser (Applied Biosystems). Nucleotide sequence data were compared with published sequences. Phylogenetic analyses was performed to establish relatedness between the outbreak strains and reference sequences. A phylogenetic tree was drawn using the aligned sequences and reference HAstVs strains obtained from the NCBI databases using MEGA5 analysis software.

Environmental investigations

Environmental Health Practitioners from the City of Tshwane conducted an environmental assessment of the caterer's facility and collected environmental surface swabs from the kitchen. Food retention samples collected for 29 and 30 October 2018 by the caterer, which included fish cakes and chicken á la King, were sent to a private food laboratory for *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* testing. Eggs collected from the caterer were also tested for *S. aureus*, aerobic bacteria, *Salmonella* spp., yeasts and moulds. Environmental assessments were also conducted at six of the ten crèches, but no environmental samples were collected.

Data analysis

Descriptive analysis was conducted using Microsoft Excel and STATA version 14. The Chi-square test was used to assess risk factors associated with illness between cases and controls.

Results

Epidemiological investigation

A total of 279 cases from all ten crèches was captured on the line list (Table 1). Where date of birth was available, 87% (235/270) cases were children and 13% (35/270) were adults. The median age among children was 3 years (range 8 months – 5 years) and the median age among adults was 30 years (range 19-62 years). All adult cases were females, and females accounted for 52% (112/216) of cases among children. The epidemic curve suggests a propagated outbreak in which the causative agent is transmitted by person-to-person contact (Figure 2).

Table 1: Proportion of human astrovirus (HAstV) cases across ten crèches, City of Tshwane, Gauteng Province, South Africa, November 2018.

Crèche branches	Adults n (%)	Children n (%)	Age unknown	Grand Total
Branch A	4 (7)	44 (82)	6 (11)	54 (19)
Branch J	7 (15)	39 (85)		46 (16)
Branch I	9 (21)	33 (77)	1 (2)	43 (15)
Branch C		36 (97)	1 (3)	37 (13)
Branch G	4 (13)	26 (84)	1 (3)	31 (11)
Branch H	5 (21)	19 (79)		24 (9)
Branch E		16 (100)		16 (6)
Branch B	3 (21)	11 (79)		14 (5)
Branch D	2 (22)	7 (78)		9 (3)
Branch F	1 (20)	4 (80)		5 (2)
Grand Total	**35 (13)**	**235 (84)**	**9 (3)**	**279**

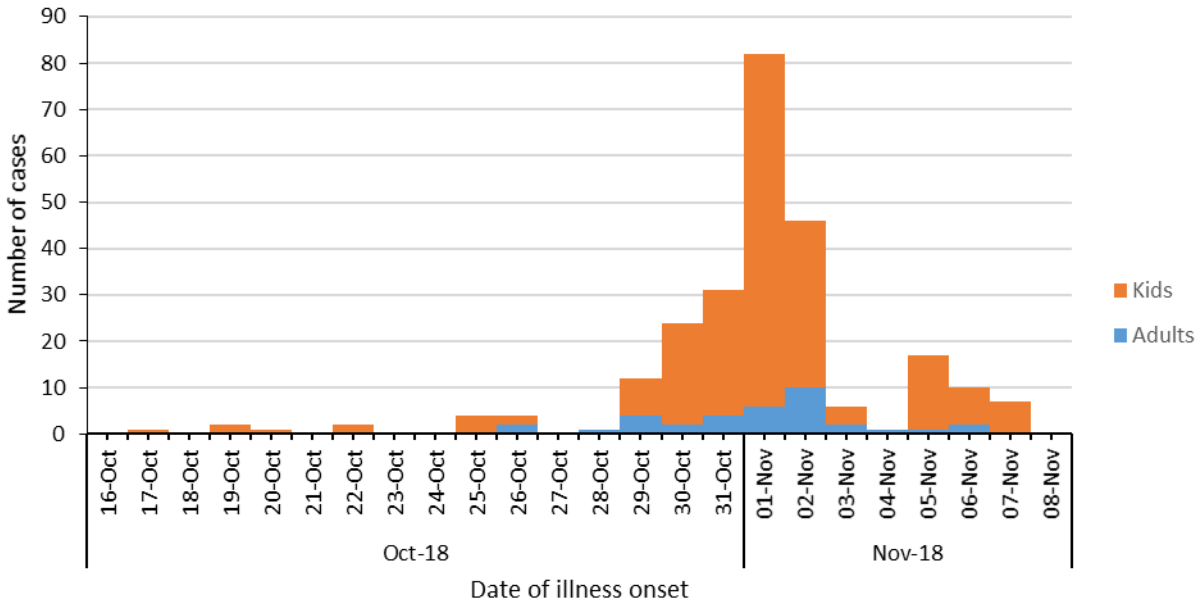

Figure 2: Epidemiological curve of human astrovirus (HAstV) cases by date of illness onset stratified by age group, Gauteng Province, South Africa, November 2018.

A total of 21 questionnaires was completed for adult cases (n=13) and controls (n=8). These included nine assistant teachers, six teachers, two managers, three caregivers and a health-hygiene assistant. The most common symptoms amongst cases were diarrhoea (92%, 12/13), abdominal cramps (92%, 12/13), nausea (46%, 6/13) and vomiting (23%, 3/13). The daily functions of most adults involved close contact with the children (90%, 19/21), while the remaining adults performed management functions and had limited or no contact with the children. The caterer reported that food is prepared every morning from 04h00 and transported to all the crèches before 10h00. The food includes lunch meals, afternoon snacks and snacks for the following day. Food is transported in cooler units with thermometers for temperature control and is reheated to 70 °C by the health-hygiene personnel at the crèches.

