

# Surveillance for Norovirus and Enteric Bacterial Pathogens as Etiologies of Acute Gastroenteritis at U.S. Military Recruit Training Centers, 2011–2016

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An estimated 179 million cases of acute gastroenteritis (AGE) occur each year in the U.S. and AGE is commonly reported within both training and deployed U.S. military populations. Beginning in 2011, the Operational Infectious Diseases (OID) laboratory at the Naval Health Research Center (NHRC) has undertaken routine surveillance of four U.S. military training facilities to systematically track the prevalence of AGE and to establish its etiologies among U.S. military recruits. Employing both molecular and standard microbiological techniques, NHRC OID routinely assays for pathogens of direct military relevance, including norovirus genogroups I and II, *Salmonella*, *Shigella*, and *Campylobacter*. During its initial surveillance efforts (2011–2016), NHRC OID identified norovirus as the primary etiology of both sporadic cases and outbreaks of AGE among trainees.

Acute gastroenteritis (AGE) is defined as the rapid onset of diarrheal disease, with or without accompanying symptoms, such as nausea, vomiting, fever, or abdominal pain. Annually, AGE causes 1.8 million deaths worldwide in children younger than 5 years old.<sup>1,2</sup> In the U.S. alone, despite public health advances to improve food, water, and sanitation, AGE remains a major cause of morbidity and hospitalization, accounting for more than 1.5 million outpatient visits, 200,000 hospitalizations, and 300 deaths annually.<sup>3–5</sup> Furthermore, an estimated 179 million cases occur annually in the U.S. among people who do not seek medical attention.<sup>5</sup> AGE is one of the most common ailments affecting travelers and military populations. Polymerase chain reaction (PCR), along with other diagnostic techniques, have been utilized to identify both bacterial and viral causations, with norovirus (NoV) being identified as the primary etiologic agent.<sup>6,7</sup>

Among U.S. Department of Defense (DoD) military populations and other international military units, NoV has been implicated or suspected as the causative agent in several AGE outbreaks.<sup>8,9</sup> Results of an anonymous cross-sectional survey of U.S. military personnel deployed to Southwest Asia from 2003 through 2004 found that 76.8% of personnel stationed in Iraq and 54.4% in Afghanistan had experienced at least one episode of diarrhea.<sup>6</sup> It was found that diarrhea was most often associated with time spent off the regional base and with eating local food.<sup>6</sup> Additional studies have shown that among deployed U.S. military personnel, the bacteria *Salmonella*, *Shigella*, and *Campylobacter* were responsible for 5%–17%, 3%–7%, and 6%–10% of reported cases of gastroenteritis, respectively.<sup>7</sup> NoV incidence rates have been estimated to account for approximately 3% of all diarrhea within the same population.<sup>10</sup> As a result of crowding and subsequent ease of transmission, AGE

due to NoV has been estimated to spike to over 70% during shipboard outbreaks.<sup>9</sup> These studies clearly showed that bacterial and viral etiologies, with an emphasis on *Salmonella*, *Shigella*, *Campylobacter*, and NoV have a significant impact on the morbidity, medical resources, and operational effectiveness of deployed troops.<sup>11,12</sup> Given the unpredictability and disruptive effect of infectious gastroenteritis on U.S. military training, readiness, and operational performance, AGE surveillance efforts are crucial to understanding the etiology and epidemiology of AGE within these “at-risk” populations.

The impact of these pathogens is also recognized in DoD recruit training populations. The enteric disease surveillance study implemented by the Naval Health Research Center Operational Infectious Diseases (NHRC OID) offers a unique opportunity to focus on this specific, high-risk AGE population. Recruit populations offer several advantages for studying highly communicable infectious diseases. First, recruit trainees live within a “crowded” environment (paramount for sustained transmission dynamics for infectious diseases). Second, trainees have excellent healthcare facilities at their disposal and are required to seek care when ill. Finally, military populations have demonstrated excellent research study participation and compliance (within the framework of approved institutional review board [IRB] protocols), allowing the collection of enrollment and follow-up data to be almost fully complete. For etiologic identification, NHRC OID has developed and implemented a sophisticated enteric diseases surveillance program with the capability to identify NoV and several significant bacterial pathogens in human clinical samples.

