SHIPBOARD IMPACT OF A PROBABLE NORWALK VIRUS OUTBREAK FROM COASTAL JAPAN

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Abstract. Norwalk virus has been implicated in shipboard diarrheal disease outbreaks throughout Asia. A large outbreak of suspected Norwalk virus was investigated on a U.S. Naval aircraft carrier following the clinical recognition of 450 cases of gastroenteritis over a 2-week period (September 14–28, 1997) during coastal exercises. A random sampling of 44 cases from 450 personnel who sought medical attention was compared with 19 controls. Junior enlisted sailors and marines comprised 97% of all cases. There was no evidence of shipboard geographic clustering of cases. Furthermore, no single food type was associated with illness on the basis of comparative analysis (cases versus controls). Principal case signs and symptoms reported included watery stools (89%), nausea (82%), and vomiting (77%). Anecdotal reports indicated > 50% of the cases received rehydration therapy. An absence of fever was also noted in 32% of the cases and only 5% had blood in their stools. The mean duration of illness was 37 hr, with a range of 3–96 hr. Laboratory findings based on reverse transcription–polymerase chain reaction and Southern hybridization methods showed that 21 (72%) of 29 patients had evidence of the UK2 prototype of the Norwalk virus. A cross-sectional study of 131 crew members from the ships population (n = 4,200) showed an attack rate of 44%. Attack rate is a variant of an incident rate applied to a narrowly defined population observed for a limited period of time, such as during an outbreak. The numerator is people who get sick and the denominator is people (population) at risk. An extrapolation of these findings suggests as many as 1,806 sailors may have been affected during the outbreak, of which only 26% (of the 57 outbreak related cases) where identified from sick call records. There was no difference in the mean ages between outbreak and non-outbreak affected crewmen, or geographic clustering based on berthing or work spaces. Outbreak-related cases reported signs and symptoms of watery-stools (79%), nausea (65%), and vomiting (47%). The mean duration of illness was 28 hr, ranging from 2 to 96 hr. Thirty-one percent of outbreak affected cases reported a sick call visit. Loss of work was reported by 39% of the outbreak affected population. This report documents the epidemic potential of Norwalk virus and the associated impact on fleet operational readiness. Additionally, that this outbreak occurred against a background of 3 other consecutive gastroenteritis outbreaks onboard the same ship (March 1997, February/March 1998, and June 1998), all sharing the same clinical and epidemiologic profiles, suggests possible shipboard persistence of Norwalk virus over time, despite periodic ship-wide disinfection efforts.

Norwalk virus (NV) or Norwalk like-viruses (NLV) have been implicated in outbreaks of viral gastroenteritis worldwide.4 Outbreaks involving NV and NLV have been best documented in North America and Europe. In Asia, NLV infection was detected in gastroenteritis outbreaks reported from Japan and Australia.2,3 Unfortunately, limited diagnostic capabilities have made it difficult to ascertain the importance of NV- and NLV-related gastroenteritis outbreaks in most developing areas.5 The occurrence of epidemic NV and NLV transmission has been repeatedly described in shipboard settings.6 A review of cruise ship outbreaks involving travel to U.S. ports from international areas, from 1986 through 1993, identified NV in 29% (9 of 31) of outbreaks investigated. Notable was the large proportion of gastrointestinal cases recognized from 31 investigated shipboard outbreaks7 attributed to NV and NLV infections: 37% of 8,227 gastrointestinal cases. The epidemic potential of NV in shipboard environments was also demonstrated in a large outbreak of acute gastroenteritis that affected 13% of a 4,500 crew onboard a U.S. aircraft carrier during a trans-Atlantic cruise from the United States to the Mediterranean.8

Outbreaks of NV and NLV are characterized by explosive diarrhea and vomiting, in the absence of high-grade fever.9 Generally self-limiting, gastrointestinal symptoms associated with NV and NLV infections usually range from 24 to 48 hr in duration, although clinical gastroenteritis described during a Mediterranean cruise ship outbreak of NLV lasted from 24 hr to 5 days.9 The estimated incubation period for NV and NLV ranges from 24 to 48 hr, although human volunteer studies suggest incubation may be as short as 10 hr.1 Fecal-oral transmission via contamination of water and food supplies has been identified as the principal mechanism associated with epidemic spread of NV and NLV. Consumption of shellfish, particularly oysters, has been attributed in a number of outbreaks as the likely source.10-13 Also, person-to-person spread has been reported in shipboard and long-term facility outbreaks, reflected in propagated epidemic curves.8,9,11 The window of disease communicability in humans, the only known reservoir, begins with the acute disease phase and lasts up to 48 hr post-gastroenteritis symptoms, affording ample opportunity for on-going exposures among groups sharing the same living and working environment over time, as in military shipboard communities.

