Gastroenteritis in US Marines during Operation Iraqi Freedom

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Background. Approximately 83,000 US Marines participated in the opening phase of Operation Iraqi Freedom in Spring 2003. A Navy Preventive Medicine laboratory was set up in Ad Diwaniyah, Iraq, to provide clinical diagnostic support for Marine medical units during a period of repositioning in south-central Iraq.

Methods. Specimen collection boxes were sent to >30 primary care medical stations handling 500–900 personnel each. The laboratory had capability to detect many different disease agents, especially those causing febrile illness. Diarrheal stool diagnostic evaluation included plating and biochemical identification, antigen serologic testing, fluorescent antibody antigen detection, disk diffusion antimicrobial susceptibility testing, enzyme immunoassay, and reverse-transcribease polymerase chain reaction for norovirus (NV). Confirmation and sequencing work for NV was done at Cincinnati Children’s Hospital Medical Center (Ohio).

Results. By far the most common reason for infectious disease sick call visits was gastrointestinal illness; no other symptoms had equivalent impact. An enteropathogen was detected in 57 (44%) of 129 stool samples, with NV detected in 30 stool samples (23%) obtained from 14 different battalion or similar-sized units; next in frequency were Shigella flexneri and Shigella sonnei, which were isolated from 26 stool samples (20%) obtained from 15 units. Sequencing the NV RNA polymerase gene demonstrated that NV strains represented 7 genetic clusters, including 2 strains from genogroup I and 5 from genogroup II. Ciprofloxacin was effective in vitro against most bacterial agents, but neither doxycycline (which was taken daily as the antimalarial prophylaxis dose) nor trimethoprim-sulfamethoxazole were effective.

Conclusions. Multiple strains of Shigella species and NV predominated, probably because they do not require a large inoculum to cause infection. Otherwise, personnel remained free of infectious illness during this phase of the conflict, because other infectious agents were rare or absent.

Infectious diseases have long been problematic to deployed US military ground forces. During recent operations in the Middle East and Somalia, food and water sources were tightly controlled, and medical intervention measures, such as administration of vaccines and antimicrobial prophylaxis regimens, have been successfully implemented to reduce disease incidence. When such measures are not followed, even the preventable diseases again become an issue [1–3]. Iraq has many of the diarrheal disease agents typically found in the Middle East, including Vibrio cholerae in some areas. Norovirus (NV), a common cause of viral gastroenteritis outbreaks in military units, was undocumented in Iraq but was presumably present, because it was a cause of outbreaks of infection in the military in nearby Saudi Arabia during the 1991 Gulf War [4, 5]. Other disease threats endemic in Iraq include malaria (mostly due to Plasmodium vivax), arboviruses, brucellosis, leishmaniasis, leptospirosis, infection with rickettsial agents, schistosomiasis, Q fever, hepatitis A and B, and typhoid fever [6, 7].

On 20 March 2003, Operation Iraqi Freedom commenced, with US and allied military entering Iraq. The ground component included ~83,000 Marines of the First Marine Expeditionary Force. The Marines quickly
moved north through the Tigris-Euphrates floodplain to Bagh- dad and Tikrit, but during the time period covered in this ar- ticle (24 April through 1 June 2003), they were redeploying south of the capital. Marines generally chose to sleep in open buildings, without electricity or windows and/or screens. The weather was rapidly warming up to 40°C, with occasional dust storms. Sanitation conditions were poor, because camps were not well established and were often overcrowded. Unscreened burn-barrel shelters were the most common latrine models, and male urination was handled by use of pipes inverted into sand pits. Hand washing was encouraged with placement of water bags (Lister bags) and waterless alcoholic gel dispensers near food facilities and latrines. Food came in prepackaged individual meals, later replaced by precooked bulk trays, which were all brought from the United States. Filth flies were abundant both in the latrines and dining facilities. Drinking water was from reverse-osmosis– and chlorine-treated river water or regionally imported bottled water only. Personnel were not allowed to acquire food in town, although this rule was broken more than once, usually for grilled chickens.