Study participation was strictly voluntary for all recruits who agreed to enroll from 12 May 2011 through 30 September 2016. Only presumptive infectious AGE patients were sought and enrolled as cases; those trainees who were deemed to have non-infectious AGE (e.g., dehydration, heat- or exercise-related AGE), were excluded from enrollment after initial evaluation.

The case definition of AGE was a trainee presenting for medical care after having experienced three or more episodes of diarrhea or vomiting in the preceding 24 hours, or a combination of episodes of diarrhea (two or more) or vomiting (two or more) accompanied by additional gastrointestinal-related symptoms (two or more), such as nausea or abdominal cramps. Since 2011, the standardized surveillance network has included Marine Corps Recruit Depot, San Diego, CA (initiated May 2011); Marine Corps Recruit Depot, Parris Island, SC (June 2011); Recruit Training Command, Great Lakes, IL (October 2011); and U.S. Army Training Center, Fort Leonard Wood, MO (April 2012).

This surveillance research was approved by the NHRC IRB in 2011. Following consent and enrollment, all enrolled trainees with AGE were asked to provide a stool sample. Stool samples were preserved in Cary-Blair transport medium and Campy-thioglycollate medium. Recruits unable to provide a stool specimen were asked to self-administer two rectal swabs, which were preserved in universal transport medium (UTM) and Cary-Blair medium. All inoculated media and remaining unpreserved stool were stored at 4°C. Collected specimens were shipped to the NHRC OID laboratory under refrigerated conditions on a weekly basis.

Each training site had a dedicated NHRC OID research assistant to conduct surveillance. Research assistants normally collected up to 10 specimens per week but could have collected up to three specimens per day during an AGE outbreak. An AGE outbreak was defined as an “unusual” (relative to weekly averages) number of recruits with AGE symptoms clustered by both time

and place. More practically, an outbreak for the purposes of surveillance was defined as two or more associated cases of diarrhea and/or vomiting within a 24-hour period.

The NHRC OID laboratory is accredited by the College of American Pathologists and the DoD Clinical Laboratory Improvement Program. Additionally, NHRC OID has been certified to participate in the CaliciNet National Norovirus Outbreak Network. The Centers for Disease Control and Prevention (CDC) launched CaliciNet in 2009 to collect information on NoV strains associated with gastroenteritis outbreaks in the U.S. Participation is limited to federal, state, and local public health laboratories in the U.S. The NHRC OID laboratory is currently the only DoD laboratory participating in the program and has been certified to do so since 2011.<sup>13</sup>

### Data Management

The NHRC OID laboratory maintains a relational database containing demographic and epidemiologic information collected from case report forms as well as laboratory results. Summary statistics are frequently generated on all relevant variables, including demographics, symptoms, disposition, and functional outcomes. Enrollment and specimen collection may occur on a daily basis at each field site; laboratory testing occurs at least once per week.

### Laboratory Processing

#### *Detection of Salmonella, Shigella, and Campylobacter*

Stool specimen swabs received in Cary-Blair transport media were streaked onto tryptic soy agar with 5% sheep blood, MacConkey media, and xylose lysine deoxycholate agar plates. Inoculated plates were incubated for a total of 48 hours at 37°C, with primary analysis at 24 hours. Suspected *Salmonella* and *Shigella* colonies were isolated and identified using the BD Phoenix Automated Microbiology System (Becton Dickinson).

Swabs received in Campy-thioglycollate media were streaked onto Campy-CVA agar plates and incubated under microaerophilic conditions for 72 hours at 42°C. Suspected *Campylobacter* colonies were

isolated and identified at the genus level by the following characteristics: cellular morphology, reaction on Gram staining, oxidase, and hippurate analysis. Single-swab samples received in Cary-Blair media were used to streak all of the aforementioned plates. Incubation times, temperatures, and subsequent analyses were identical to the procedure outlined above.