This investigation was intended to characterize and describe the impact of a gastroenteritis outbreak in a shipboard population involving probable NV or NLV transmission.

METHODS

The outbreak. Following shipboard reports of temporal clustering involving 450 cases of gastroenteritis between September 14 and 28, 1997, an investigation was initiated on September 27 aboard the affected U.S. Navy aircraft car-
shipboard with a crew of 4,200 sailors and marines. Cruising in Japanese coastal waters during the actual outbreak period, the ship had last made landfall during a port visit to Otaru, Japan on September 5. Anecdotal data relayed via the ship’s Senior Medical Officer suggested a likely viral etiology associated with disease occurrence (nausea, vomiting, abdominal cramping, and watery diarrhea) in the absence of fever. Notable was the high proportion (> 50%) of the cases requiring rehydration therapy. Forty-nine percent (221 of 450) of outbreak-related disease cases were sufficiently debilitated to be assigned as sick in quarters (SIQ) for 24 hr. Evidence of person-to-person spread was reflected in the gradual increase in clinically recognized SIQ cases, peaking on days 9 and 10 of the 14-day outbreak period, as shown in the epidemic curve presented in Figure 1.

Case-control study. A random sampling of 44 male (43 enlisted and 1 officer) outbreak cases (out of 450) were selected for study inclusion, regardless of SIQ status. Additionally, the last 19 cases were age and grade/rank matched with 19 (18 enlisted 1 officer) healthy controls having no reported gastroenteritis (nausea, vomiting, abdominal cramping, or watery diarrhea). Demographic, clinical, and (possible) risk-related data was obtained using a standardized questionnaire. The mean age of all study cases was 24 years, ranging from 19 to 44 years, and 24 years for control subjects, ranging from 19 to 39 years. Voluntary, written, informed consent was obtained from participants prior to enrollment in the study. This study was reviewed and approved by the U.S. Naval Medical Research Unit No. 2 (NAMRU-2) Committee for the Protection of Human Subjects and Ethical Review Board.

Laboratory testing. Stool specimens were obtained from 29 of the 44 case subjects. A sample of each specimen was placed in 1) Cary-Blair transport medium for bacteriologic evaluation, 2) 10% formalin for parasitologic examination, and 3) frozen in liquid nitrogen for molecular analysis. The clinical samples were then transported from the Naval Station in Yokosuka, Japan to NAMRU-2 in Jakarta, Indonesia. Stool samples were cultured at NAMRU-2 for the common bacterial etiologic agents of diarrhea: Salmonella spp., Shigella spp., Campylobacter spp., and Vibrio spp. Stool was also screened for the presence of heat-labile enterotoxin (LT)– and heat-stable enterotoxin (ST)–producing Escherichia coli using a monosialoganglioside GM1 ELISA developed by Svennerholm and others. Additionally, formalin-preserved stool samples were examined microscopically for the presence of ova and parasites: Giardia lamblia, Entamoeba hartmani, Entamoeba histolytica, Entamoeba coli, Endolimax nana, Iodamoeba buetschlii, Dientamoeba fragilis, Cryptosporidium, Cyclospora, Chilamastix mesnili, and Trichomonas intestinalis. Specimens were examined for presence of rotavirus by a commercially available ELISA (Rotazyme; Abbott Laboratories, North Chicago, IL).

Molecular analyses were conducted using the reverse transcription–polymerase chain reaction (RT-PCR) assay and Southern hybridization with probes. The RT-PCR was performed with 2 different primer sets, designated G-1 and G-2, to amplify NV prototype UK2 and NLV prototypes UK1,
UK3, and UK4, respectively. Southern hybridization of the RT-PCR-amplified products with 4 different probes enabled identification of specific prototypes of NV and NLV. Probe P2-A was used to identify the NLV UK1 prototype (Taunton agent). A mixture of several probes, designated P1-A, were used to identify NV UK2 prototype, probe P1-B was used to identify NLV prototype UK1, and probe P2-B was used to identify NLV prototype UK3/4. There are no true measures of validity as to the sensitivity and specificity associated with the RT-PCR Norwalk virus detection assay. Previously low detection rates using the RT-PCR resulted from the considerable nucleotide sequence diversity among NLVs with distinct antigenicity. However, the composition of the RT-PCR primers used in this study was 2 broadly reactive primer sets and 4 internal oligonucleotide probe sets that provided for improved detection of genetically diverse NLVs.

