# Noroviruses : a Challenge for Military Forces.

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## Abstract

**For military forces, the control of infectious acute gastroenteritis constitutes an old, constant and unsolved concern. Recent epidemiological studies suggest that the common bacterial causes are being overtaken by viruses. Norviruses are the most alarming group and norovirus outbreaks in military forces are regularly reported. Illness is generally mild and characterised by acute vomiting and diarrhoea, which lasts for a few days on average, but may be severe and potentially life-threatening in subjects who are already dehydrated due to daily activity. Moreover, outbreaks may diminish operational effectiveness. Prevention of norovirus infection currently relies on strict application of personal and collective hygiene rules including isolation of the cases, to the greatest possible extent. Although noroviruses are frequently mentioned as the cause of gastroenteritis outbreaks in troops deployed overseas, laboratory diagnosis is rarely done. So their real burden in military forces remains unclear and further epidemiological studies are required to determine the full impact of norovirus gastroenteritis on troops.**

## Introduction

Infectious diseases constitute an old, constant and unsolved problem for military forces. History is littered with episodes in which these diseases have decided of the outcome of battles, by causing more casualties than battlefield injuries. In the Battle of Valmy in September 1792, the French victory was facilitated by the outbreak of dysentery in the Prussian troops [1,2]. Nowadays, acute gastroenteritis continues to be an important cause of morbidity among military personnel. In 2009, more than 6000 cases of acute gastroenteritis were reported among French troops, most of them being observed in the personnel deployed overseas [3]. In a survey of healthy US service personnel, 77% of those deployed in Iraq and 54% in Afghanistan reported diarrhoea at some time during deployment [4]. Even if the illness is generally mild, it may still diminish operational effectiveness. Recent experiences suggest that the common bacterial causes are being overtaken by viruses. Of 11 outbreaks that have occurred in deployed British troops during 2002-2007, ten (91 %) had a proven viral cause and of 84 enteropathic viruses identified, 61 (73 %) were noroviruses [5]. These viruses are now considered as a major cause of gastroenteritis outbreaks in military populations.

## Biology and epidemiology of noroviruses

### *Virological characteristics*

*Norovirus* is a separate genus in the *Caliciviridae* family and includes viruses previously denoted as "Norwalk-like virus" or "small round structured viruses". Noroviruses are non-enveloped viruses with a single positive-strand RNA genome. They are quite numerous and five distinct branches named genogroups (G) are recognised. Within genogroups, noroviruses are further subdivided further into genotypes, of which presently more than 40 are recognised. Infections in humans have been described for viruses from GI, II and IV, whereas GIII and GV viruses have been found so far in animals only (cattle and mice, respectively). This great diversity of strains is attributed both to the accumulation of point mutations associated with error-prone RNA replication and to recombination between two related viruses [6,7].

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### *Epidemiologic features*

Noroviruses are the leading cause of non-bacterial gastroenteritis outbreaks in children and adults worldwide, and probably represent the second most common aetiological agent of sporadic gastroenteritis in young children after rotavirus [6,7]. They appear to be near-perfect pathogens and several of their characteristics enhance their ability to spread (Table I). They can be transmitted through faeco-oral route, direct person-to-person spread, contaminated food or water, or contact with contaminated surfaces [8].

### *Host susceptibility and immunity*

Susceptibility to norovirus is mostly related to histo-blood group antigens. Noroviruses bind to ABH histo-blood group antigens (HBGAs) that are expressed on the surface of mucosal surfaces. The combination of the strain-specific binding and the variable expression of HBGA receptors may explain the varying host susceptibility observed. However, given the diversity of norovirus strains, someone who is resistant to one strain may be susceptible to another. Infection induces only a short-term homotypic immunity (6 – 14 weeks). Therefore, given the genetic variability of noroviruses, individuals are likely to be repeatedly infected throughout their lifetime [9,10].

