

**Waterborne outbreak of gastroenteritis on the KwaZulu-Natal Coast, South Africa, December 2016/January 2017**

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Running title: Waterborne outbreak of gastroenteritis in SA

## Summary

An unexpected increase in gastroenteritis cases was reported by healthcare-workers on the KwaZulu-Natal coast, South Africa, January 2017 with >600 cases seen over a 3-week period. A case-control study was conducted to identify the source and risk factors associated with the outbreak so as to recommend control and prevention measures. Record-review identified cases and controls and structured-telephonic interviews which were conducted to obtain exposure history. Stool specimens were collected from 20 cases along with environmental samples and both screened for enteric pathogens. A total of 126 cases and 62 controls were included in the analysis. The odds of developing gastroenteritis were 6.0 times greater among holiday-makers than residents (95%CI: 2.0-17.7). Swimming in the lagoon increased the odds of developing gastroenteritis by 3.3 times (95%CI: 1.06-10.38). Lagoon water samples tested positive for norovirus (NoV) GI.6, GII.3 and GII.6, astrovirus and rotavirus. Eleven (55%) stool specimens were positive for NoV with eight genotyped as GI.1 (n=2), GI.5 (n=3), GI.6 (n=2) and GI.7 (n=1). A reported sewage contamination event impacting the lagoon was the likely source with person-to-person spread perpetuating the outbreak. Restriction to swimming in the lagoon was apparently ineffective at preventing the outbreak, possibly due to inadequate enforcement, communication and signage strategies.

**Keywords:** Gastroenteritis, Diarrhoea, Norovirus, Waterborne, KwaZulu-Natal, Lagoon, Recreational water

Word count: 200

## Introduction

Globally, open water sources such as lakes, rivers, seas and lagoons, provide communal recreational resources for swimming and other water sports. While serving as either official or unofficial recreation resources, rivers and lagoons are often also used for personal hygiene in low resource settings [1]. Inadequate treatment of sewage effluent or leakage of sewage into these water sources leads to the introduction of common waterborne pathogens including enteric viruses [2]. Therefore, many open water sources may pose a considerable health risk to the population utilizing them [3].

A study in the Eastern Cape Province, South Africa (SA), detected rotaviruses and enteroviruses in the Buffalo River as well as dams supplied by this river [4]. Enteroviruses, rotaviruses (RVs) and adenoviruses (AdVs) were also detected in the Umgeni River, KwaZulu Natal (KZN) Province. The majority of these viruses were confirmed through molecular characterisation to be of human origin, suggesting faecal contamination of the river [5].

Although many enteric viruses have been involved in waterborne outbreaks, Norovirus is a major concern. Norovirus was implicated in gastroenteritis waterborne outbreak in China, due to probable sewage contamination of a drinking water well [6].

Noroviruses (NoVs) have been detected in several rivers in Gauteng [7] and a recent quantitative microbial risk assessment indicated a high risk of NoV infection when swimming in contaminated rivers [8]. These studies highlight the health risk posed by noroviruses in open water sources in SA.

In SA, several factors contribute to surface water contamination. These include poor operational state of wastewater treatment infrastructure, inadequate capacity of wastewater treatment facilities especially in rapidly expanding informal settlements or poorly planned towns and insufficient monitoring for wastewater treatment compliance with available guidelines [9, 10].

On 5 January 2017, the Outbreak Response Unit (ORU) of the National Institute for Communicable Diseases (NICD), SA, was alerted to a high number of persons falling ill with gastrointestinal symptoms (diarrhoea and/or vomiting) on the coast of KZN Province. The alert from health authorities in the KZN Province indicated that between 14 December 2016 and 05 January 2017, 690 people presented with gastrointestinal symptoms at a private hospital on the KZN south coast. Over 600 cases were observed while 350 or less were expected for the period. Other surrounding health facilities, including public sector hospitals, did not observe this increase.

Based on the available information at the time, which included local newspaper and online articles that reported a sewage spillage into a nearby lagoon; the investigating team hypothesized that contamination of a coastal lagoon could have led to the outbreak. An epidemiological investigation was conducted to describe the extent of the outbreak, identify the source of the outbreak and identify associated risk factors so as to recommend control and prevention measures.

