

Norovirus outbreak in a district general hospital – new strain identified

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Summary

Introduction: Media reports about Norovirus outbreaks, especially in hospitals and nursing homes, have accumulated in the past years. The reasons for this increasing problem are manifold: resistance against common disinfectants, a high level of contagion (a dose of less than 100 particles may be infective); variability of the virus-genome documented by polymerase chain reaction-studies, as well as further factors concerning modern life-style.

The following study describes and analyses a Norovirus outbreak in the SRO-Hospital Langenthal from November 25, 2003, to December 31, 2003.

Patients and methods: The case definition included sudden vomiting and diarrhoea, abdominal cramps, fever below 38.5 °C and recovery after 48 hours. Stool cultures were taken and tested for bacteria (*Campylobacter jejuni*, salmonella and shigella species) and rotaviruses. Stool and vomit specimens were then tested using Real Time Polymerase Chain Reaction (RT-PCR) for noroviruses using different primer sets. Epidemiological data were gained such as transmission modus from person-to-person or food-borne.

Results: A total of 77 persons were affected by the viral disease. Two incidence-peaks of gastroenteritis were noted. The first peak happened in the geriatric ward and the second in the geographically distinct department of internal medicine.

The existence of a new norovirus strain of genogroup II/4 has been confirmed by the CDC Enteric Viruses Branch in Atlanta, GA. The measures to be taken are pointed out.

Conclusions: A norovirus gastroenteritis outbreak of such intensity and extent as described had not thus far occurred in the hospital's history. The strains newly found by sequencing are associated with genogroup II/4. This genogroup caused a striking increase of gastroenteritis outbreaks in Europe in the year 2002 including atypical spring and summer peaks. This new variant is supposed to be more virulent and environmentally more stable than previous strains [21]. Preventive measures that are in agreement with the published guidelines were taken before the viral pathogen was identified by RT-PCR.

The obvious emerging problem of norovirus infections in our society and the economic data analysing the costs caused by lost bed days and staff absence make epidemiological investigation of outbreaks and application of molecular tests more important. They are the basis for determining transmission routes and characterising the different strains in order to improve preventive strategies.

Key words: norovirus; hospital-outbreak; polymerase chain reaction; strain

Introduction

Noroviruses (formerly called “Small Round Structured Viruses”, SRSV, or “Norwalk-like Viruses”) are the prototype of caliciviruses (chalice = cup / beaker from latin *calix*) [1]. They are single-stranded, non-enveloped RNA-viruses and measure 28–35 nm; only Picorna-Viruses are smaller (25 nm). The virus was first discovered in 1968 in Norwalk County, Ohio [2]. Electron mi-

croscopy (EM) shows a characteristic chalice-shaped surface [3, 4]. In the early nineties, the genome was identified and sequenced by reverse-transcriptase polymerase chain reaction [5]. This represents the most sensitive diagnostic method.

Clinical picture

After a short incubation time lasting 12–48 hours, vomiting, usually in a projectile manner, diarrhoea and abdominal cramps begin. The illness is mostly self-limiting within 12–60 hours. In fact, recognition of a viral gastroenteritis outbreak can reliably be based on clinical symptoms and epidemiological characteristics alone as proposed by Kaplan et al. [6]. The transmission happens via the oral route. Aerosols are produced during projectile vomiting. Environmental contamination is common and due to its high resistance the virus can survive on surfaces for several weeks. The contagiousness is very high and a virus dose of 10–100 particles may be enough to cause a new infection [7]. The acid-stable viruses pass the stomach undamaged. The small intestine mucosa shows inflammatory changes and the epithelial cells develop blunting of the villi, shortening of the microvilli, dilation of the endoplasmic reticulum, swollen mitochondria, and intracellular oedema [8, 9]. Microscopic recovery of intestinal mucosa usually occurs within 14 days.

