FOOD AND DRINKING WATER HYGIENE AND INTESTINAL PROTOZOA IN DEPLOYED GERMAN SOLDIERS

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This report analyzes the occurrence of *Cryptosporidium* spp., *E. histolytica*, and *G. intestinalis* in stool of returnees from military deployments and the impact of hygiene precautions. Between 2007 and 2010, stool samples of 830 returnees that were obtained 8–12 weeks after military deployments in Afghanistan, Uzbekistan, the Balkans, Democratic Republic of the Congo/Gabonese Republic, and Sudan and 292 control samples from non-deployed soldiers were analyzed by PCR for *Cryptosporidium* spp., *E. histolytica*, *G. intestinalis*, and the commensal indicator of fecal contamination *E. dispar*. Data on hygiene precautions were available. The soldiers were questioned regarding gastrointestinal and general symptoms. Among 1122 stool samples, 18 were positive for *G. intestinalis*, 10 for *E. dispar*, and no-one for *Cryptosporidium* spp. and *E. histolytica*. An increased risk of acquiring chronic parasitic infections in comparison with non-deployed controls was demonstrated only for *G. intestinalis* in Sudan, where standardized food and drinking water hygiene precautions could not be implemented. Standard food and drinking water hygiene precautions in the context of screened military field camps proved to be highly reliable in preventing food-borne and water-borne chronic infections and colonization by intestinal protozoa, leading to detection proportions similar to those in non-deployed controls.

Keywords: deployment, field camp, food hygiene, water hygiene, parasite, real-time PCR

Introduction

About 20,000 German soldiers are deployed each year outside Germany, mostly for 2 to 6 months. Deployment areas comprise subtropical and tropical areas with low local hygiene standards. Accordingly, screening analyses of returnees for infectious agents including intestinal protozoa have been established.

In the northern hemisphere, incidences of parasitic infections in general and of protozoan infections with *Entamoeba histolytica*, *Giardia intestinalis*, or *Crypto-*

sporidium parvum in particular have declined steadily over recent decades. According to the German National Reference Centre for Infectious Diseases “Robert Koch Institute” (RKI), about 4000 cases of *G. intestinalis* and 1000 cases of *C. parvum* are annually reported in Germany. There is no obligation to report amoebic infections in Germany. A growing proportion of protozoan infections arise from visits to countries with lower hygiene standards [1].

Diseases due to these protozoan organisms are distributed worldwide and are especially common in areas with

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poor sanitary conditions or insufficient water hygiene [1, 2]. Most human infections occur not only by direct fecal-oral transmission but also through contaminated water [3–5]. Due to their high environmental resistance, *Cryptosporidium* spp. and *Giardia* spp. in particular have caused several large water-borne outbreaks in the past, when water treatment was mainly focused on chlorination and additional treatment such as filtration was inadequate [2, 5–7]. Food may also represent a hazard if it is contaminated by food producers and distributors.

A multiplex real-time PCR was used to estimate chronic infestations of stool with *E. histolytica*, the non-pathogenic *E. dispar*, *G. intestinalis*, and *Cryptosporidium* spp. in samples from German soldiers who presented for routine returnee examination 8–12 weeks after a deployment in a foreign country (Afghanistan, Uzbekistan, the Balkans, Democratic Republic of the Congo/Gabonese Republic, and Sudan) and in samples from non-deployed soldiers who served as controls to assess potential threats from these pathogens (Table 1) for deployed soldiers and the effectiveness of established hygiene procedures and preventive measures. Additionally, associations of gastrointestinal symptoms and parasite detections were analyzed.

Methods

Study population

Soldiers who underwent a returnee medical examination 8–12 weeks after a deployment in subtropical or tropical countries between February 2007 and December 2010 and who were without current diarrhea were invited to provide stool samples. This 8–12 weeks interval was chosen to ensure microscopic detection of worm eggs in case of helminth infections. Soldiers without any foreign deployment for at least the 12 months preceding a scheduled routine medical examination provided stool samples as references for comparison.