## **Materials and Methods**

### Study setting

The outbreak occurred within an urban community on the south coast of KZN Province during December 2016 and January 2017. The community had an estimated population size of 26785 during the 2011 census [11]. However, the population can reportedly double during the festive seasons as inland residents travel to the coast, especially during Christmas holidays in December to January. Along the community beach there is a lagoon which connects a local river to the Indian ocean (Figure 1).

### Study design

A case-control study was conducted, with a case defined as a person of any age who presented to the private hospital's emergency department between 19 December 2016 and 6 January 2017 with an acute onset of diarrhoea and/or vomiting. Controls were defined as a

person of any age who presented at the same hospital during the same period with acute onset of respiratory illness or musculoskeletal problems, e.g. trauma.

Data collection

### *Epidemiological*

Hospital patient records were reviewed to identify cases and controls. Due to time constraints of the visit it was not possible to review patient files for all 760 cases; a decision was made to obtain data from between 30% – 50% of the cases who presented at the hospital between 19 December 2016 and 6 January 2017. With respect to the controls, information regarding the total number of persons presenting on the days between 19 December 2016 and 6 January 2017 was not known. A decision was then made to review 150 patient files of the controls without stratifying by day.

Structured telephonic interviews were conducted to collect the exposure history within the five days prior to onset of illness, from both cases and controls using a semi-structured investigation form. Cases and controls who could not be contacted telephonically were sent a Google Form (<https://www.google.com/forms/about/>) link which allowed them to complete the investigation form electronically. The investigation form included questions on the demographics of participants, clinical symptoms (if present) and laboratory test results (if known). In addition, questions on exposure variables such as swimming in the sea/lagoon/rivers, ingestion of water and food items from different sources were included.

### *Laboratory investigations*

Clinical specimens

Twenty acute phase stool specimens were collected from cases presenting to the hospital between 06 and 10 January 2017 and sent cooled to the Centre for Enteric Diseases (CED), NICD, for testing. Nucleic acid was extracted from stool specimens with the QIAamp Fast DNA Stool kit (Qiagen, Hilden, Germany) using a modified method and screened using a custom Taqman Array card (Thermo Fischer, Carlsbad, CA) with Ag-Path-ID One-Step RT-

PCR reagents [12-14]. The arrays screened for viruses (RV, NoV GI and GII, AdV, astrovirus (AstV), enterovirus, sapovirus (SaV) and oral polio virus 1, 2 and 3), bacteria (*Aeromonas* spp., *Bacteroides fragilis*, *Clostridium difficile*, *Campylobacter jejuni* & *coli*, *Escherichia coli* (*E.coli*) including Enteroaggregative *E.coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E.coli* (EIEC) & *Shigella* spp., Shiga-toxin producing *E. coli* (STEC), *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Salmonella* spp. and *Vibrio cholerae*) and parasites (*Ancylostoma* spp., *Ascaris lumbricoides*, *Cryptosporidium* spp., *Cryptosporidium hominis*, *Cryptosporidium parvum*, *Cyclospora cayentanensis*, *Enterocytozoon bieneusi*, *Entamoeba histolytica*, *Encephalitozoon intestinalis*, *Giardia* spp., *Giardia A*, *Giardia B*, *Isospora belli*, *Necator americanus*, *Strongyloides stercoralis*, *Trichocephalus trichiuris*).

#### Environmental samples

There were reports of a sewage spillage into a local river on the 19 December 2016. Water samples were collected from the lagoon and a nearby wastewater treatment works discharge (final effluent) which drains into a river that flows into the lagoon (Figure 1). Samples of lagoon water (1 L and 10 L) and wastewater discharge (590 mL) were collected on 17 January 2017 and sent, in a cooler box with ice packs, to the Department of Medical Virology, University of Pretoria, for analysis for microbial indicator organisms and enteric viruses. Samples were received and analysed within 48 hours of collection (19 January 2017). On arrival the pH and temperature of the samples were measured and samples were stored at 4°C until processing.

#### *Microbial indicator analysis*

Water samples were analysed >24 and <48 hours after collection. The samples were tested for total coliforms, thermotolerant (faecal) coliforms by membrane filtration and selective media, namely m-Endo Les Agar and mFC Agar, as per SANS 5221:2006 Ed 4.2. Samples were tested for *E.coli* using m-ColiBlue24® broth [15]. Results were expressed as colony forming units (cfu) per 100 mL.