Three Navy Preventive Medicine–Mobile Medical Augmen- tation Readiness Teams (PM-MMARTs) were deployed to sup- port Operation Iraqi Freedom. Part of their mission was to determine the risks and causes of infectious diseases, using surveillance and a clinical diagnostic laboratory to directly de- tect which agents were causing illness. PM-MMART 5 was set up in the central Iraqi city of Ad Diwaniyah, which is ~175 km south of Baghdad. This location was ideal to support the ~30 First Marine Expeditionary Force battalions, each with >900 personnel, which were located in cities of central Iraq. Although the laboratory had the capability and mission to iden- tify many infectious disease pathogens, including traditional agents of biological warfare, this article focuses on cases of gastroenteritis, because those represented by far the major dis- ease impact on the troops.

MATERIALS AND METHODS

Specimen collection. PM-MMART 5 arrived in Ad Diwaniyah on 21 April 2003 and set up at an abandoned university campus. Within a few days, it began dispersing boxes containing col- lection and transport supplies for stool, blood, and other clinical specimens to 30 First Marine Expeditionary Force Battalion Aid Stations or other medical stations in the area. (In this article, “units” are defined as battalions or similar groups.) This included all units located from north of Nasiriyah to the south- ern outskirts of Baghdad. Specimens were collected by First Marine Expeditionary Force Battalion Aid Station personnel and stored at room temperature until transportation was ar- ranged to get them to PM-MMART 5, usually ≤24 h later. Acute-phase blood specimens were to be collected from all febrile patients, with fever defined as a temperature of ≥38.3°C (∼101°F) measured sublingually; convalescent-phase blood specimens were to be collected from febrile patients for whom no enteric pathogens were identified. Stool samples were col- lected from any patient with diarrhea, regardless of whether fever was present. If the First Marine Expeditionary Force Bat- talion Aid Station location was >1 h distant from Diwaniyah, then stool samples were divided into 3 transport vials (Ecofix, Enteric Plus, and a clean vial [Meridian Diagnostics]), whereas local First Marine Expeditionary Force Battalion Aid Station specimens were brought directly to the laboratory in the original collection cup. No other medical data were collected from the patients, although PM-MMART 5 did collect Disease and Non-Battle Injury reports from Diwaniyah-area units. All First Marine Expeditionary Force personnel were ordered to take doxycycline (100 mg q.d.) as prophylaxis against malaria.

Stool specimen diagnostic evaluation. All stool samples were cultured on MacConkey and XLD (xylose lactose de- oxycholate) agar plates. Samples of watery stool were also plated on thiosulfate-citrate–bile salts–sucrose agar, and samples of stool with gross blood or fecal leukocytes (as determined by direct methylene blue stain microscopy) were plated on Sorbitol-MacConkey agar to screen for enterohemorrhagic Escherichia coli and on sheep blood agar to screen for Campylobacter species with use of a membrane-filter swim-through procedure [8]. Lactose nonfermenters were screened with tri- ple-sugar iron agar and motility-indole-lysine biochemical agar tubes and were speciated using API 20E (bioMérieux). Sal- monella and Shigella isolates were confirmed with Wellcolex latex serology kits (Oxoid). For 4 patients, suspected E. coli colonies were tested for heat-labile enterotoxin using the Ox- oid VET-RPLA kit and for heat-stable enterotoxin using Oxoid St-EIA. Giardia and Cryptosporidium species were identified with a Merifluor fluorescent antibody kit (Meridian) and direct methylate-iodine-formalin stain (Meridian). NV was identi- fied by use of an RT-PCR assay (Robust RT-PCR kit; MJ Re- search) and 289/290 primers, as well as by use of an antigen-capture EIA [9, 10]. Both assays include specific reagents developed by the reference laboratory at Cincinnati Children’s Hospital Medical Center (Ohio). Bacterial isolates were tested against commonly deployed antimicrobial agents with use of the disk diffusion assay on Mueller-Hinton Agar.

NV strain characterization. After they were returned to San Diego, stool samples were shipped on dry ice to one of the authors (X.J.) at Cincinnati Children’s Hospital Medical Center for confirmation and further strain characterization. A modified degenerate primer set in the same region of the 2 original primers that contained 2 reverse primers (289H and 289I) and 4 forward primers (290H, 290I, 290J, and 290K) was used, which also produced a 319-bp product [11]. Sequences of individual NV strains were determined by direct sequencing of the RT-PCR products following purification of the amplicons.
from agarose gels after electrophoresis analysis. Sequences of some strains also were determined after cloning of the RT-PCR products if the DNA bands were weak in the gels. In brief, the DNA products were cloned into the pGEM-T vector (Promega), in accordance with the manufacturer’s protocol. Positive clones were identified by PCR screening. The cloned cDNA was sequenced using M13 forward and reverse primers by the chain termination method on an ABI PRISM 3700 capillary sequencer (Applied Biosystems). All clones were sequenced at least twice.