Overall, stool samples from 1122 soldiers were screened during their first visits. Of the 1122 examinees, a total of 830 were returnees from foreign deployment, comprising 316 Afghanistan returnees, 107 Uzbekistan returnees, 71 Balkans returnees, 144 Congo/Gabonese Republic returnees, and 192 Sudan returnees. No further geographical discrimination was performed. The other 292 soldiers were control subjects who underwent a routine examination without a previous deployment to a foreign country.

Interview

All volunteers were questioned during their examination concerning general health disturbances that are typically associated with enteric diseases. The features comprised abdominal complaints/diarrhea, stool numbers of ≥ 3 per day, occurrence of bloody stool, abdominal pain, nausea and vomiting, abdominal cramping, strong meteorism,

and fever. They were further asked whether they had been medically treated prior to the stool examinations. With the exceptions of age and sex (Table 2), no further data about the volunteers were collected for the study to ensure the participants' privacy.

Diagnostic workflow

PCR procedure

DNA was isolated from fresh native or frozen (-20°C) stool samples (200 mg) using a QIAamp[®] DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A previously described real-time multiplex PCR assay for *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* spp. [8] was validated and established on a multi-channel RotorGene RG-6000-5 HRM Cycler (Qiagen, Hilden, Germany) at the Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany. A specific probe for the detection of *E. dispar* was included [9].

In short, the PCR reaction mix consisted of 12.5 μl HotStarTaq master-mix (Qiagen), 2.5 μl DNA template, 3.5 μl 25 mM MgCl_2 (leading to a concentration of 5 mM MgCl_2), 0.75 μl primer mix, and 3.5 μl H_2O . The cycle condition comprised an incubation step at 95°C for 15 min, followed by 40 cycles of denaturation (15 s, 95°C), annealing (30 s, touchdown during the first nine cycles from 64°C to 60°C in 0.5°C steps), and elongation (30 s, 72°C). The positive controls included DNA of *E. histolytica* strain HM-1:IMSS (ATCC 30459), *E. dispar* strain HX-2: CDC (ATCC 30931), *G. intestinalis* strain Portland 1 (ATCC 30888), and *C. parvum* Tyzzer (genomic DNA from *C. parvum* strain Iowa, ATCC Pra-67DTM).

If several stool samples had been provided by the same soldier, only PCR results from the first stool sample were included, thus excluding follow-up control samples after treatment.

Statistics

Statistical analysis, including odds ratios according to the approximation of Woolf and Fisher's exact test (two-sided, with Yates's continuity correction), was performed with GraphPad Instat[®] Version 3.06 (GraphPad Software, Inc., La Jolla, CA, USA) to investigate associations between the areas of deployment as well as subjective symptoms and parasite detection during initial examinations. Significance was accepted at $p < 0.05$. Follow-up examinations were excluded to avoid the inclusion of copy strains.

Food and drinking water hygiene precautions during deployments

The rationale of food and water hygiene for soldiers during missions is based on European general principles and

Table 1. Known occurrence of intestinal parasites in or near the analyzed areas of deployment

Study	Year of publication	Affected country (region)	Affected population	Detection of <i>Cryptococcus</i> spp. (%)	Detection of <i>G. intestinalis</i> (%)	Detection of <i>E. histolytica</i> (%)	Detection of <i>E. dispar</i> (%)
Babiker et al. [13]	2009	Sudan (Khartoum)	Food handlers	Not assessed	9.7	4.3% without differentiation of <i>E. histolytica</i> and <i>E. dispar</i>	
Saeed and Hamid [14]	2010	Sudan (Omdurman Area)	Food handlers	Not assessed	20.5	2.6% without differentiation of <i>E. histolytica</i> and <i>E. dispar</i>	
Ekdahl and Andersson [15]	2005	Afghanistan	Emigrants	Not assessed	3.8	Not assessed	Not assessed
Haghighi et al. [16]	2009	Iran* (Zahedan)	Patients with gastrointestinal complaints	Not assessed	10.1	0	0.34
Nasiri et al. [17]	2009	Iran* (Karaj City, Tehran province)	Locals, partly refugees	Not assessed	3.8	0.021	Not assessed
Kheirandish et al. [18]	2011	Iran* (Lorestan Iran, Khorramabad)	Bakery workers	Not assessed	3.7	Not assessed	Not assessed
Gryseels and Gigase [19]	1985	Democratic Republic of Congo (Kinshasa)	People drinking water from wells	Not assessed	21	19% without differentiation of <i>E. histolytica</i> and <i>E. dispar</i>	
			People drinking water from water pipes	Not assessed	12	10% without differentiation of <i>E. histolytica</i> and <i>E. dispar</i>	
Wumba et al. [20]	2007	Democratic Republic of Congo	AIDS patients	9.7	Not assessed	Not assessed	Not assessed
Nikolic et al. [21]	1995	Serbia	School children	Not assessed	22.24	Not assessed	Not assessed
Nikolic et al. [22]	1998	Central Serbia (16 regions)	School children	Not assessed	6.8	0.02	Not assessed
Quamile et al. [23]	2010	Kosovo (Mitrovica)	Children with diarrhea	0	40	Not assessed	Not assessed