The immune response to the microbe is complex and is not well understood. There is some short-term immunity lasting days to weeks, whereas no long-term immunity is known following a single infection [10]. Frequent exposure may stimulate a sustained immunity and genetic traits are likely to play an important role [11]. The unequal immunological response to the pathogen is rather explained by local immune responses than by circulating antibodies.

Diagnostic methods

Since no culture medium permitting *in vitro* growth of caliciviruses has so far been found, electron microscopy (EM), enzyme linked immunoassay (ELISA) and real-time polymerase chain reaction (RT-PCR) represent important diagnostic tools.

EM has fundamental historical meaning, is fairly simple and inexpensive. However, the direct detection requires a high viral load of about 10^6 particles per ml and is therefore rather insensitive. Specimens taken 48 hours after symptoms have ceased usually remain negative [12]. ELISA testing reaches a sensitivity of about 64% in stool samples, whereas false positive results are rare with a specificity of 96%. This technique is almost as reliable as electron microscopic analysis, which has a specificity of 98%. Immunoassays fail to detect the large genetic diversity of the virus [13]. ELISA studies show variable results when compared with PCR based tests. Nevertheless, it is a suitable, cheap and simple method to define a gastroenteritis outbreak on a routine basis in order to take the necessary preventive steps.

RT-PCR is the gold-standard in calicivirus diagnostics. It represents the most sensitive method. Stool specimens may turn positive until two weeks after infection. Furthermore, vomit, food- and water-sources, where virus is found in smaller quantities, can also be tested [6]. Still, the risk of contamination resulting in false positive results is substantial. The continuous development of new primer pairs permits identification of the rapidly changing viral strains and thus, to follow up the chain of infection during an outbreak.

Patients and methods

The SRO Hospital in Langenthal, Switzerland is a 229 bed secondary care centre with internal medicine, surgical, orthopaedic, obstetrics & gynaecology, geriatric, oncology, haemodialysis and intensive care units. Its referral area amounts to 100'000 inhabitants.

On November 27, 2003 the hospital infection preventive service noted an unusually large number of patients with vomiting and diarrhoea in the geriatric ward of the hospital, which triggered the initial steps in outbreak investigations. The case definition included sudden vomiting and diarrhoea, abdominal cramps, fever below 38.5 °C and recovery within 48 hours. Stool cultures were taken and tested for bacteria (*Campylobacter jejuni*, *Salmonella* and *Shigella* species) and rotaviruses. Stool and vomit specimens were then tested using RT-PCR for noroviruses in the cantonal laboratory of Basel-Landschaft in Liestal using the primer sets BN 11 and BN 12 for genogroup I, and BN 21 and BN 22 for genogroup II, respectively [14], as well as in the cantonal laboratory of St. Gallen using Primer COG1F 20Mer and 22Mer for

genogroup I, and Primer COG2F 26Mer and 21Mer for genogroup II, respectively [15]. We then investigated a subunit of the specimens at the Centers for Disease Control and Prevention (CDC) in Atlanta GA, USA, using two different primer sets.

Stool specimens submitted to CDC were initially tested by RT-PCR with degenerate primers targeted to a 172-base pair region of the polymerase gene (Region B), using previously described methods [16]. To better characterise detected noroviruses, particularly those indistinguishable in the Region B sequences, selected specimens were also tested for norovirus by using primers targeted to a 277-base pair region of the capsid gene (Region C) [17]. All detected sequences were analysed by use of the GCG suite of programs [18] to create a phylogram, by which the similarity or divergence of strains from different cruise-ship and land-based outbreaks could be compared.

Furthermore, epidemiological data were gained, such as transmission modus from person-to-person.