### *Viral recovery and analysis*

Viruses were recovered from the 10 L lagoon water sample using a glass wool adsorption-elution method followed by secondary concentration [16]. Viruses in the 1 L lagoon water and 500 mL wastewater discharge (final effluent) sample were concentrated to a final volume of 10 mL using PEG8000/NaCl precipitation [15].

Total nucleic acid was extracted from 1 ml of the virus concentrates using the NucliSENS®EasyMag® instrument (BioMerieux, Marcy, l'Etoile, France) according to manufacturer's instructions and eluted into 100 µL. Selected enteric viruses were detected in one-step quantitative reverse transcriptase – polymerase chain reaction (qRT-PCR) monoplex assays using the Quantifast® Pathogen RT-PCR + IC Kit (Qiagen, Hilden, Germany), 5 µL nucleic acid and published primers and Taqman probes for AstV, enteroviruses, hepatitis A virus, NoV GI, NoV GII, RV and SaV [17-25]. The molecular amplification and qPCR detection of AdV was done using the TaqMan® Environmental Master Mix 2.0 (Applied Biosystems, Foster City, CA) and primers and Taqman probe [26].

Enteric viruses were isolated in cell culture from 4 ml of the virus concentrate as described previously [7, 27]. Briefly, the recovered virus concentrates were treated with antibiotics and antimycotic. Thereafter, monolayers of the human hepatoma cell line PLC/PRF/5 (ECACC 85061113) and an African Green Monkey cell line (BGM: ECACC 90092601) in 25cm<sup>2</sup> cell culture flasks were inoculated, in duplicate, with 1 ml of the virus concentrate and incubated at 37°C. Seven days post-infection the cell cultures were blind passaged onto monolayers of the same cell type and incubated at 37°C for a further 7 days after which the infected cells were harvested for molecular analysis for enteroviruses and AdVs. In addition, the harvested cell cultures were passaged onto monolayers of the Vero African Green Monkey cell line (ECACC 84113001) in cell culture tubes and monitored daily for a cytopathic effect. After a further 7-day incubation the infected cell cultures, on flying coverslips, were stained with haematoxylin and eosin and examined for virus specific inclusion bodies.

Irrespective of whether CPE was evident or not, total nucleic acid was extracted from 200 µL of all the harvested cell culture extracts using the NucliSENS®EasyMag® instrument (BioMerieux) according to manufacturer's instructions and eluted into 50 µL. Enteroviruses were detected in one-step real time RT-PCR monoplex assay using the Quantitect Probe RT-PCR kit (Qiagen), 5 µL nucleic acid and published primers and Taqman probes for enteroviruses [18]. The molecular amplification and real time PCR detection of AdV was done as described above.

#### *Norovirus genotyping*

Norovirus GI and GII strains were genotyped based on the partial capsid sequence (320 bp, Region C) [15]. Partial capsid amplicons were cloned in the ClonJET™ vector (Thermo Scientific, Waltham, MA) and randomly selected clones were sequenced using vector specific primers and the ABI PRISM BigDye1 Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems). Genotypes were assigned with the online Norovirus Genotyping Tool version 2 [28]. Norovirus strains from lagoon water and gastroenteritis cases were compared using Neighbour-joining phylogenetic analysis in MEGA6 [29]. The sequences obtained in the study were submitted to GenBank under the following accession numbers: MG662693-MG662704.

#### Data analysis

Questionnaire results were exported from Google Forms into MS Excel (Microsoft Corp., USA), cleaned and exported into Stata v.14 (StataCorp., USA) for data analysis. The outbreak was described by time, place and person. Characteristics of cases and controls were described and analysed using appropriate significance tests (e.g.  $\chi^2$  test). The incubation period of the implicated predominant pathogen was calculated by determining the number of hours between peaks on the epidemic-curve.

Univariable analysis was conducted using a logistic regression model for each exposure variable and binary outcome variable of cases or controls, thereby enabling calculation of



odds ratios (ORs) as a proxy for risk. Stratified analysis was conducted on exposure variables with the largest ORs for illness in the univariable analysis to explore whether other variables were confounding the associations. Variables associated with illness or protection were included at  $P < 0.2$  in a multivariable logistic regression model to derive adjusted ORs.