#### *Detection and characterization of NoV genogroups GI/II*

RNA was extracted from 20% saline suspensions of stool or directly from UTM using the QIAamp® Viral RNA Mini Kit (Qiagen Inc., Valencia, CA), according to the manufacturer’s instructions. Bacteriophage MS2 virus (ZeptoMetrix) was added to the fecal suspension prior to extraction. Eluted RNA extractions were assayed for the presence of norovirus using a multiplex real-time RT-PCR assay developed by CDC for simultaneous detection of human NoV GI/GII, as well as an MS2 virus internal amplification control.<sup>13</sup> Strain identification was performed in accordance with CDC protocols and uploaded to the CaliciNet surveillance database.<sup>13</sup>

## RESULTS

During the 5-year surveillance period, approximately 3% of all recruits across the four training sites were diagnosed with AGE by a clinician. Although 1,940 subjects provided informed consent and were enrolled, four enrollees failed to provide samples, leaving 1,936 samples collected and submitted to the NHRC OID laboratory for analysis (**Table**). Among the population experiencing AGE, rates of consent to participate in surveillance and contribution of research samples varied between sites. The highest average rate of participation among those approached to enroll was seen at Marine Corps Recruit Depot San Diego (47%), the lowest at Fort Leonard Wood (22%), though variation was seen across years at all sites. A minor seasonal trend was observed across all sites, with the highest participation occurring during winter months, suggestive of a positive association with AGE activity. Etiologic agents were

**TABLE.** Cumulative acute gastroenteritis (AGE) surveillance data, by recruit training site, May 2011–September 2016

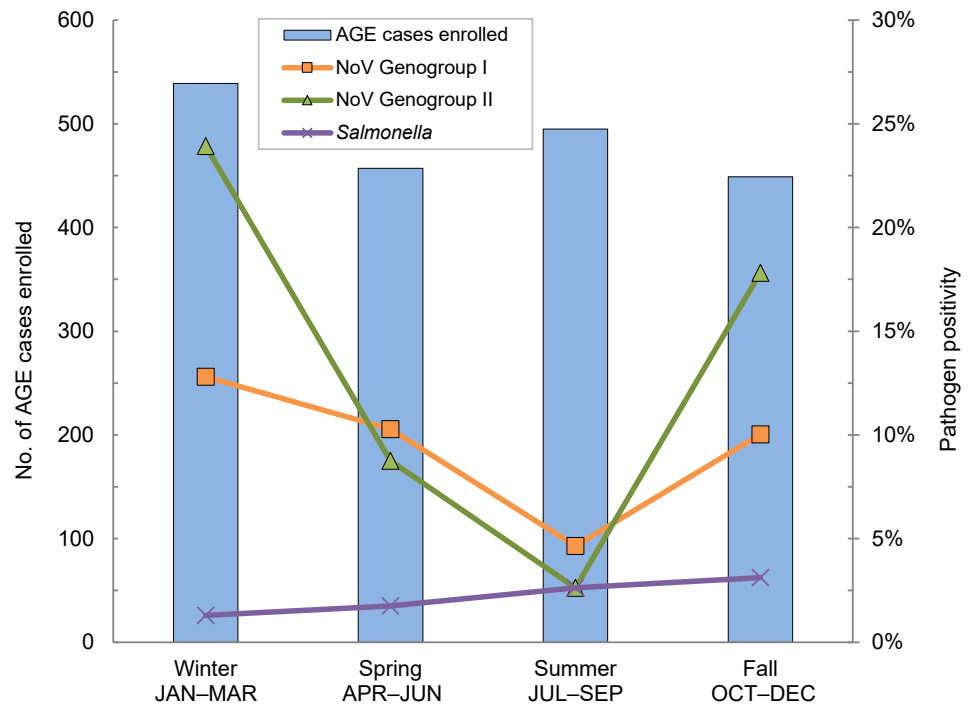
Recruit training site	Total cases of clinician-diagnosed AGE	Total no. of recruits on site	No. of cases approached to enroll	No. of samples collected	Participation rate (%)	No. of norovirus positive	No. of <i>Salmonella</i> positive	No. of <i>Shigella</i> positive	No. of <i>Campylobacter</i> positive
Recruit Training Command, Great Lakes, IL	6,940	198,279	2,283	458	20.1	75	1	0	2
Marine Corps Recruit Depot, San Diego, CA	2,365	84,796	1,061	502	47.3	102	25	0	0
Marine Corps Recruit Depot, Parris Island, SC	2,605	95,703	1,411	427	30.3	121	13	0	0
U.S. Army Training Center, Fort Leonard Wood, MO	2,604	97,331	2,442	549	22.5	148	3	1	2
Totals	14,514	476,109	7,197	1,936	26.9	446	42	1	4

identified in 491 (25%) samples. NoV GI/II accounted for 90% of all positive testing results. Bacterial agents (*Salmonella*, *Shigella*, and *Campylobacter*) accounted for the remaining 10% (Table).