The following sequences, published in GenBank, were used in phylogenetic analysis based on that of Vinje et al. [12]: genogroup I, Chiba (AB042808) GI/4, Desert Storm GI/3, and Norwalk (M87661) GI/1; genogroup II, Hawaii (U07611) GII/1, Lordsdale (X86557) GII/4, Melksham (X81879) GII/2, Mex7076 (AY579431), Saitama U25 (AB039780) GII/8, and VA207 (AY038599) GII/9. Saitama U25 was used for GII/8 because RNA polymerase data for Amsterdam are not available from public databases. Mex7076 has been characterized at Cincinnati Children’s Hospital Medical Center, and according to the phylogenetic analysis of the whole capsid sequences, it represents a new genetic cluster within genogroup II NVs (unpublished data). Multiple alignments of nucleotide sequences were created using Omiga software, version 2.0 (Oxford Molecular). Aligned sequences were edited in GeneDoc, version 2.5, obtaining a 210-bp consensus length [13]. Distance trees were constructed by the UPGMA clustering method of Molecular Evolutionary Genetics Analysis, version 2.1, with Jukes-Cantor distance calculations [14]. The confidence values of the internal nodes were obtained by performing 125 bootstrap analyses.

RESULTS

Collection of specimens and clinical data. The PM-MMART laboratory received its first specimen on 24 April 2003, and a total of 129 stool samples were collected, representing 33 units scattered across south-central Iraq. Clinical information was sparse, because no units made log entries during the major combat phase; however, discussions with medical care givers suggested that a viral etiology for gastroenteritis predominated during the early part of the conflict, with large outbreaks of nausea, vomiting, and diarrhea that lasted 24–48 h. These began shortly after the fall of Baghdad on 9 April and continued through the first weeks of May, when cases of febrile dysentery prevailed. Most units were in little or no contact with each other during this period. Some were front-line fighting units, whereas others were support units that arrived in Iraq at approximately the same time as PM-MMART 5.

Disease etiology and location. An enteropathogen was detected in 57 (44%) of 129 stool samples. NV was detected by RT-PCR in 30 stool samples (23%) obtained from patients in 14 units (figure 1), and either Shigella sonnei or Shigella flexneri was isolated from 26 stool samples (20%) from 15 units; in only 1 unit were both Shigella species found (table 1). All 5 stool samples that yielded Campylobacter isolates were from different units. A total of 43 of 109 stool samples had fecal leukocytes present, indicating inflammatory diarrhea. Among the stool specimens with an agent identified, 24 were positive for NV only, 22 were positive for Shigella only, and 4 were positive for another single agent (including a case of Cryptosporidium infection). The other cases included 6 mixed infections with NV and either Shigella or Campylobacter species and 1 case in which a single Salmonella arizonae colony mixed with abundant Campylobacter species. Although NV-positive stool samples were collected up through the last week of May, the number of such samples had decreased from the earlier periods.

Antimicrobial susceptibility testing. Doxycycline resistance was found in all but 1 Shigella isolate, although the number of doxycycline-susceptible Campylobacter strains may indicate that compliance with antimalarial therapy prevented many more cases of Campylobacter infection. Resistance to trimethoprim-sulfamethoxazole was similarly common. On the other hand,
all *Shigella* species were susceptible to ciprofloxacin, whereas all *Campylobacter* species were resistant to this agent. All 3 cephalosporins—especially ceftriaxone—inhibited the bacterial enteric pathogens isolated, except for *Campylobacter* species.