## **Results**

### Epidemiological results

As of 10 January 2017, 760 persons presenting with gastrointestinal symptoms had been seen at the hospital. Historic data of diarrhoeal admissions at the hospital showed that this was above the expected number of admissions for the time period (see Supplementary Figure S1).

### *Descriptive epidemiology*

A total of 311 cases and 126 controls were identified. Questionnaire responses were obtained from 157 (50%) cases and 62 (49%) controls. Of the 157 cases, 142 (90%) responded to the telephonic interview and 15 (10%) completed an online investigation form. Responses from controls were obtained through telephonic interview only.

Of the 157 cases, 53% were females. The median age for the cases was 21 years (interquartile range (IQR) 10-40 years). The age group most affected was <1 to 10 years, with 27% of cases in this age group. There was no significant difference between the cases and controls in terms of age and gender distribution. However, there was a difference in terms of resident type, the control group had more permanent residents than visitors compared to the cases ( $p = 0.001$ ) (Table 1).

The most common symptoms were vomiting (85%; 133/157), diarrhoea (73%; 115/157) and abdominal cramps (31%; 49/157). The incubation period of the implicated predominant pathogen was 24-72 hours. Where data was available on the onset and resolution of gastroenteritis ( $n=68$ ), the average duration of illness was 3.6 days (Figure 2).

### *Exposures associated with illness*

Four variables were significantly ( $P \leq 0.05$ ) associated with either increased or decreased odds of becoming ill in our univariable analysis. These included: being a visitor to the coast (OR 5.3, 95% CI: 2.1-13.5), drinking municipal water at the place of residence (OR 0.5, 95% CI: 0.2-0.9), drinking bottled water (OR 2.8, 95% CI: 1.4-5.3) and swimming in the lagoon (OR 3.6, 95% CI: 1.2-10.9) (Table 2).

In the final multivariable analysis model, the following positive associations were noted: holiday-makers/visitors had 6.0 times greater odds of developing gastroenteritis than the residents (95% CI: 2.0-17.6) and people who swam in the lagoon had 3.3 times greater odds of developing gastroenteritis compared to those who did not swim in the lagoon (95% CI: 0.1-10.3). In addition, people who drank bottled water had 2.4 times greater odds of developing gastroenteritis compared to people who drank municipal water (95% CI: 1.1-4.8). Conversely, people who ate at restaurants had 0.5 times lesser odds of developing gastroenteritis compared to those who did not eat at restaurants. In our univariable analysis, eating at restaurant was not a significant factor associated with illness (Table 3). The goodness of fit test demonstrated that the model predicted results were not significantly different than the actual observed results, indicating adequate fit ( $p = 0.65$ ).

#### Laboratory results

##### *Stool/Clinical specimens*

Of the 20 clinical specimens tested, 11 (55%) tested positive for NoV; nine were positive for NoV GI while two were positive for NoV GII. *Aeromonas*, enterovirus and EPEC were also detected in one NoV GI-positive specimen and STEC in another NoV GI-positive specimen.

##### *Environmental samples:*

The total coliform and thermotolerant (faecal) coliform counts in the lagoon water were  $2.39 \times 10^4$  and  $9.5 \times 10^3$  cfu/100 mL, respectively. The *E. coli* level was  $1.93 \times 10^3$  cfu/100 mL.

Enteric viruses, namely AstV, NoV GI, NoV GII and RV, were detected by direct analysis of the lagoon water, with the wastewater discharge testing positive for NoV GII. After

amplification in cell culture, AdVs were detected in the wastewater discharge, by PCR in the infected cell culture extracts. Reoviruses were isolated from, and identified by typical reovirus eosinophilic cytoplasmic inclusions [28] in cell cultures infected with the wastewater discharge suggesting the presence of potentially infectious viruses.

#### *Norovirus characterisation:*

Eight NoV GI-positive specimens could be genotyped and GI.1 (n=2), GI.5 (n=3), GI.6 (n=2) and GI.7 (n=1) strains were detected. Based on BLAST-n analysis, the GI.1 strains were most closely related to strains detected in China (KM246902.1, 100% identity) and Taiwan (KT732279.1, 93% identity) between 2011 and 2015. The GI.5 strains were 97-99% identical to strains that had been detected in surface water and sewage in SA between 2010 [6] and 2015 [13]. The GI.6 viruses were closely related to strains that circulated in China (KU724081, 99% identity) and Russia (KY210910, 99% identity) in 2015/2016. The GI.7 genotype had previously been detected in a child hospitalised with gastroenteritis in Johannesburg, SA in 2011 (KR904267, 96% identity).

