Case series study of travelers’ diarrhea in U.S. military personnel at Incirlik Air Base, Turkey

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Abstract

Military personnel with travelers’ diarrhea (n=202) while deployed to Incirlik, Turkey from June to September 2002 were evaluated for pathogen-specific immune responses. Serologic and fecal IgA titers to ETEC antigens (CS6, CS3 and LT) were quite low. In contrast, subjects with Campylobacter had high serologic and fecal IgA responses.
Introduction

Acute infectious diarrhea affects up to 60% of short-term international travelers and is one of the most common medical problems for military troops deployed abroad (23, 24, 26). Recent estimates of diarrheal incidence in troops in Afghanistan and Iraq have been as high as 49% per month. (21, 28, 32). Despite safe and effective treatment regimens (27, 33), most military personnel with diarrhea do not seek care (21, 23, 32), suggesting that primary prevention is important in mitigating disease burden. Additionally, since widespread chemoprophylaxis is not recommended (2, 3, 10), and given the rapid development of antibiotic resistance, vaccine development is an important long-term strategy in reducing the diarrhea burden among deployed U.S. military personnel. To support vaccine development, increased understanding of immune responses in infected personnel is needed. A prospective case-series study was conducted at a U.S. Air Force Air Base in Incirlik, Turkey to characterize immune responses in subjects presenting with diarrheal symptoms.
Materials and Methods

A case-series study was conducted at Incirlik Air Base, located in southeastern Turkey (1). During the study period (June to September 2002), all on-base US military personnel or their adult dependents reporting for medical care due to diarrhea were eligible to enroll. Following informed consent, participants underwent a standard clinical evaluation and provided baseline blood and stool samples. Subjects returned to the clinic to provide stool and blood samples 3, 7, 14 and 28 days after enrollment.

Stools were cultured using standard procedures for the isolation and identification of common enteric bacterial species causing diarrhea. Hippurate hydrolysis was used to differentiate Campylobacter isolates into C. jejuni and non-C. jejuni (Hardy Diagnostics, Santa Maria, CA). As previously reported, the GM1 enzyme-linked immunosorbent assay (GM1-ELISA) and a competitive inhibition ELISA were utilized to identify the heat labile (LT) and the heat stable (ST) toxins of enterotoxigenic Escherichia coli (ETEC) (25, 31). Toxin-expressing E. coli colonies were characterized for the presence of surface colonization factors using an immunodot blot method employing monoclonal antibodies against the CFs (6, 34). Enzyme immunoassays (EIA) were used to evaluate stool samples for rotavirus (Rotaclone, Meridian Diagnostics, Inc., Cincinnati, OH) norovirus (15), Cryptosporidium, Giardia, and Entamoeba histolytica (29). Additional ova and parasite screening was performed by light microscopy.

Immunology assays were performed on a subset of subjects based on sample availability. For Campylobacter, IgA and IgG levels to a glycine extract (GE) antigen of C. jejuni strain 81-176 were determined by ELISA (4, 5). Similarly for ETEC, serologic responses to the B subunit of the native heat-labile toxin (LT) and colonization factors
(CF) CS3 and CS6 (chosen based on previous epidemiological studies) were evaluated by ELISA (11, 14, 16, 19, 30). The antibody titers represented the geometric mean of duplicate determinations on different days. Reciprocal endpoint titers < 5 were assigned a value of 2.5 for computational purposes. Stool samples were aliquoted and frozen at -70°C at the U.S. Air Force hospital at the Incirlik Air Base. All specimens were shipped to the NAMRU-3 laboratory processing and secretory IgA determination (17, 18). Immune response was defined as a ≥ 4-fold rise over baseline titer. Immunology data were compared using a repeated measures analysis of variance with infection as the between subject factor (i.e., Campylobacter, CS6 ETEC, CS3 ETEC and LT ETEC) and sample collection time-points as the repeated factor. Statistical analyses were conducted with SAS version 8.2 for Windows (SAS Institute, Inc., Cary, North Carolina) using a two-tailed alpha of 0.05.
Results

Initial presentation. A total of 202 subjects met the inclusion criteria and were enrolled. The majority were male (89%), Caucasian (76%), enlisted personnel (83%) on deployment for either Operation Northern Watch (73%) or Operation Enduring Freedom (17%). Pathogens were identified in 53% (n=108) of cases with ETEC (n=82) and Campylobacter (n=25; including 5 ETEC co-infections) being the most common. The most common ETEC toxin type was ST (76%) followed by LT (13%) and LTST (11%). CS6 was the predominant colonization factor (40%) followed by CS1CS3 (20%). Multiple ETEC phenotypes were identified from 6 (7%) subjects with ETEC infections. No CF was detected in 17%.