Laboratory findings

1. Clinical samples

a) Screening assays

Of the 13 specimens received, seven (7/13, 54%) tested positive for HAstVs, five (5/13, 39%) were positive for adenovirus, one (1/13, 8%) was positive for sapovirus and one (1/13, 8%) was positive for norovirus GI. Of the seven HAstV-positive specimens, four were detected as mixed viral

infections and the remaining three were single HAstV infections. All specimens were negative for enteric bacteria and parasites. Five specimens were negative for all pathogens screened.

b) RT-PCR amplification and Sanger sequencing

Only six of the seven HAstVs detected could be amplified for further characterisation. Sanger sequencing reactions were performed using these six RT-PCR products. Of these, nucleotide sequence contigs could be constructed for three strains and all three were identified as HAstV genotype 8 (HAstV-8) by comparison with BLAST

c) Phylogenetic analysis

The results for analysis of the capsid region showed that the strains detected from the outbreak were highly similar to each other (99%) and grouped together in a single clade with the highest similarity to a HAstV -8 strain identified in Hungary (AJ620757). Furthermore, this reference strain was most closely related to a HAstV-8 isolate identified previously in SA in 1998. Figure 3 shows the phylogenetic tree drawn using nucleotide sequence similarities from the capsid region of the genome. The three HAstVs identified as genotype 8 were collected from two of the crèches where the gastroenteritis outbreaks occurred.

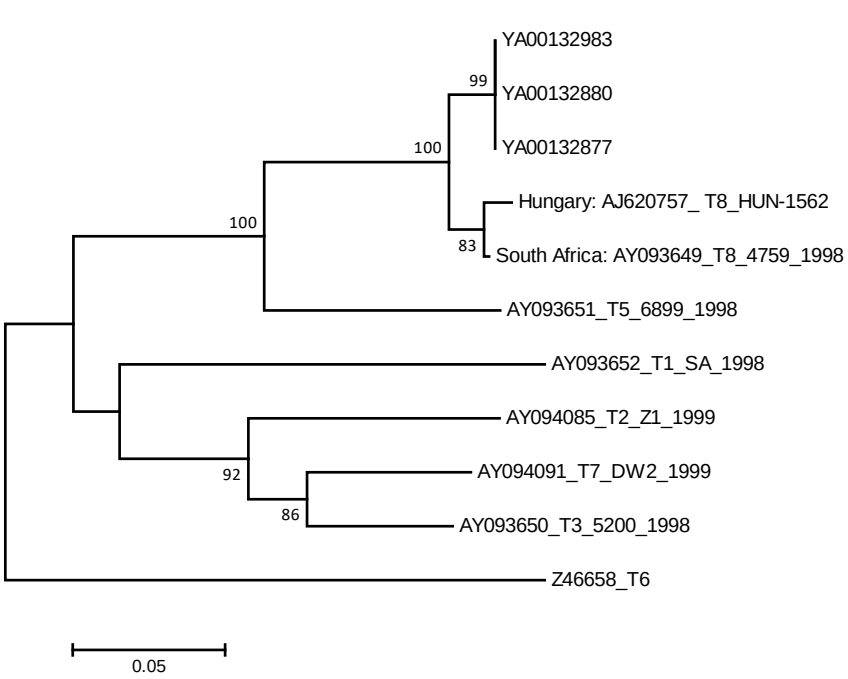

Figure 3: Molecular characterisation of human astrovirus identified in human stool specimens showing the clustering of related outbreak strains (YA00132983, YA00132880, YA00132877) and similarity to other human astrovirus type 8 strains (Hungarian and South African). Outbreak specimens highlighted in brackets.

Environmental findings

The caterer reported that the retention samples they had submitted were negative for all pathogens that were tested. Although environmental and food hygiene practice at most of the crèches was satisfactory, several concerns were noted. Thermometers were not always available for temperature monitoring on food delivery, and Certificates of Acceptability and health certificates were not available at some of the crèches. At one crèche, temperatures of delivered food and refrigerators were not recorded for more than a month prior to the onset of the outbreak.

Discussion

In this outbreak, children and adults from multiple crèches in a childcare facility chain presented with gastroenteritis. A caterer was identified as the only common link between the crèches. The epidemiological curve and analysis of the questionnaire data shows that the adult cases were likely secondary infections following contact with ill children. Interviewed adults reported that they typically consume food from their home-prepared lunch boxes and rarely eat the pre-prepared food from the caterer. The most plausible route of transmission is likely from contaminated food to the crèche children, and then person-to-person spread from the children to adult employees.

The molecular results suggest that the HAstV-8 strain identified from the three stool specimens was from a single source. The strain detected in one child was identical to the HAstV strain detected in the two teachers. The child and one teacher were from Branch J and the other teacher was from Branch I. The distance between the two crèches is approximately 152 km, which further supports the hypothesis that contaminated food from the caterer was the likely source of infection.

Food handlers were reluctant to provide stool specimens, so by the time they consented and specimens were collected the outbreak was over and the likelihood of detecting HAstV extremely low. Astrovirus is able to survive on inert surfaces and fomites[18], which may lead to contamination of the food preparation environment and food items. Unfortunately, environmental surface swabs were not tested for HAstV. Food retention samples from the food supplier that were sent for testing were negative for foodborne pathogens, but were also not tested for HAstV.

Further molecular analysis of the HAstV-positive strains was restricted by the quality of specimens submitted for testing and the delay in specimen receipt.

This investigation highlights the importance of collecting good quality clinical specimens during an outbreak. It also serves as an important reminder that food specimens (and, often, clinical specimens) are not routinely tested for viral enteric pathogens and so many viral foodborne infections and outbreaks are missed.

Conclusion

This was an HAstV-8 associated foodborne outbreak spread by a common food source and further propagated by person-to-person transmission.

Acknowledgements

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