Norovirus genotypes GI.6, GII.3 and GII.6 could be characterized from NoVs detected in the lagoon water samples. The GII.3 strains were closely related to viruses that were detected in children with gastroenteritis in SA in 2013 (KR904473, 97% identity) as well as NoVs detected in wastewater (MF182296, 97% identity) in the Free State Province in 2016. The GII.6 strains in contrast were related to strains that circulated in China (KP3355058, 99% identity) and South Korea (KX764803, 99% identity) in 2014/2016. Norovirus GI.6, with 99% identity over a 299 nucleotide region of the capsid gene, was identified in both the lagoon and two gastroenteritis cases (Figure 3).

## **Discussion**

In this outbreak, gastroenteritis was associated with swimming in the lagoon, drinking bottled water, and being a visitor to the South Coast. Eating at restaurants seemed to be a protective factor against illness. The diverse pathogens and multiple NoV strains detected

were consistent with faecal contamination of the water sources [30]. Norovirus GII was also found in the final effluent from the wastewater treatment works.

Previous studies have suggested an association between gastroenteritis and swimming in recreational water [31-33]. A Danish study showed that healthy athletes who swam in sewage-polluted coastal water were five times more likely to get gastroenteritis compared to those who swam in non-polluted conditions [31]. In the current study, swimming in the lagoon was significantly associated with gastroenteritis. The water was analysed for microbial indicators and despite testing being performed >24 hours and <48 hours after collection, the thermotolerant (faecal coliform) count was  $>2 \times 10^3$ . According to the South African Water Quality Guidelines for recreational water, these counts indicate an increased risk of gastrointestinal illness. Likewise, the *E. coli* detected in the lagoon water was 4.8 times above the recommended <400 cfu/100 mL and indicated an increased risk of health effects [34].

During telephonic interviews, it was established that the residents of the South Coast did not swim in the lagoon as the water was perceived to be contaminated. In fact, of the 34 people who swam in the lagoon only 1 (3%) was a resident of the South Coast (Table 3). In addition, there were reports of a sewerage spill into the river and warning signs were placed near the lagoon prohibiting people from swimming. These data support the hypothesis that the lagoon was the source of the outbreak among holiday-makers on the KZN Coast.

Sewage waste can harbour several enteric pathogens including NoV, AstV, and RV. NoV has been previously implicated in waterborne gastroenteritis outbreaks [30, 31]. Even though the sewage spill occurred almost one month before the lagoon was sampled, the flow out of the lagoon into the ocean is limited (see Figure 1) and norovirus has been shown to remain detectable and infectious for at least 61 days in groundwater [35]. It is therefore possible that the pathogens from the spill would still be detectable and infectious after one month.

Considering that only 16% of the study participants indicated swimming in the lagoon, the outbreak was probably perpetuated by person-to-person transmission [36]. The transmission

of NoV occurs through direct person-to-person contact or through indirect contact including contaminated food, water, or environment surfaces [37]. At the time of the outbreak, many people were visiting the South Coast for their holidays. As a result, the beach area next to the lagoon, flats, hotels, guest houses, other communal places of residence, and recreational places were most likely overcrowded. This would provide a conducive environment for transmission of NoV.

Activities such as shaking of hands, hugging each other, caring for the sick, and preparing food for friends and families can all facilitate the spread of infection. Norovirus can survive on surfaces such as toilet seats, floors or even walls and remain stable for longer periods, making the spread of infection difficult to prevent [38].

Norovirus diversity in individuals involved in an outbreak is considered an indication of waterborne exposure [30]. The detection of multiple genotypes provides supporting evidence that the outbreak originated in the contaminated lagoon. Furthermore, NoV GI.6 strains (99% identical) were identified in the lagoon and in two individuals with gastroenteritis providing another potential link between swimming in the lagoon and contracting disease.

Norovirus has an incubation period of 12 to 48 hours, with the illness duration of 12 to 72 hours [39]. In severe cases, duration of illness can be prolonged; this is commonly seen among the very old and very young [40]. The NoV incubation period and duration of illness are consistent with our findings in this study.