Results

A total of 77 persons (28 patients and 49 health-care workers, HCW) were affected in two different buildings (figure 1). In building 1, including the geriatric wards, the oncology department and a public restaurant, 39 cases were reported (18 out of 49 patients corresponding to 37%, 20 out of 45 HCW corresponding to 45%, and 1 person working in the restaurant). The first peak in gastroenteritis incidence was reached on November 29. Building 2, the main building of the hospital, includes the internal medicine, intensive care, surgery, orthopaedic and obstetrics & gynaecology departments. In this building 38 persons (ten out of 60 patients corresponding to 17% and 28 out of 70 HCW corresponding to 40%) were affected. A second peak was noted on December 9.

On November 25, 2003 (day 1), a female patient on the geriatric ward fell ill with gastroenteritis. One day before, she was visited by a person, who had just recovered from gastroenteritis. This person probably was the index patient. On day 2 a health care worker, who had nursed this patient suffered from projectile vomiting and diarrhoea. She also had contact with patients and staff from the floor above (also part of the geriatric unit). On day 3, nine patients became ill, including three patients from the upper floor. Day 4 showed another seven sick persons, four of whom were HCW. Counting ten new gastroenteritis cases on day 5, this day marked the first peak in incidence. The public restaurant, one floor below had to be closed because of an infected staff-member.

The sick HCW were sent home for a minimum of 48 hours and the sick patients were iso-

lated and cohorted. Movements were reduced to a minimum and wearing masks, gloves and gowns was prescribed for both, HCW and visitors. Hand disinfection was mandatory with a product that contains 95% ethanol, which shows optimal activity against feline calicivirus which is a surrogate marker for norovirus [19, 20]. Surface disinfection was done daily.

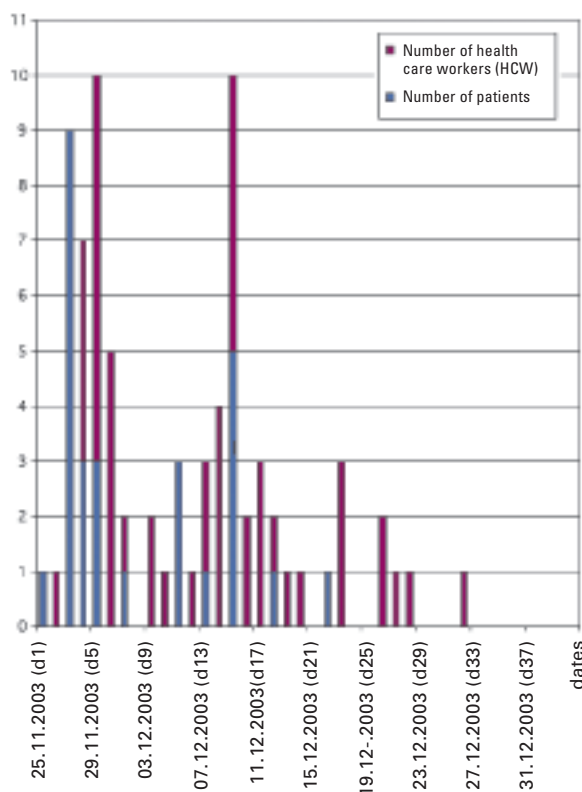
On the following days the gastroenteritis incidence slowly waned. On day six a HCW from building 2 fell ill one day after she had visited her mother, who was a resident of the geriatric ward. New cases in building 1 only sporadically happened after day 6. The last sick person in this building was noted on day 18. By day 11, three HCW working in building 2 had fallen ill, when the first two patients of this building suffered gastroenteritic symptoms. The second peak in incidence of gastroenteritis was reached on day 15, which showed ten new cases in one day. The only patient who died was an 81-year old woman, who had been hospitalised two days before due to unstable angina. Although suffering from gastroenteritis in the end, a cardiac complication was suspected as cause of death on day 12. The last sick person in building 2 was noted on day 33.

Whenever possible, stool and vomit specimens were collected and analysed. Four out of a total of 18 samples (13 patients and 5 HCW) investigated in the cantonal laboratory of Basel-Landschaft in Liestal tested positive for norovirus genogroup II (GG II). All were from geriatric patients and had been collected at the beginning of the first outbreak in building 1 (on November 25th and on 28th, twice on November 27th). There were no other enteric pathogens than norovirus found. Two out of the 18 samples were vomit specimens, both tested negative.