Cross-sectional study. Standardized questionnaires were randomly distributed to sailors and marines at their work stations, located on 10 of the aircraft carrier’s decks, representing both forward and aft areas of the ship. Data pertaining to demographics, outbreak related experience, and possible factors contributing to risk was collected from 131 of the all-male crew (119 enlisted and 12 officers), during the last days of the purported outbreak (September 27–29, 1997). The age range among study participants was 19–51 years, with a mean of 28 years.

Environmental water sampling. Environmental sampling of water was conducted from 32 water tanks that provided for all shipboard potable water needs. The absence or presence of coliforms was determined using the standard membrane filter technique. Additionally, free available chlorine (FAC) were measured from 27 strategic potable water distribution points throughout the ship over a 30-day period (September 1–30, 1997). The method used for identifying FAC levels was standard chromometric testing. Trace (0.1 ppm/FAC) FAC levels at the furthest point along the water distribution system were considered the minimal acceptable measure.

RESULTS

Case-control study. Epidemiology. The mean age of the 44 study cases was the same as for the 19 controls: 24 years, ranging from 19 to 44 years and 19 to 39 years, respectively. Enlisted sailors accounted for 98% of the case subjects, of which 98% were of junior enlisted grades E-6 and below. There was no significant difference in proportional representation by job category: when job groupings were collapsed into 2 principal categories, 55% of cases were involved in aviation-related occupations and 43% were involved in non-aviation jobs.

There was no correspondence in food and drink consumption and gastroenteritis, as determined through case comparisons with controls. Uncooked vegetables and fish elicited the highest odds ratios of 2.0 and 2.5, respectively, although neither represented a statistically significant finding ($P > 0.01$, by chi-square test). Notable was the high proportion of cases who ate in the forward galley (78%) within 24 hr of onset of symptoms compared with the aft galley (16%). As expected from the earlier findings, the proportion of cases reported to have eaten in the chief petty officer (CPO) (3%), and officer (3%) galleys was negligible. Unfortunately, comparative data from the control populations was not collected for risk ascertainment. Finally, analysis of living spaces, including work and berthing areas, showed no evidence of shipboard geographic clustering of cases.

Clinical. Principal case signs and symptoms attributed to the outbreak of gastroenteritis included nausea (82%), vomiting (77%), and watery stools (89%). Thirty-two percent of case patients presented with fever, and only 5% had blood in their stools. The mean duration of illness was 37 hr, with a range of 3–96 hr. Outbreak related gastroenteritis among case patients accounted for 42 days lost, or 764 (lost days)/1,000 patient days, with 69% of the patients reported ≥1 lost days, ranging from <1 (recorded as 0) to 3 days.

Laboratory findings. Norwalk virus gene sequences were detected in 72% of the cases (21 of 29) assayed by the RT-PCR. Hybridization of the amplified PCR products and the P1-A probe confirmed that the amplified nucleic acid contained sequence of the UK2 prototype of NV. Figure 2 shows a Southern blot of RT-PCR-amplified products hybridized with the UK2 NV prototype probe.

All stool specimens were negative by culture for Campylobacter spp., Shigella spp., and Vibrio spp. Two stool samples, both positive for NV, were culture positive for Salmonella groups C and D. Stools were also negative for the

Figure 2. Results of a Norwalk virus reverse transcriptase–polymerase chain reaction (RT-PCR). A, electrophoresis of RT-PCR products on an agarose gel stained with ethidium bromide. B, Southern hybridization using digoxigenin-labeled oligonucleotide probe sets SR65d, SR69d, and SR63d (P1-A probe). Lanes 2–10 show G1 products of representative stool samples. Lane 1, 2-kb ladder marker; lane 11, negative control; lane 12, positive control. The arrow indicates the position of the 123-basepair fragment.
presence of LT- and ST- producing *E. coli*, parasitic pathogens, and rotavirus.