Drinking bottled water was also significantly associated with gastroenteritis during the outbreak. During December 2016, holiday-makers on the KZN Coast generally drank bottled water as there were water shortages at the time. Therefore, drinking bottled water may have been a confounder in this case. If the outbreak was caused by bottled water contamination either at processing or factory level, the outbreak would have affected a larger number of people, including local residents, as supermarkets sell the water on a large scale to consumers. However, sharing bottled water could have facilitated the virus spread.

During the outbreak, eating at a restaurant was significantly associated with not becoming ill. Restaurants are more likely to have policies in place to ensure proper personal hygiene among staffs and in food handling compared to ordinary people. A study in the Netherlands showed that enhanced hygiene practices significantly reduced person-to-person transmission of NoV [36]. This could explain the protective effect associated with eating at restaurants seen during the outbreak.

Limitations encountered in the study included, refusal of participation by cases and controls during telephonic interviews. There was a limited number of cases in the study due to lack of or incorrect contact details and recall bias could also not be ruled out. Our control group had more permanent residents than visitors compared to the cases; this may have resulted in an over or under estimation of the odds ratio. However, despite the bias, results from environmental and clinical investigations support both the source (lagoon) and cause (NoV) of the outbreak. Failure to collect clinical specimens by the healthcare workers from the cases was also a limitation in this study. Municipal drinking water was not tested for bacterial indicators; this is also a limitation in our study.

## **Conclusion**

The outbreak investigation showed that gastroenteritis was associated with swimming in the lagoon. The lagoon was reportedly contaminated by the wastewater treatment works effluent which drained into the river flowing into the lagoon. Our findings also indicate that the outbreak was further propagated through person-to-person transmission resulting in the high number of cases seen. We recommend that better signage, communication and enforcement should be put in place to restrict swimming in the lagoon in the event of sewage spills. Emphasis should also be put on proper hygiene practices within the residential and recreational areas among holiday-makers. Inspections of the wastewater treatment infrastructures should also be conducted frequently to prevent any leakages of raw sewage into water sources such as rivers.

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### **Conflict of interest**

The authors declare no competing interests.

### **Ethical Standards**

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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## 2 Tables and figures

Table 1: Description of the study participants included in the analysis, KZN 2016/17.

Variables	Cases (%)	Controls (%)	P value *
<b>Age (Years)</b>			
0 – 10	42/157 (27)	15/61 (25)	0.802
11 – 20	35/157 (22)	17/61 (28)	0.501
21 – 30	25/157 (16)	4/61 (7)	0.103
31 – 40	17/157 (11)	4/61 (7)	0.380
>40	38/157 (24)	21/61 (34)	0.256
<b>Gender</b>			
Female	82/156 (53)	25/62 (40)	0.331
Male	73/156 (47)	37/62 (58)	0.333
<b>Resident type</b>			
Permanent	8/155 (5)	14/62 (23)	<b>0.001</b>
Visitor	147/155 (95)	48/62 (77)	0.365

\*Significance of the  $X^2$  Pearson statistic, Significant P-value  $\leq 0.05$

Table 2: Results of the univariate logistic regression analysis of factors potentially associated with illness, KZN 2016/17.

Exposure variables	N	Cases	Controls	OR (95% CI)	P value
<b>Resident type</b>					
Permanent	22	8 (36)	14 (64)	Ref	
Visitor	195	147 (75)	48 (25)	5.3 (2.1-13.5)	<b>&lt;0.001</b>
<b>Shared a toilet with more than 3 people</b>					
No	9	5 (56)	4 (44)	Ref	
Yes	194	150 (77)	44 (23)	2.7 (0.7-10.5)	0.147
<b>Ate at a restaurant</b>					
No	123	93 (76)	30 (24)	Ref	
Yes	95	63 (66)	32 (34)	0.6 (0.3-1.1)	0.133
<b>Ate at a fast-food outlet</b>					
No	181	130 (72)	51 (28)	Ref	
Yes	35	24 (69)	11 (31)	0.8 (0.3-1.8)	0.697
<b>Drank municipal water at place of residence</b>					
No	147	112 (76)	35 (24)	Ref	
Yes	69	42 (61)	27 (39)	0.5 (0.2-0.9)	<b>0.021</b>
<b>Drank bottled water</b>					
No	57	31 (54)	26 (46)	Ref	
Yes	157	121 (77)	36 (23)	2.8 (1.4-5.3)	<b>0.002</b>
<b>Swam in the lagoon</b>					
No	176	118 (67)	58 (33)	Ref	
Yes	34	30 (88)	4 (12)	3.6 (1.2-10.9)	<b>0.019</b>

OR, Odds ratio; CI, Confidence interval, Significant P-value  $\leq 0.05$

Table 3: Results of the multivariable logistic regression analysis of factors potentially associated with illness, KZN 2016/17.