**NV strain comparison.** NV strains were compared by sequencing the RNA polymerase genes of 21 strains, which could be grouped into 2 genogroup I (GI) and 5 genogroup II (GII) genetic clusters on the basis of sequences of known strains (figure 2). This is not a definitive classification of strains and clusters, because it is based on a fairly short sequence. Three strains (031952, 031967, and 031973) had nucleotide sequence identities of 89%–100% with each other and 89%–97% with Saitama U25. Three strains (031931, 032040, and 032041) that had a 99% identity with each other had a 90% identity with Mex706, which is a new cluster representative within GI1. Two strains (031933 and 031966) that had a 98% identity with each other had a 90% identity with VA207; 3 strains (031948, 032000, and 032046) that had a 96%–98% identity with each other had 91%–93% identity with Melksham. Three strains (031945, 031962, and 031998) that had 96%–100% identity with each other had an equal 83% identity with the Hawaii and Lordsdale viruses and 91%–94% identity with a Spanish GIIb strain (AJ487789). One strain (032036) had 80% identity with the Desert Shield virus (GI/3), and 6 strains (031944, 031951, 031955, 031957, 031980, and 032047) that had 95%–100% identity with each other had 87%–93% homology to the Chiba virus (GI/4).

The occurrence of NV strains, by unit and timing. Military units were arbitrarily designated 1–14; units 12–14 did not have a strain sequenced from their single specimen. One strain (032040) did not have a unit identifier with the specimen. Cluster GI/4 contained 5 strains (4 of which were from the same battalion) collected in late April 2003 and 1 strain (from a different unit) collected in late May. Units 2 and 3 had 2 different strains detected at the same time, but most units had only 1 strain detected during the same 2- or 3-week period. On the other hand, all but 1 strain were found in multiple units, with GI/3 being detected from only 1 specimen. According to pairwise distance analysis, the intrACLuster distances for all of the strains on the distance tree ranged between 0 and 0.088. The intercluster distance was referred by 2 well-characterized genetic cluster representative strains, Hawaii and Lordsdale, and was 0.112. Strains 031967 and 032036 separated from any of the strains with greater distances than that between Hawaii and Lordsdale (>0.118 and >0.195, respectively). Future study is necessary on the capsid sequences to decide whether these 2 strains represent new genetic clusters.

**DISCUSSION**

The results for First Marine Expeditionary Force reinforce experience elsewhere; despite strict control of food and water sources, certain enteropathogens that have very low inoculum requirements (e.g., *Shigella* species and NV) caused a significant number of cases in a highly unsanitary environment. Filth flies may account for part of their spread, along with person-to-person contact. *Shigella* species was the most common enteropathogen in First Marine Expeditionary Force personnel in Somalia in 1992–1993, where lack of access to local sources of food and water kept exposure to other causes of traveler’s diarrhea low, and NV testing was not applied there. For those agents that require a larger inoculum, heat-labile and heat-stable enterotoxins were not found at all, whereas *Salmonella* and *Campylobacter* were rare and probably linked to roast chicken meals acquired in town. As expected, most of the isolates encountered were resistant to doxycycline, so the number of enteric cases prevented by daily doxycycline antimalarial prophylaxis is not known. Infection with other drug-susceptible pathogens, such as *Leptospira* species, may also have been prevented by doxycycline therapy. Ciprofloxacin, another common

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**Table 1.** Major findings from 129 stool specimens obtained from soldiers in Operation Iraqi Freedom and percentage of bacterial isolates resistant to antimicrobial agents, as determined by disk diffusion assay.

<table>
<thead>
<tr>
<th>Agent</th>
<th>No. of agent-positive patients</th>
<th>No. of units with agent-positive patients</th>
<th>Percentage of drug-resistant isolates, by drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Doxycycline</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>13</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>13</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td><strong>Campylobacter species</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td><strong>Other bacteria</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>All bacteria</td>
<td>35</td>
<td>26</td>
<td>86</td>
</tr>
<tr>
<td>Norovirus</td>
<td>30</td>
<td>14</td>
<td>NA</td>
</tr>
</tbody>
</table>

**NOTE.** Doxycycline, 30 μg in disk; ciprofloxacin, 5 μg; trimethoprim-sulfamethoxazole (TMP-SMZ), 1.25 μg trimethoprim; cefoxitin, 30 μg; cefazolin, 30 μg; and ceftriaxone, 30 μg. NA, not applicable.

<sup>a</sup> Fifty-six stool samples were tested for *Campylobacter* species.