Because of a lack of positive samples in building 2 we contacted CDC in Atlanta in order to follow up the chain of infection that was clinically and epidemiologically suspected. Twelve frozen samples (6 of each outbreak) were then sent to CDC. Five samples tested positive. Four of them could be related to genogroup II cluster 4 (reference strain: Bristol virus). Three sequences were identical and have been assigned a unique sequence identifier (SeqID) of G204040148 (figure 2). One sequence differed from the predominant strain and was assigned a SeqID of G204040149. Strains with these SeqID have not been previously reported in the database of Calicinet Region C Dendrogram. The last and only specimen of the outbreak in building 2 that tested positive, could not be sequenced despite three attempts, which makes a false positive test probable.

Figure 1

Norovirus-Outbreak 25.11.2003–31.12.2003: Epidemic curve shows two peaks of case incidence. First peak in building 1, second peak in building 2. Total number of persons with gastroenteritis: 77.



Conclusions

We report a norovirus gastroenteritis outbreak in a Swiss district general hospital that is unsurpassed in its intensity and extent so far. The epidemic curve showed a two-peak-shape explained by two geographically separated buildings. The clinical and epidemiological data made a similar causative agent very likely since the chain of infection could be verified by movement of infected persons, especially in-between the two floors in building one as well as between departments. All patients showed a similar clinical picture including acute vomiting and diarrhoea, no fever higher than 38.5 °C and recovery after 48 hours (excepting the one person mentioned above). Diagnostic testing using RT-PCR could not confirm this hypothesis, since the only positive test in the outbreak in building 2 was probably false positive. Despite the considerable variability of the virus, a mutation within one week undetectable by the same primer sets is very unlikely. Thus, possibly two different outbreaks occurred.

Two strains of norovirus so far unknown could be detected in collaboration with the Enteric viruses branch of CDC in Atlanta. Both strains belong to cluster 4 of genogroup II. Genogroup II/4 with a consistent mutation in the polymerase gene as with the predominant emergent norovirus vari-

ant that caused a striking increase of gastroenteritis outbreaks in nine other European countries in the year 2002 [21]. Furthermore, in four countries (Great Britain, the Netherlands, Germany and Finland) atypical spring and summer peaks in outbreaks were noted. This new variant is presumed to be more virulent and environmentally more stable than previous strains.

Person-to-person contact and environmental persistence are presumably the predominant routes of transmission, since all ill persons detected had previous contact to either, the areas involved or to other infected patients. Person-to-person transmission is estimated to be the primary route of infection before food borne transmission (81 vs. 13%) [22].