### *Clinical features and treatment*

After an incubation period of 12 to 48h, the disease in adults often begins with nausea and vomiting, followed by abdominal cramps, fever in up to 50 % of the cases, non-bloody diarrhoea, and constitutional symptoms such as headache, chills and myalgias. These symptoms typically disappear within 24-72 h. The illness, which is generally mild, may be severe and life-threatening in groups

| Features | Observation | Consequences |
|---|---|---|
| Low infectious dose | < $10^2$ viral particles | Permits droplet or person-to-person spread, secondary spread, or spread by food handlers |
| Prolonged asymptomatic shedding | Up to 2 weeks | Increased risk for secondary spread or problems with control regarding food handlers |
| Environmental stability | Survive up to 10 ppm chlorine, freezing, and heating to 60°C | Difficult to eliminate from contaminated water; virus maintained in ice and steamed oysters |
| Substantial strain diversity | Multiple genetic and antigenic types | Repeated infections by multiple antigenic types; may limit sensitivity of diagnostics |
| Lack of lasting immunity | Disease can occur with re-infection | Childhood infection does not protect from disease in adulthood; difficult to develop vaccine with lifelong protection |

***Table 1. Characteristics of noroviruses facilitating their spread [7].***

at high risk as mentioned below. The treatment is oral rehydration with fluids and electrolytes, if the patient is alert and able to drink, or with intravenous fluids, if vomiting and dehydration are severe. Anti-motility and anti-secretory agents can be useful in situations where a person's performance is critical [6,7].

### *Detection methods*

Norovirus diagnostic tests are performed mainly on stools, but vomits can be used too. For epidemiological studies, noroviruses research can be performed on water, food, or environmental specimens in order to identify the source of infection. Collection and storage conditions for samples are listed in Table 2 [11].

Two major types of assays for diagnosing noroviruses in biological samples have been developed. One detects the viral antigens by recombinant enzyme-linked immuno-assays (EIAs) and the other detects the viral RNA. Various commercial stool EIAs have been developed. They are easy to use and highly specific, but sensitivity may vary based on the diversity of the circulating strains in the population; so they are not recommended to search for norovirus in cases of sporadic gastroenteritis, but are a useful tool for diagnosing noroviruses in outbreak analysis. Performed on several samples collected from different patients suffering from gastroenteritis, these tests are sufficient for aetiologic confirmation if at least one sample is positive. Real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) assays are now the reference method for detecting noroviruses in clinical, food and environmental samples, to make adjustment for the great diversity of strains, a cocktail of primers is required [12,13].

| Specimen | Important considerations |
|---|---|
| Stool | Timing: collect specimens as soon as possible from affected individuals (if possible, during the acute phase of illness). Liquid diarrheal stool is the best sample to use.<br><br>Number and quantity: if possible, specimens from >10 ill persons should be obtained during the acute phase of illness. Bulk samples (ie. 10-50 ml of stool placed in a stool cup or urine container) are preferred.<br><br>Storage and transport: store specimens at 4°C for 2 to 3 days. For a longer time of storage, freeze specimens at –20°C or 80°C. |
| Vomitus | Specimens of vomit can be collected to supplement the diagnostic yield from stool specimens during an investigation.<br><br>Recommendations for collection, storage, and shipment are the same as those for stool specimens. |
| Environmental specimens | Suspected contaminated food and water samples should be obtained as early as possible and stored at 4°C before to being submitted for RT-PCR analysis.<br><br>Environmental swabs are an additional tool for the detection of norovirus in outbreak settings. |

***Table 2. Collecting specimens for norovirus diagnosis [11].***

## Lessons from outbreaks

The first norovirus gastroenteritis outbreak in troops was observed during Operation Desert Shield and led to the description of the eponymous strain [14-15]. Since then norovirus outbreaks in military personnel have regularly been reported in the literature (Table 3) [5,16-23].