<b>Variables</b>	<b>Adjusted OR</b>	<b>95% CI</b>	<b>P value</b>
<b>Resident type</b>			
Permanent	Ref		
Visitor	6.0	2.0-17.6	<b>0.001</b>
<b>Ate at a restaurant</b>			
No	Ref		
Yes	0.5	0.2-0.9	<b>0.032</b>
<b>Drank bottled water</b>			
No	Ref		
Yes	2.4	1.1-4.8	<b>0.015</b>
<b>Swam in the lagoon</b>			
No	Ref		
Yes	3.3	0.1-10.3	<b>0.039</b>

OR, Odds ratio; CI, Confidence interval, Significant P-value  $\leq 0.05$

Figure 1: Satellite image of the beach front, showing the lagoon, beach and the river which connects to the lagoon, KZN, South Africa.



Figure 2: Epidemic-curve showing the number of cases by date of illness onset, KZN 2016/17.

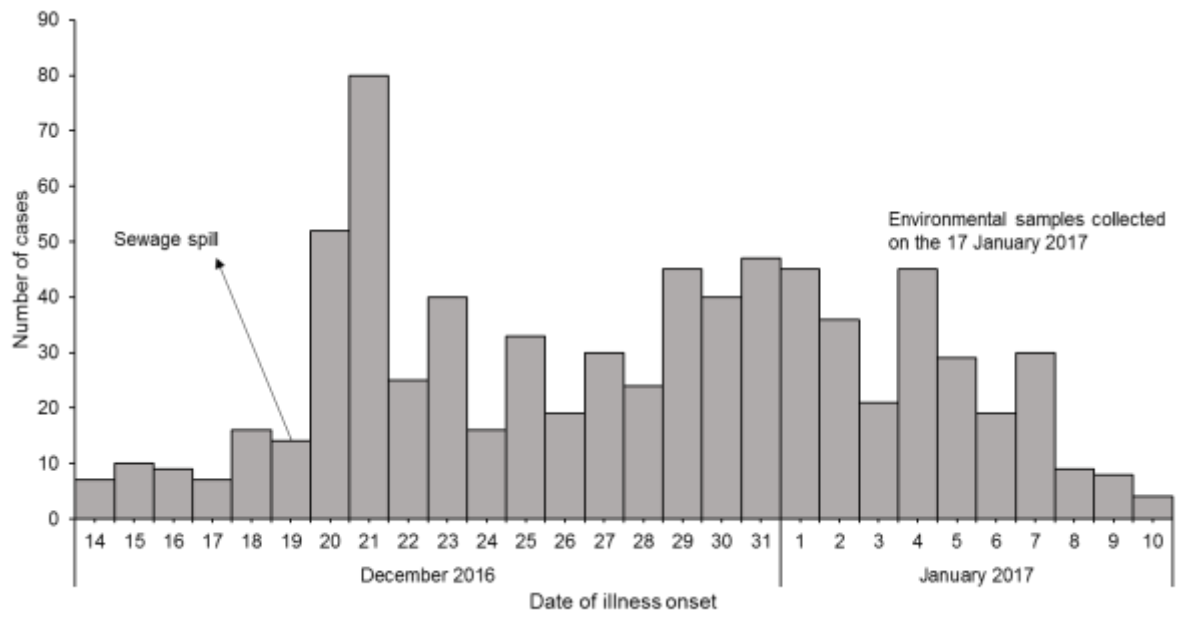




Figure 3: Neighbour-joining phylogenetic analysis of partial capsid sequences (299 bp) derived from gastroenteritis cases and lagoon water with norovirus GI reference strains. Strains indicated in bold are from the outbreak investigation. Bootstrap support (1000 replicates) of >70% is shown. The scale bar represents the number of base substitutions per site.