Immune response. Serologic responses to glycine extract of C. jejuni strain 81-176 were more common in subjects with Campylobacter (IgG = 56%; IgA = 72%; IgM = 72%) than in those without (IgG = 4%; IgA = 7%; IgM = 5%) (all p<0.001). Peak responses were observed 14 days after initial presentation and IgG persisted through day 28 (Figure 1). Similarly, fecal IgA (secretory IgA) responses peaked on day 14 and were significantly higher (p<0.001) in Campylobacter cases (Geometric Mean Titer (GMT) = 1,281) than non-cases (GMT = 16).

ETEC anti-CF (CS3 and CS6) serologic responses (IgA and IgG) were more common in subjects with homologous CF-expressing ETEC compared to those without. Serologic titers (IgG and IgA) increased significantly over baseline titers (repeated measures p<0.001) with peak IgA titers on day 7 and IgG titers increasing through day 28 (Figures 2 and 3). Anti-CF fecal IgA, anti-CF serologic IgM and anti-LT serologic responses were not indicative of infection.
To our knowledge, this is the first study to evaluate the short-term (1-month) kinetics of the immune response in persons with travelers’ diarrhea secondary to ETEC and Campylobacter infection. As reported previously, we confirmed that C. jejuni infections are capable of inducing a robust mucosal and systemic immune response (7, 8, 20). However, it is unknown if the magnitude of these titers would prevent subsequent infection.

Infection with CS3 and CS6 ETEC induced homologous anti-CF serologic responses. However, maximum titers and response rates were quite low. In contrast, a recent publication of CS6 immune responses in Bangladeshi patients hospitalized with CS6 ETEC showed robust IgA and IgG titers (22). One fundamental difference in these two studies is the study population. The results presented here represent a U.S. traveler population in an ETEC-endemic setting. The study by Qadri et al enrolled persons hospitalized with diarrhea in Dhaka, Bangladesh, an ETEC hyper-endemic region. The robust responses in these subjects may represent a primed and booster effect indicative of prior infection.

This is supported by Coster et al who fed CS6 ETEC to a generally naïve study population (9). The authors showed similar serologic titers to those presented here. Additionally, ASC levels reported by Coster were lower than those presented by Qadri (anti-CS6 IgA Geometric Mean ASCs 2/10^6 PBMC and 430/10^6 PBMC respectively).

Disease severity may also have diminished the magnitude of CF-specific responses. Previous studies evaluating immune responses following Shigella infection show a positive correlation between disease severity and humoral responses (12, 13). Of
the subjects in the study by Qadri, 85% were moderately or severely dehydrated, and 100% were hospitalized. In contrast, none of our subjects were hospitalized, and only 4% received intravenous rehydration. Additionally, early antibiotic treatment in our study may have reduced the immune response. However, the rapid treatment times did not result in low responses to Campylobacter glycine extract for *C. jejuni*-infected subjects. This may be due to longer eradication times despite symptom resolution for Campylobacter infection or a difference in host responses to an invasive pathogen like Campylobacter versus a non-invasive pathogen such as ETEC.

This study was limited to cases severe enough to cause an individual to seek care. This represents a subset of infected persons potentially skewing the immunology results. Additionally, lack of a control group in our study limits the ability to make strong inferences. Future studies should evaluate pathogen-specific immune responses in a population-based setting utilizing appropriate controls.
Acknowledgements

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References


Figure 1. Serum IgA and IgG responses to glycine extract by initial microbiology findings

- IgA: Campylobacter + (n=21)
- IgA: All other illness (n=109)
- IgG: Campylobacter + (n=21)
- IgG: All other illness (n=109)
Figure 2. Serum IgA and IgG responses to CS6 by initial microbiology findings

*One subject excluded due to repeat infection on day 7 with CS6 ST ETEC
Figure 3. Serum IgA and IgG responses to CS3 by initial microbiology findings

- IgA: CS3 + ETEC (n=19)
- IgA: All other illness (n=110)*
- IgG: CS3 + ETEC (n=19)
- IgG: All other illness (n=110)*

*One subject excluded due to repeat infection on day 7 with CS2, CS3 LTST ETEC