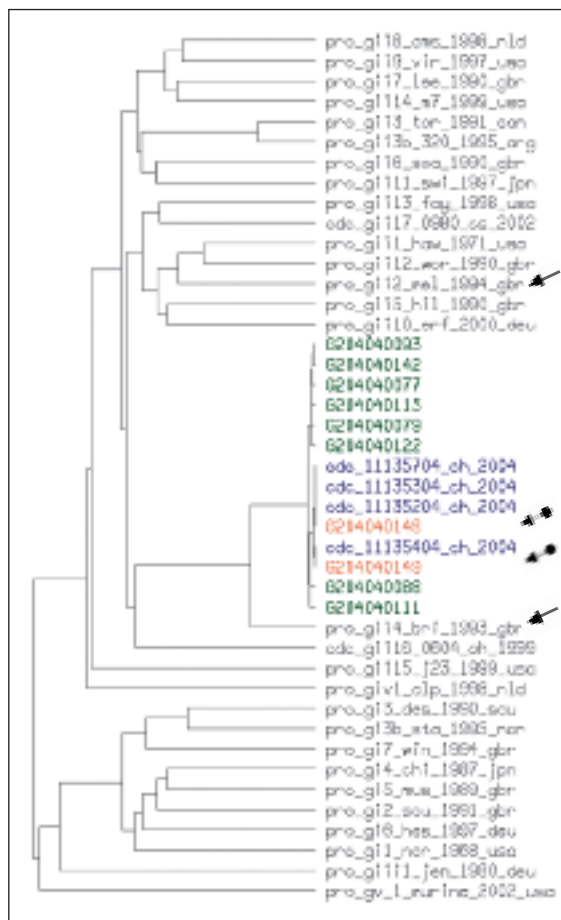
A package of measures to interrupt the chain of infection was immediately imposed by the hospital infection preventive service when the first case occurred, even before the diagnosis was validated [23]. However, vigorous application of all published guidelines was not always feasible. Some HCW returned to work earlier than 48 hours because of severe staff shortage, which was accepted, as otherwise the care of patients would have been seriously jeopardised. Compliance with hand washing and disinfecting for at least 30 seconds was not always strictly enforced. A few visitors did not show adequate understanding to accept the published instructions. Isolation and cohorting of patients stretched the limits of our infrastructure. Nonetheless the outbreaks could be contained and, fortunately, there were no patients from the oncology department situated one floor lower in building 1 suffering from gastroenteritis. Immunocompromised persons are known to be at risk for severe chronic secretory and osmotic diarrhoea after being infected by caliciviruses [24].

British data confirm that outbreaks can be contained significantly more effectively when adequate measures are taken, including prompt closure for new admissions. The costs for the British National Health Service in lost bed-days plus staff absence were estimated around 115 million Pounds Sterling (corresponding to 175 Mio EUR or 267 Mio CHF) in one year [25]. Geriatric and general medical care units are at increased risk for nosocomial gastroenteritis, as are institutions with greater number of beds and short duration of stay [26].

Increasing numbers of beds in nursing homes, changing lifestyle habits including eating more food handled by a variety of potentially infected people and rising consumption of fresh vegetables and fruit, as well as greater population movement through enhanced travel options make our society more vulnerable to viral gastroenteritis. The same measures that reduce the incidence of bacterial gastroenteritis may be ineffective in the case of caliciviruses, because these are resistant to chlori-

Figure 2

Calicinet Region C Dendrogram. Genetic relationship of Region C sequences of noroviruses associated with the outbreaks. Sequence names ending with ... ch_2004 are associated with this outbreak. Other names are reference sequences, identified by genogroup (gi-iv) and cluster (1-17). The three letters in the middle of the sequence refer to the first letters of the prototype strain for that cluster. Therefore pro_gii2_mel_1994_gbr refers to the gii cluster 2 prototype strain Melksham (■—). Three sequences of the outbreak were identical and have been assigned a unique sequence identifier (SeqID) of G204040148 (●—●). One sequence differed from the others by 1 base pair in region C and was assigned to SeqID of G204040149 (●—●). Strains with these SeqID's have not been previously reported. Reference strain: Bristol strain (■—).



nation and freezing, they persist in the environment and need vanishingly small doses to be infective. All these points suggest norovirus outbreaks are becoming an emerging problem that is not only explained by improved surveillance systems and diagnostic tools [27]. Recent advances in finding a cell culture system for noroviruses may allow assessment of efficacy of various disinfectants [28]. Epidemiological investigation of outbreaks and application of molecular tests will be of growing importance for determining transmission routes and characterising the different strains in order to improve effective preventive strategies.

We are indebted to Marlise Wüthrich (head of hospital hygiene) for collecting epidemiological data, Jürg Grimbichler, (Cantonal Laboratory Basel-Landschaft, 4410 Liestal, Switzerland) and Jürg Schmid (Cantonal Laboratory St. Gallen, 9001 St. Gallen, Switzerland) for PCR-Analyses.

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