On the whole, norovirus outbreaks have both high attack rates (~50%) and high secondary transmission rates (~30%). Primary cases are frequently associated with consumption of contaminated water or food, whereas secondary and tertiary cases most frequently result from person-to-person transmission. Outbreak spread is facilitated by the enclosed living quarters and the low level of personal hygiene, especially observed during the initial phase of deployment. Consumption of fresh fruit and vegetables purchased from local distributors is an important risk factor for outbreaks in deployed troops [23].

If the illness is generally mild, it may still diminish operational effectiveness. In March 2003, during the second week of the Iraq War, a norovirus gastroenteritis outbreak was notified in British troops deployed there; it coincided with the delivery of locally produced fresh rations (including salad and fruit). In one month,

| Army | Setting (date) | Number of cases | Key features | Reference |
|---|---|---|---|---|
| U.S. | Naval aircraft carrier (1995) | 585 | Outbreak spread facilitated by close-living conditions. | 16 |
| U.S. | Training centre – Texas (1998) | 99 | Foodborne outbreak with food handler as the point source. Importance of hygiene measures with isolation of ill food handler up to 7 days after recovery. | 18 |
| U.S. | Naval aircraft carrier (1999) | 450 | Observation of three consecutive norovirus outbreaks. High environmental resistance of norovirus. | 17 |
| British | Deployed troops in Afghanistan (2002) | 29 | Outbreak of unusual severity. Increased risk of severe outcome in subject who is already dehydrated due to daily activity. | 19 |
| British | Deployed troops in Iraq (2003) | 1340 | Outbreak probably due to delivery of locally produced fresh rations and secondary outbreak amongst hospital staff. Suggested banning fresh rations during military operations. | 23 |

***Table 3. Examples of published reports of norovirus outbreaks in military forces.***

1340 soldiers had gastroenteritis, of whom 975 required admission to the 200-bed British military field hospital. The average length of stay in hospital was 1.65 days and bed occupancy reached > 90%. The outbreak spread to the hospital staff and 382 of 500 (76%) developed gastroenteritis. The diminution of operational capabilities and the disorganisation of the health chain may have had a greater effect if fights had been more intense [23].

Another outbreak of unusual severity has been reported in other British soldiers in Afghanistan. After a short incubation period, 29 British soldiers and staff of a field hospital became acutely ill with vomiting, diarrhoea, and fever. The first three patients presented with severe acute illness characterized by headache, neck stiffness, photophobia, obtundation, and gastrointestinal symptoms, which made the initial diagnosis elusive. The third patient's illness was complicated by disseminated intravascular coagulation. Two of these patients required ventilatory support in the field hospital's intensive care unit. This report suggests that someone who is already dehydrated due to daily activity, although otherwise be in robust health, has an increased risk of severe outcome when infected with norovirus [19].

## Options for control

Prevention of norovirus infection essentially relies on strict application of personal and collective hygiene rules: a) wash hands using soap and hot water for at least 20 seconds; b) stringent clean-up of toilets, food preparation facilities, and living quarters ; c) drink bottled water and d) eat food thoroughly cooked. Alcohol-based hand gels must not be used as they may be relatively ineffective against noroviruses [24]. Military units must avoid fresh rations during military operations until adequate hygiene measures have been fully implemented and inspected [5].

Occurrence of a norovirus gastroenteritis outbreak requires rapid implementation of measures to limit its spread. Management can be adapted from guidelines for the management of outbreaks in healthcare settings [11]. Measures revolve around enforcing the general hygiene measures outlined above and isolating, to the greatest extent possible, patients until 48 hours after all symptoms have resolved. This time period must be increased for food handlers (up to 7 days) in order to prevent norovirus transmission by food. Moreover, washroom facilities must be specified to symptomatic patients. The end of the outbreak is notified 72 hours after the disappearance of clinical signs in the last case. These measures can be applied only with difficulty to deployed troops and controlling a norovirus outbreak remains a major challenge.