**Cross-sectional study. Epidemiology.** Reported survey findings from 131 members of the ship’s crew showed an attack rate of 44% (57 outbreak related cases and 74 non-cases). Attack rate is a variant of an incident rate applied to a narrowly defined population observed for a limited period of time, such as during an outbreak. The numerator is people who get sick and the denominator is people (population) at risk. An extrapolation of this measure as applied to the ship’s population translates into an estimated 1,806 case-subjects of a crew of 4,200 sailors. The mean age of outbreak cases (28 years, ranging from 19 to 54 years) was the same as for non-outbreak cases (28 years, ranging from 19 to 47 years). There was no evidence of outbreak case clustering related to berthing or work spaces. Enlisted personnel comprised 95% of the outbreak cases (82% of whom where E-6 and below) and 88% of non-outbreak cases (89% of whom where E-6 and below); the proportion of outbreak affected sailors by enlisted ranking ranged from 29% to 50%.

**Clinical.** The principal signs and symptoms reported by the outbreak affected study population were watery stools (79%), nausea (65%), muscle aches (56%), stomach cramps (53%), and vomiting (47%). Blood in the stools was identified by < 2% of the case respondents. The mean duration of illness was 24 hr, ranging from 2 to 96 hr, with a mode of 24 hr. Thirty-one percent of case subjects reported (as validated by clinic records) at least 1 visit to sick call. The percentage of sailors seeking medical attention for outbreak-related gastroenteritis decreased significantly ($\chi^2 = 10.360$, $P < 0.01$) as rank seniority increased. Of enlisted personnel with outbreak-related illness, 62% (8 of 13) of E3s and below reported to sick call compared with 28% (8 of 29) of E-4s through E-6s, and none (0 of 10) of E-7s through E-9s. Loss of work was reported by 39% of the outbreak cases: 77% missed ≤ 1 day, 18% missed 1–2 days, and 5% > 2 days.

**Environmental study.** All 32 water samples obtained from the ship’s water storage tanks were negative for coliform bacteria. Three-hundred eight water samples, collected from 27 representative locations from three of the ship’s decks, all had traces of FAC: a mean of 0.42 ppm/FAC (ranging from 0.1 to 2.0) on the second deck, 0.48 ppm/FAC (ranging from 0.1 to 2.0) on the third deck, and 0.38 ppm/FAC (ranging from 0.1 to 1.0) on the fourth deck.

**DISCUSSION**

In the absence of measurable baseline data for descriptive trend analysis, the large number of gastroenteritis episodes during the outbreak window clearly highlights epidemic occurrence. The duration and the attack rate associated with this outbreak are compatible with other shipboard (NV or NLV) gastrointestinal outbreak investigation results when presented in aggregate fashion: 14 days versus 4–15 days and 44% versus an overall mean of 31% per cruise, respectively. This contrasts sharply with the epidemiologic picture of an NLV outbreak reported on another aircraft carrier, in which the duration in the occurrence of cases was considerably longer (5 weeks) and the attack rate lower (15%).

This probable NV outbreak could not be attributed to exposure associated with the last port visit, which occurred 9 days prior to the first clinically recognized gastroenteritis cases, thereby exceeding the maximum incubation period. Also, there was no evidence of geographic clustering of cases relative to berthing or work areas. A delay in (cross-sectional) survey collection activities, not carried out until the end of the outbreak (September 27–29, 1997), precluded reliable recall data of food and drink consumption for risk analysis. Failure in tracing point source and mechanisms associated with transmission may be attributed to the small sampling of clinically recognized cases (10% of 445 patients) and high ratio of cases to controls (2.3:1); case-control investigation was initiated late in the outbreak, resulting in lost follow-up opportunities. There was, however, no evidence of specific food, drink, or galley contamination related to the outbreak, based on case and control comparative findings. In contrast, likely contributing risks have been identified in most investigations of previously reported NV or NLV outbreaks. These include inadequately prepared oysters, consumption of raw oysters, water consumption from a contaminated well at a tourist restaurant, and consumption of contaminated water by canoeists following capsizing.4,10,11,17,18 In cruise ship-specific outbreaks of NV or NLV, crowded sleeping quarters, inappropriate food handling, hygiene, and storage, consumption of eggs, fresh fruit, lobster, and chicken salads were all found to be associated with increased exposure-related risk.3–9

All potable water samples collected from the ship were negative for coliforms. Unfortunately, water samples from potable water exit sources, such as sinks, fountains, and showers, were not collected and tested for coliform contamination. Therefore, possible contamination along the water delivery route could not be evaluated. Examples of water attributed exposure have been documented from cruise ship NV or NLV outbreaks.6,7 While chlorine levels were sufficient for killing bacterial pathogens, the resistance of NV and NLV to environmental disinfection and decontamination may require higher FAC levels to prevent and/or control outbreaks caused by NV or NLV. This finding was highlighted by the failure of chlorination in preventing an explosive outbreak transmission of NLV from a sink contaminated with infectious vomit from a kitchen assistant at a wedding reception.9