*Iran was chosen due to its close proximity to Afghanistan

Table 2. Distribution of age and sex of the study participants

	<i>n</i>	Percent male	Mean age	SD	Median age (y)	Age range (y)	Interquartile range (25–75th centile) (y)
Home medical examination	292	97.6	28.2	8.6	25.2	18.8–61.8	22.2–30.7
Afghanistan	316	95.5	30.1	7.3	27.8	20.6–54.7	25.0–32.5
Uzbekistan	107	99.1	34.4	8.6	32.0	23.3–52.4	26.9–41.7
Balkans	71	94.3	26.7	6.5	24.8	19.3–49.4	22.7–28.8
Congo/Gabonese Republic	144	93.7	33.7	8.3	32.2	21.3–60.7	27.2–37.5
Sudan	192	100	39.9	7.1	39.8	28.4–65.9	33.8–44.9
	1122	97.0	31.8	9.0	29.5	18.8–65.9	24.6–37.4

n = number. SD = standard deviation

requirements of food law, European procedures regarding food safety (Regulation EC No 178/2002), and the German food and feed law (“Lebensmittel- und Futtermittelgesetzbuch,” LFGB). Whenever possible, the production and delivery of food and drinking water is undertaken by German soldiers or under their direct supervision. The operators of dining and water treatment facilities have to implement HACCP (hazard analysis and critical control points) systems from delivery to disposal. The military and civilian staffs of facilities supplying food and water receive instruction in accordance with the German infection prevention law (“Infektionsschutzgesetz,” IfSG). The HACCP systems must include not only cleaning and disinfection measures in conjunction with food production but also handling procedures.

Food and drinking water control is the responsibility of military public health officials such as veterinarians or hygiene officers. Laboratory surveillance of delivered and prepared food as well as treated water prior to release is regularly carried out. Laboratory surveillance in general focuses on hazards that endanger the mission, especially on infectious diseases. Local staffs are screened for pathogens according to relevant directives from the hygiene department.

Deployed soldiers are instructed only to consume safety-approved drinking water. If no reliable water treatment is possible, they are allowed to drink only bottled water. The consumption of food from the country of deployment is basically forbidden, unless producers are audited and approved by military food specialists.

Nevertheless, these hygiene standards can only be maintained for large enough deployments. Individual military observers who operate independently have to supply themselves from local markets. Although training and education in basic hygienic measures is implemented before deployment, these soldiers are more endangered. Eating only cooked, washed, or peeled foods and drinking only boiled water is generally strongly recommended, but this is often unfeasible.

Ethical approval

The project was approved by the ethics commission of the Medical Doctor’s Association of Hamburg, Germany. There were no ethical concerns.