## What next ?

The real norovirus gastroenteritis burden in military forces remains unclear. Although they are frequently mentioned as the cause of gastroenteritis outbreaks in troops deployed overseas, their laboratory diagnosis is rarely done. Indeed, diagnostic assays are usually unavailable in deployed microbiology laboratories and clinical specimens are rarely sent to reference laboratories; thus Kaplan's criteria are generally used in order to identify norovirus as a cause of outbreak : (1) vomiting in more than half of affected persons, (2) mean (or median) incubation period of 24 - 48 hours, (3) mean (or median) duration of illness of 12 - 60 hours, and (4) absence of bacterial pathogen in stool cultures [25]. These criteria are validated to be highly specific and reasonably sensitive in discriminating norovirus outbreaks from other aetiologies, but their use may induce an under- or over-estimation of the burden of disease for norovirus [26]. Further studies using laboratory confirmation are required to determine the full impact of norovirus gastroenteritis on deployed troops. A solution can be to deploy a Rapid Deployable Outbreak Investigation Team (RDOIT) in case of outbreak. This team has all the techniques necessary for a rapid identification of enteropathogens including noroviruses. If these tests can not be introduced in the deployed microbiology laboratories, then the use of Whatman™ FTA™ papers to sample and transport specimens to reference labs appears to be a solution of choice by avoiding storage at +4°C or –20°C (Box 1). Indeed, stool samples collected in this manner can be stored at ambient temperature for up to 11 weeks before analysis with molecular-based assays [27]. The isolation of strains during these studies would also improve our knowledge of norovirus molecular epidemiology in different parts of the world, which is a key step in the development of a vaccine.

Prevention of infection or illness by vaccination could be particularly useful. Recombinant virus-like particles expressed either in a baculovirus or transgenic plants have been shown to be safe and immunogenic when administrated orally to volunteers. However, many challenges to developing noroviruses vaccines still exist, including an incomplete understanding of the immune correlates of protection, the lack of persistent long-term and

**Sample application**

1. Dilute the stool sample 1:100 into sterile water.
2. Decant at ambient temperature for 15 min.
3. Apply 150 µL of diluted sample per sample area of FTA™ cards. To prevent cross-contamination, apply one sample per FTA™ card.
4. Dry overnight before storing FTA™ card in an envelope at ambient temperature (sample's stability: up to 11 weeks)

**Extraction**

1. Put a 6 mm disk of the FTA™ card in a tube
2. Add 140 µL of sterile water and mix thoroughly for 30 seconds.
3. Extract RNA with classical procedures.

***Box 1. Protocol for the collect, transport and extraction of stool specimens on Whatman™ FTA™ papers [27].***

cross-prospective immunity and the existence of multiple genetic and antigenic types of viruses. Given the continuing and rapid evolution of the virus, an annual process of strain selection, similar to that of the influenza virus, might be needed to match the vaccine with circulating norovirus strains [28,29]. Finally, the recognition that noroviruses bind to HBGAs has opened up an area of research looking for compounds that inhibit this binding process as possible future therapeutics [9,10].

## Conclusion

Noroviruses are now recognised as being the leading cause of gastroenteritis outbreaks. They are a military concern and norovirus outbreaks in military forces are regularly reported. The illness, which is generally mild, may be severe and life-threatening in someone who is already dehydrated due to daily activity. Moreover, outbreaks may diminish operational effectiveness, but the burden of norovirus disease for the army remains unclear. Although noroviruses are frequently mentioned as the cause of gastroenteritis outbreaks causing agents, their laboratory confirmation is rarely done. Indeed, diagnostic assays are usually unavailable in deployed microbiology laboratories and clinical specimens are rarely sent to reference laboratories. Further epidemiological studies are required to determine the full impact of norovirus gastroenteritis on troops. The isolation of strains during these studies would also improve our knowledge of norovirus molecular epidemiology in different parts of the world, which is a key step in the development of a vaccine.

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