Self-administered rectal swabs accounts for approximately 40% of all specimens collected during the surveillance period. Of those, only 15.6% of all swabs collected yielded a pathogen-positive result, compared to 32.5% of all stool samples tested (data not shown). All four *Campylobacter* were isolated from stool, while 10 times as many *Salmonellae* were isolated from stool as from rectal swabs, and three times as many NoV were detected from stool as from rectal swabs (data not shown). Although comparable numbers of samples (449–539) were cumulatively collected from trainees during all seasons of the year, sporadic NoV cases and outbreaks were most prevalent during the fall (28%) and winter (37%) versus the spring (19%) and summer (8%) months (Figure 1). *Salmonella* was identified at a lower, consistent rate across total enrollments, regardless of the season (Figure 1). NoV GI was most commonly identified in specimens collected during 2011, while NoV GII was the predominant genotype in circulation among all recruit training sites in 2012–2015 (Figure 2).

As of 30 September 2016, a total of 18 NoV GI- and 26 NoV GII-associated outbreaks had been identified across all sites since surveillance began in 2011 (Figure 3).

**FIGURE 1.** Cumulative numbers of acute gastroenteritis (AGE) cases enrolled<sup>a</sup> and percentage distribution of pathogens identified among U.S. military recruits, all sites, by season, May 2011–September 2016



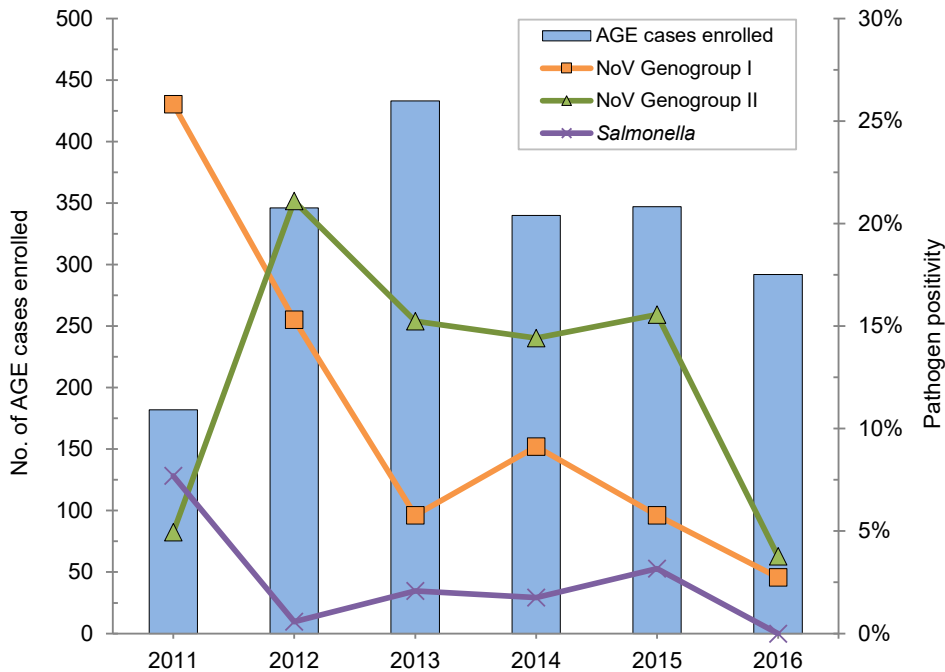
<sup>a</sup>Four enrollees failed to provide samples.