Table 3. PCR results of stool examinations (initial stool samples only)

	<i>n</i>	<i>G. intestinalis</i>		<i>Cryptosporidium</i> spp.		<i>E. histolytica</i>		<i>E. dispar</i>	
		Positive	%	Positive	%	Positive	%	Positive	%
Home medical examination	292	2	0.7	0	0	0	0	1	0.3
Afghanistan	316	4	1.3	0	0	0	0	3	1.0
Uzbekistan	107	1	0.9	0	0	0	0	0	0
Balkans	71	0	0	0	0	0	0	1	1.4
Congo/Gabonese Republic	144	2	1.4	0	0	0	0	2	1.4
Sudan	192	9	4.8	0	0	0	0	3	1.6
	1122	18		0		0		10	

n = number

Results

Distribution of parasites in the study population as assessed by PCR

In total, 18 samples were positive for *G. intestinalis* and 10 for *E. dispar*, while *Cryptosporidium* spp. and *E. histolytica* were not detected.

The *G. intestinalis* detections were distributed as follows among the returnees: 9 (4.8%) of 192 Sudan returnees, 4 (1.3%) of 316 Afghanistan returnees, 2 (1.4%) of 144 Congo/Gabonese Republic returnees, and 1 (0.9%) of 107 returnees from Uzbekistan tested positive. *E. dispar* colonizations were detected in 3 Sudan returnees (1.6%), in 3 Afghanistan returnees (1.0%), in 2 Congo/Gabonese Republic returnees (1.4%), and in 1 returnee from the Balkans (1.4%).

In contrast, among the soldiers without history of overseas deployment, stool samples from 2 (0.7%) of 292 examinees were positive for *G. intestinalis* in PCR. *E. dispar* DNA was detected in stool of 1 (0.3%) control subject (Table 3).

Associations of protozoan detection and areas of deployment

We calculated the odds ratios stratified by country of deployment for *G. intestinalis* and *E. dispar*, using the routine medical examination results from the soldiers without a history of deployment as reference. The risk of *G. intestinalis* infestation was more than seven times higher in returnees from Sudan compared with soldiers without deployment to foreign countries ($p = 0.009$). None of the other odds ratios reached a significance level of $p < 0.05$, even for the comparison of all deployed and all non-deployed soldiers (Table 4).

Associations of parasite detection and observed symptoms

Among the 18 soldiers with *G. intestinalis*-positive PCR results and with a history of deployment, 11 (61%) had reported abdominal complaints and/or diarrhea. The most typical symptom was strong meteorism, which was reported by 8 soldiers (44%). *G. intestinalis*-positive PCR results were found 12 times more often if the soldier had suffered from meteorism. Significant associations were also seen for the detection of *G. intestinalis* and a history of fever, nausea and vomiting, and stool frequencies of ≥ 3 stools per day. Association with abdominal cramping only just failed to reach significance (Table 5).

In contrast, there were no significant associations between *E. dispar* detection and any of the assessed clinical symptoms (Table 5).

Local hygiene situation

The German Armed Forces hygiene standards for food and drinking water were maintained during the deployments in Afghanistan, Uzbekistan, the Balkans, and the Congo/Gabonese Republic. Compared with larger missions with their own supply and maintenance, the military observers in Sudan had to operate separately from the German Armed Forces infrastructure. This included independent self-supply with non-controlled food and drinking water in the respective country of deployment.

Discussion

A continuous risk assessment by standardized monitoring of returnees from deployments in subtropical or tropical countries contributes to evaluation of both individual risk and preventive measures.

Table 4. Prevalence ratios for *G. intestinalis* and *E. dispar* comparing samples from foreign country deployment returnees with examinations of non-deployed soldiers

	<i>G. intestinalis</i>				<i>E. dispar</i>			
	Positive	OR*	95% CI	<i>p</i> value†	Positive	OR*	95% CI	<i>p</i> value†
Examinations of non-deployed soldiers ($n = 292$)	2	Ref.	–	–	1	Ref.	–	–
Afghanistan ($n = 316$)	4	1.86	0.34–10.23	0.69	3	2.79	0.29–26.98	0.62
Uzbekistan ($n = 107$)	1	1.37	0.12–15.25	1.00	0	–	–	–
Balkans ($n = 71$)	0	–	–	–	1	4.16	0.26–67.33	0.35
Congo/Gabonese Republic ($n = 144$)	2	2.04	0.28–14.66	0.60	2	4.10	0.37–45.61	0.25
Sudan ($n = 192$)	9	7.13	1.52–33.39	0.009	3	4.62	0.48–44.76	0.31
Foreign deployment vs. home (830 vs. 292)	16 vs. 2	2.85	0.65–12.48	0.18	9 vs. 1	3.19	0.40–25.30	0.47