<sup>b</sup> Putative enteroinvasive *Escherichia coli*, 2 cases; *Salmonella* arizonae, 1 case; and *Plesiomonas* shigelloides, 1 case.
Figure 2. Distance tree of norovirus polymerase gene sequences detected in the US First Marine Expeditionary Force in Iraq, April through May 2003. Strains are listed by identification numbers, followed by arbitrary numbers for military units of the strains isolated, with the question mark indicating that the unit was not recorded. The reference strains included in the comparison are in bold.

antimicrobial used empirically by First Marine Expeditionary Force Battalion Aid Station personnel, was highly effective in vitro against all but the *Campylobacter* isolates. Enteropathogens were identified in dozens of separate First Marine Expeditionary Force units. Unlike during the Persian Gulf War, when a few strains of *S. sonnei* caused large outbreaks of infection in military dining facilities, in Iraq, there was no area-wide occurrence of infection with any 1 strain, although the relatively small units described in this article tended to have the same species of *Shigella* strain isolated. Characterization of the *Shigella* isolates is currently underway elsewhere.

NV was shown to be a cause of morbidity in US personnel in Operation Iraqi Freedom, just as it is in US sailors and marines worldwide. Since 1999, we have documented 24 outbreaks of viral gastroenteritis on the largest US Navy ships and a dozen more on numerous smaller vessels and in shore units, and we have confirmed that NV was responsible in almost every incident in which specimens were submitted for analysis (S.A.T., unpublished data) [15]. Even with weak or anecdotal data, a conservative estimate would be that thousands of cases of NV illness occurred in the First Marine Expeditionary Force in April through May 2004. Although the symptoms are short-lived, the effects of an outbreak can affect a unit undergoing the physical demands of near-continuous combat. Confirmation of NV in these outbreaks can ease psychological worries about chemical and biological agent use, which was expected on a daily basis during this time. An outbreak in a coalition military hospital in Bagram, Afghanistan, in 2002 caused con-
cern about such an attack, because laboratory confirmation of NV was not possible in theater [16]. The present report probably represents the first confirmation of NV gastroenteritis in a wartime theater using a field laboratory. Even when an outbreak of nausea, vomiting, and/or diarrhea is presumed to be due to NV, there are problems in containing spread of the virus, especially in the environment faced by the troops in Iraq.

It is not surprising that several distinct NV strains were detected, because the units had little contact with each other during their movements. Units were often affected by multiple strains during this period, and multiple strains coexisted in a few units. Three amphibious ships reported outbreaks of viral gastroenteritis shortly after re-embarking Marines to return to the United States in late May 2003 (S.A.T., unpublished data). Although none of the subjects had specimens collected, and although the units were not the same as those reported above, it would appear that the Marines brought the strains aboard the ships. Viral gastroenteritis in Iraq has not gone away: an outbreak affected Dutch troops patrolling the southern border of Iraq in July 2003 [17], as well as US Marines in central Iraq in June 2004 (S.S.S., personal communication). Although this is probably the first recorded confirmation of NV in Iraq, that virus would seem to be ubiquitous in the country, with several strains that included both major genogroups. No specimens were collected from Iraqis to characterize strains from the civilian population. In the United States, GI strains predominate, and one such strain, Farmington Hills, was detected in 41% of 27 outbreaks nationwide and on cruise ships [18]. One of the genetic clusters detected in this article, GGIIb, has been reported as the emerging genotype in Europe [19]. In our experience, US Navy ships that experience outbreaks of NV gastroenteritis after visiting Asian ports are more likely to have GI strains.

Although the PM-MMARTs were deployed to Operation Iraqi Freedom primarily because of the perceived threat of chemical and biological warfare agents, infectious gastroenteritis proved to be a larger health concern. PM-MMART 5 demonstrated the value of having a clinical infectious disease/public health surveillance laboratory in close proximity to the echelon of care normally given to these cases, so results of etiology and drug susceptibility studies could be useful in implementing countermeasures in a timely manner. The laboratory was equipped to identify etiologic agents of many diseases besides gastroenteritis; however, there were very few patients who presented to the aid stations whose cases did not involve enteric symptoms as the major complaint. The lack of solid denominator data and attack rates during the major maneuver campaign were the result of the inability to be in constant touch with the various units, which also had priorities other than patient data entry. Once the units settled into garrison camps and began reporting surveillance data, they indicated overall illness attack rates were higher than background levels previously recorded in their US home garrisons. Aid stations are not supplied for collection of clinical specimens, so the laboratory must be further equipped to send supply boxes to them. The mission of the PM-MMART is to act as a public health laboratory, to gather laboratory-based evidence about which pathogens are prevalent, and to recommend possible interventions, but not to provide a clinical service to every patient in the theater. A better method is needed to collect accurate data on disease incidence in military personnel during maneuver warfare.

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