#### EDITORIAL COMMENT

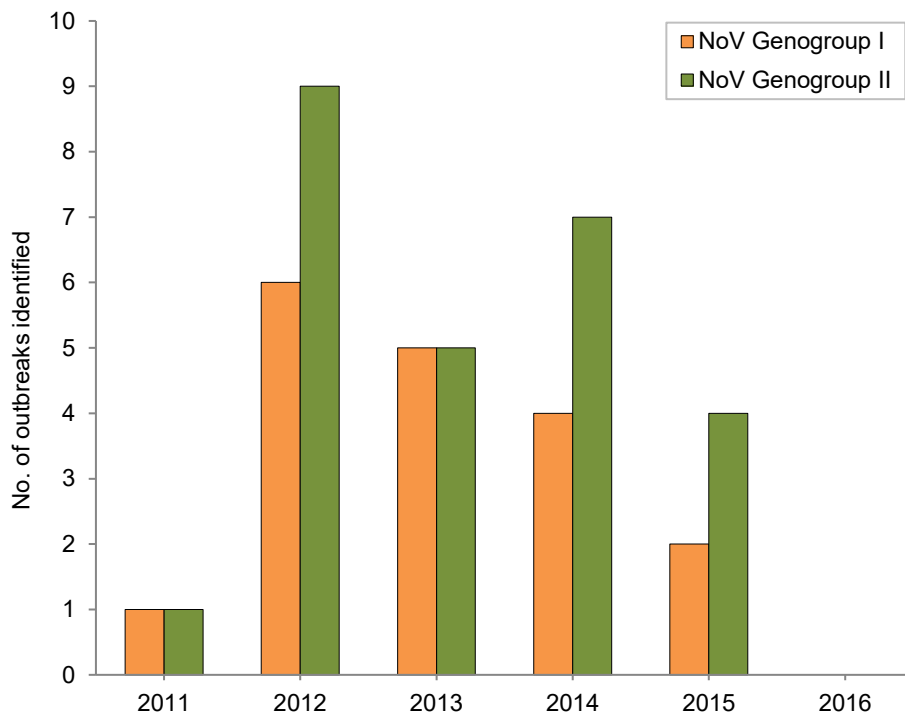
The enteric disease surveillance program was established to describe the epidemiology of AGE and NoV among U.S. DoD military recruits. Historically, most AGE

outbreaks have been attributed to bacterial pathogens. However, surveillance data spanning from 2005 to 2012 have identified viral agents as the most common etiology in AGE outbreaks in military operational settings, with NoV the most commonly identified.<sup>10</sup> Surveillance data collected by

**FIGURE 2.** Numbers of acute gastroenteritis (AGE) cases enrolled and percentage distribution of pathogens identified among U.S. military recruits, all sites, by year, May 2011–September 2016



**FIGURE 3.** Numbers of norovirus outbreaks<sup>a</sup> identified by Naval Health Research Center Operational Infectious Diseases among U.S. military recruits, all sites, May 2011–September 2016



<sup>a</sup>Norovirus genogroup and genotype were reported to the CaliciNet national surveillance database per Centers for Disease Control and Prevention protocol.

NHRC OID support these findings. In this study, the observed trends relating to NoV seasonality, outbreak trends, and prevalence of GII circulation correspond to what has been previously described in the literature.<sup>14,15</sup> The reason for the dramatic drop in NoV burden in 2016 is unknown.

Among the enrollees with AGE studied in this report, etiologic agents were not identified by routine surveillance testing in approximately 75% of the total collected specimens. This pathogen recovery rate is low, compared with previous studies in which causative organisms were identified in approximately 45%–50% of all symptomatic cases of AGE.<sup>16,17</sup> The dissimilarity in NHRC OID’s findings during the 2011–2016 surveillance period could be attributed to a variety of factors, including the study population, specimen type, testing methodologies, and pathogenic targets. The cited studies focus on long-term traveler and/or deployed military populations. Recruit populations are generally restricted to their respective facilities for the duration of their training and are not exposed to as many sanitation- and poverty-related AGE risk factors as the populations monitored in cited studies. Additionally, all trainees enrolled in the study met the required AGE case definition. The fact that such a high percentage were found to be pathogen-negative by the current methodologies could be indicative of the need to minimize the collection of self-administered rectal swabs and focus primarily on collecting and testing stool samples, the need for improved recovery methods, and/or the need for more sensitive detection techniques. Finally, low pathogen recovery rates could be due to the choice of bacterial and viral agents targeted in these initial efforts. More extensive testing of these samples would be required to determine whether other bacterial, viral, or parasitic pathogens are more prevalent than the currently targeted organisms.

Since its inception in 2011, NHRC OID’s enteric disease surveillance program’s initial surveillance efforts have succeeded in establishing a standardized method for tracking the incidence of, and determining common etiologies of, AGE among U.S. military recruits. Ultimately, data derived from this program have the potential to facilitate the development of

more targeted and effective AGE prevention and/or intervention policies and programs. Such policies and programs could help decrease the impact and burden of infectious gastrointestinal disease not only on military recruits and operational forces but on the general U.S. population as well.

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