*Odds ratio, approximation of Woolf

†Fisher's exact test, two-sided, Yates's continuity correction

Table 5. Univariate regression of symptoms that occurred either on or after the mission on the outcomes *G. intestinalis*- or *E. dispar*-positive PCR

	<i>G. intestinalis</i> (18 positive/1122)					<i>E. dispar</i> (10 positive/1122)			
	<i>n</i> (%)	<i>n</i> (%)	OR*	95% CI	<i>p</i> value [†]	<i>n</i> (%)	OR*	95% CI	<i>p</i> value [†]
Abdominal complaints/diarrhea	198 (17.7)	11 (61)	7.7	2.95–20.44	<0.0001	2 (20)	1.2	0.25–5.55	0.69
≥3 stools/day	87 (7.8)	5 (28)	4.8	1.67–13.78	0.0095	2 (20)	3.0	0.63–14.46	0.18
Bloody stool	15 (1.3)	0	†	†	†	0	†	†	†
Abdominal pain	76 (6.8)	3 (17)	2.8	0.80–9.82	0.12	0	†	†	†
Nausea and vomiting	49 (4.4)	5 (28)	9.3	3.16–27.14	0.0007	0	†	†	†
Abdominal cramps	54 (4.8)	3 (17)	4.1	1.15–14.72	0.052	0	†	†	†
Strong meteorism	71 (6.3)	8 (44)	13.2	5.04–34.67	<0.0001	1 (10)	1.7	0.21–13.25	0.48
Fever	59 (5.3)	4 (22)	5.4	1.74–17.11	0.012	1 (10)	2.0	0.25–16.22	0.42
Soldiers who have had previous treatment	62 (5.5)	3 (17)	3.5	1.00–12.58	0.072	1 (10)	1.9	0.24–15.36	0.43

n = number

*Odds ratio, approximation of Woolf

†Fisher's exact test, two-sided, Yates's continuity correction

‡None of the PCR positives reported this symptom

The occurrence of *G. intestinalis*, *Cryptosporidium* spp., *E. histolytica*, and *E. dispar* in German soldiers returning from foreign deployments was analyzed in comparison with non-deployed soldiers, taking the specific hygiene precautions during the various missions into account. Though *E. dispar* is not a pathogen, it was included as an indicator of fecal contaminations. *Blastocystis* spp., important waterborne transmitted intestinal protozoa, were not included in the study for practical reasons, because no easily implementable respective primer-probe-combination for our multiplex PCR was available. Accordingly, potential effects of *Blastocystis* spp. infestations could not be assessed.

Other diagnostic methods have been used, comprising microscopic examinations (three stool samples per patient) of iodine-stained wet mounts (Lugol's staining) after formol-ether concentration and slides that were exposed to the modified acid-fast rod staining procedure according to Kinyoun after formol-ether concentration, as well as various rapid antigen detection tests, including "Triage parasite panel" (BIOSITE Diagnostics, San Diego, CA, USA), "*E. histolytica* II" (Techlab, Blacksburg, VA, USA), and "RIDA®QUICK Parasite Combi Control" (r-Biopharm, Darmstadt, Germany) that were performed on native stool samples to confirm microscopic results, but their comparison was out of the scope of the current study. *Blastocystis* spp. and *Dientamoeba fragilis* were occasionally detected on microscopy but were not systematically assessed for the study. No further species were found.