Anecdotal reports from the ship’s Medical Department and from a review of clinic records indicate a similar gastroenteritis outbreak, relative to the September 1997 outbreak, occurred aboard this ship in March 1997, en route to Australia from Guam. Cases presented with the (now) familiar syndrome that included nausea, vomiting, abdominal cramping, and watery diarrhea, in the absence of fever, of which a significant proportion required rehydration fluids. Also, the outbreak was characterized (as in the September 1997 outbreak) by a narrow epidemic peak suggestive of a point source. An estimated 666 cases of gastroenteritis were clinically recognized (based on sick call records) at the time of this outbreak. Similarly, NLV suggestive of a NV or NLV agent were identified in 4 gastroenteritis outbreaks on consecutive weeks in 1995 aboard a cruise ship.8 Person-to-person transmission was implicated in these successive NLV outbreaks and likely resulted from infected passengers who remained on board for a second week, passing on the virus.
to newly arrived passengers. The cycle of transmission was believed interrupted on the fourth and last outbreak, following an aggressive and intensive ship-wide disinfection. In other investigations, outbreaks of viral gastroenteritis have recurred on successive cruises, highlighting the difficulty in establishing effective control measures.20–22 On the outbreak-affected aircraft carrier, person-to-person spread was an unlikely factor in linking the 2 similar gastroenteritis outbreaks, since the 2 outbreaks were separated by a gap of 7 months, contamination was likely related to maintenance (via human or other reservoir) of the virus onboard ship. A breakdown of ship systems, such as water purification malfunction, failure to reach optimal FAC levels, seepage via a faulty water line, or inadequate food storage, processing, or handling practices, could have created the necessary conditions conducive to outbreak occurrence. There were 2 additional consecutive gastroenteritis outbreaks of likely viral etiology (with similar clinical and epidemiologic profiles as the 2 preceding outbreaks) anecdotally reported on the aircraft carrier by the ship’s Medical Department in February/March and early June 1998 during and following Naval exercises in the Persian Gulf. The first was of 2-weeks duration and impacted > 150 sailors and the second was of 1-week duration and affected < 20 sailors. These outbreaks occurred 5 and 8 months after the outbreak described in this report, further supporting the assumption of continued persistence of NV or NLV virus within this shipboard environment.

Norwalk virus, identified by the RT-PCR assay and Southern blot hybridization, was implicated as the likely causative etiology in this outbreak. The NV prototype responsible for this shipboard outbreak was the same as that associated with the first NV outbreak recognized in humans reported in 1968 from Norwalk, Ohio.23 This current outbreak finding is the first time this NV prototype has been linked to an outbreak of gastroenteritis on board a U.S. Navy ship. Similar NV UK prototype strains have been found worldwide, including Australia and Hawaii in the Pacific region.3

The large number of clinically recognized cases (445) likely represented only a small proportion of the affected crew; less than one-third of the self-described outbreak cases actually sought medical attention. On another U.S. Navy ship, only 8% of the ship’s population presented to sick call with an acute episode of gastroenteritis during an outbreak of NLV, although a cumulative attack rate of 13% was estimated.8 The decision to seek medical care for gastroenteritis is likely a function of disease severity. A relationship between pre-illness antibody levels to NLV and gastrointestinal disease incidence has been reported in other outbreak findings, with low levels of antibody to NLV corresponding with a high incidence, and conversely, high levels of antibody correlating with a low incidence of disease.8 In this study, a large proportion of junior enlisted personnel (relative to senior staff) reported an outbreak-related sick call visit, suggestive of more severe illness. With fewer previous shipboard exposure opportunities to NV or NLV, these junior sailors may have been immunologically naive and thus accounted for more disease episodes involving severe illness.

The impact of this probable NV outbreak on the ship’s crew was notable, as highlighted by an extrapolated attack rate of 44%. This report attests to the epidemic potential of NV, particularly in a shipboard environment. The implication that NV or NLV accounts for many of the gastroenteritis outbreaks on board ships warrants improved prevention measures and detection capabilities, including more attention to vigorous case and environmental monitoring, timely investigative responses, and laboratory diagnostics. Moreover, heightened sensitivity to possible outbreak conditions is justified against a historical background of shipboard NLV outbreak occurrence.

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