None of the investigated stools samples were positive for *E. histolytica* or *Cryptosporidium* spp. by PCR. Compared with non-deployed controls, only returnees from deployments in Sudan showed a significantly higher risk for being chronically infected or colonized with *G. intestinalis*. In contrast to Afghanistan, Uzbekistan, the Balkans, and Congo/Gabonese Republic, German soldiers in Sudan had to feed themselves from local markets. Estimation of the local hygienic conditions for each individual case would be beyond the scope of this work. However, no increased detection of intestinal parasites was observed in soldiers from larger missions. Thus, these results indicate that even a deployment of several months in subtropical or tropical countries does not expose soldiers to an increased risk of acquiring infection with intestinal parasites if they operate out of military field camps with standardized food and drinking water hygiene conditions. These hygienic precautions comprise food hygiene, surveillance, instruction of the local staff, and water sanitation by means of filtration, reverse osmosis, and additional chlorination. Continuous laboratory examinations of drinking water prior to delivery and routine microbiological testing of purchased and produced food serve as an effective surveillance tool.

The results from the Sudan returnees indicate a higher exposure to fecal-orally transmitted as well as water-borne or food-borne parasites, because the prevalence ratio comparing *G. intestinalis* infestation in returnees from Sudan to that in non-deployed controls was more than seven times higher.

Any conclusions are limited by the fact, that there were no data about pre-existing infections among the soldiers prior to their deployments. *G. intestinalis* and *E. dispar* infestation may have occurred during foreign country deployments or within Germany, as both protozoa can chronically persist in the human intestine without causing symptoms. A bias due to acquisition of protozoa after returning to Germany has not been formally excluded but is highly unlikely considering the low detection rates in the German population. Another limitation of the study is that data on acute but self-limiting infection or colonization with the analyzed pathogens during the deployments are not available, because the study design allowed for the detection of sub-acute or chronic infestations only. This design was initially chosen for practical considerations, because chronic infestations were regarded as relevant for further spreading of parasites after deployment.

Further, an assessment of specific risk associations would go beyond the scope of this work, because no questionnaires related to sanitary behaviors and other personal risk factors were distributed. In addition, the exposure time of each individual was not assessed. The deployment periods of soldiers in the field camps ranged from 2 to 6 months while the military observers in Sudan were deployed for about 6 months.

In spite of all these limitations, the striking dominance of *G. intestinalis* in stool samples of returnees from Sudan who depended on self-supply with non-controlled food and drinking water remains undeniable.

Similar examinations of stool samples from returning travelers showed comparable distributions, with *G. intestinalis* found quite frequently (6%) among patients with diarrhea, while *Cryptosporidium* spp. and *E. histolytica* are rarely detected [10]. However, a Dutch study found a *G. intestinalis* prevalence of 9% in patients attending general practitioners, with no difference between patients with or without a history of traveling [11].

The analysis of the reported symptoms indicated that abdominal complaints and/or diarrhea, meteorism, fever, nausea and vomiting, and stool frequencies of ≥ 3 stools per day were significantly associated with *G. intestinalis* detection, as might have been expected due to *G. intestinalis*' enteropathogenic effects. However, no single symptom occurred in more than two out of three affected soldiers, so the associations were only feeble. The symptom of severe meteorism showed the strongest association with *G. intestinalis*. However, in spite of the high association, even meteorism occurred in no more than 44% of the soldiers who shed *G. intestinalis* cysts. Colonization with the non-pathogenic *E. dispar* was not significantly associated with any reported symptom, as expected. Associations with *Cryptosporidium* spp. and *E. histolytica* could not be analyzed due the lack of detection.

Regarding *G. intestinalis*, none of the analyzed symptoms was so common that its abundance would justify discontinuing screening of patients returning from deployments in subtropical or tropical areas under non-standard-

ized hygiene conditions. Though we only detected raised proportions of *G. intestinalis*, we maintained the screening for *E. histolytica* and *Cryptosporidium* spp. as well as monitoring of the local hygiene situation. Although *E. histolytica* is quite rare [10], its early diagnosis and differentiation from non-pathogenic *E. dispar* is of importance for the initiation of early treatment to prevent invasive spreading. *Cryptosporidium* spp. can cause outbreaks even in immunocompetent hosts, as observed in young German recruits [12].

Conclusions

Higher proportions of intestinal parasite detections can only be found at areas of deployment where standard German Armed Forces food and drinking water hygiene precautions for field camps cannot be maintained. If thoroughly implemented, these precautions can efficiently keep the occurrence of intestinal protozoa at the same low level as in German controls, as demonstrated for various deployment situations.

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