

## The quality of drinking water stored in canteens of field soldiers as a potential source of enteric diseases

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### ABSTRACT

Israel Defense Forces (IDF) guidelines for drinking water require the use of water only from sources that have been inspected and authorized by a medical expert. This study aimed to compare canteen water quality of two military units (infantry and armoured corps), to search for sources of possible microbial contamination and to look for any impact on gastrointestinal symptoms. Statistical analysis revealed that canteens of armoured corp soldiers were significantly more contaminated compared to those of infantry soldiers. Outdoor taps and water in trailers were found to harbour significantly higher numbers of microbial indicators compared to showers/lavatory sources; however, the numbers were much lower compared to canteens. Canteen water retention for more than one day revealed significantly increased numbers of examined microbial parameters, possibly due to secondary contamination or regrowth. Gastrointestinal symptoms were not significantly different between the two units despite the significant canteen water quality difference. An odds ratio evaluation was conducted on 45 exposure-illness combinations based on gastrointestinal symptoms, exposure and soldiers affiliation. Out of these 45 combinations only 14 resulted in odds ratio  $> 1$ , where 3 had high values (7.44, 7.46 and 11.2) suggesting a possible connection between diarrhoea and/or vomiting versus coliphages and faecal coliforms.

**Key words** | armoured corps, canteen, drinking water, exposure, gastrointestinal illness, infantry

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### INTRODUCTION

Water is one of the basic requirements of any army unit, especially under field conditions. As sources are scarce or may be tainted, military units are obliged to carry different means of water transportation. These include: water trailer, jerrycans, Lyster bags (Fritze 1920) and personal canteens. Canteens are the individual water carriers of each field soldier and during combat are the only means of drinking water supply. The Israel Defense Forces (IDF) guidelines for water supply and usage require the use of drinking water only from sources that have been inspected and authorized by either medical or army water health experts trained to test water sources (Anonymous 1991a,b; Anonymous 2003). Mekorot Water Co. is the main water supplier in Israel and provides the

IDF's permanent training bases with the same water quality as those supplied to the civilian population. Water quality standards (chemical and microbiological) are defined according to Ministry of Health regulations (Anonymous 2000).

Despite the overall high quality of water in Israel, heterotrophic plate count (HPC or total count) bacteria found naturally in ambient and potable waters will always be present in canteen water originating from the canteen itself or from the source (Begum *et al.* 2003). It should be mentioned that, in spite of the applied disinfection processes, worldwide water supply, particularly in Israel, is not sterile and may harbour a variety of bacteria (mainly non-pathogenic but some opportunistic pathogens)

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that survived disinfection and potentially regrew in pipes (Laurent *et al.* 1999; Edberg & Allen 2004). In Israel, the standard for microbiologically safe drinking water is based on the following regulations (based on indicator bacteria presence in drinking water such as: total and faecal coliforms): 1) tested water source does not contain any faecal coliforms per 100 ml, 2) tested water source does not contain > 3 coliforms per 100 ml (Anonymous 2000).

Canteen water may also contain other pathogenic or opportunistic pathogenic bacteria, or parasites introduced through secondary pollutants (Edberg & Allen 2004; Chaidez *et al.* 2008). Some of the opportunistic pathogenic bacteria isolates commonly found in water sources, such as *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Aeromonas*, *Xanthomonas*, *Legionella* and *Mycobacterium*, may cause serious diseases (Rusin *et al.* 1997). *P. aeruginosa* is a major cause of hospital-acquired infections and carries a high mortality rate (Morrison & Wenzel 1984). *Aeromonas* is occasionally associated with wound infections and is a causative agent of diarrhoea (Figueras *et al.* 2005). *Legionella pneumophila* causes 4–20% of cases of community-acquired pneumonia and has been ranked as the second or third most frequent cause of pneumonia requiring hospitalization (Ferreira 2004). The number of cases of pulmonary disease associated with the avian *Mycobacterium avium* is rapidly growing and is approaching the incidence of *M. tuberculosis* in some areas (Du Molin & Stottmeier 1986). *Moraxella* can cause pneumonia, as well as infections of the eye and the upper respiratory tract (Pavlov *et al.* 2004).

The records of the IDF medical corps reveal an average annual number of ~45,000 incidents concerning gastrointestinal (GI) disorders (Anonymous 2006). In sporadic cases of gastrointestinal illness no investigations are conducted to determine the cause and source. When two or more individuals of the same unit (squad, platoon, company and battalion) report on gastrointestinal symptoms, the medical authorities have to initiate an epidemiological investigation. The present study was initiated in order to determine canteen water quality in the IDF and examine the association between water quality and enteric diseases in two military units (infantry and armoured corps).

## MATERIALS AND METHODS

### Study tasks

To obtain information on the microbial status of water within soldiers' canteens the following tasks were performed:

1. A sanitary investigation to identify potential microbial contaminants in canteen water and their source, examination of agents that transmit contamination from source to canteen water and identifying factors facilitating the development of contamination in canteen water (i.e. days since last canteen filling).
2. Sampling canteens' water of combat soldiers and conducting laboratory tests to determine the microbial water quality.
3. Administration of questionnaires to soldiers that participated in the survey, concerning place and time of canteen filling, time of the most recent canteen use and any enteric symptoms (diarrhoea, stomach aches, vomiting, nausea) experienced by the participants.
4. Performing statistical correlation between canteens' water quality and gastrointestinal symptoms.

### Soldiers' population tested

The two selected units for the present study were from the armoured (tanks) and infantry corps. The first group (80 individuals) was selected from recruits of an armoured personnel unit (Merkava tanks battalion) with post-basic training (3 months), who recently started their professional training period (field and classroom). The second group (158 individuals) comprised paratroop recruits with post-basic training (3 months), who had recently started their advance training (mainly field with occasional classroom). Both groups were located in permanent bases with field deployment as requested by the training schedule. The study lasted from January to August 2002. The age of the recruits in both groups ranged from 18 to 21, and all were found healthy and combat fit by the IDF. Soldiers with any sick leave permit were excluded from the study.

### Canteens

IDF military canteens (1l volume) are made of three components: canteen main body, cap and cap rubber sealer

**Table 1** | Summary of canteens' sampling tours for two units: infantry and tank regiments

No. of sampling day	Date	Sampling location	No. of soldiers whose canteens were tested	No. of filling sources tested
1	January 4th	Armoured battalion (tanks)	27	6
2	January 18th	Infantry	25	4
3	February 20th	Armoured battalion (tanks)	30	ND
4	March 3rd	Infantry	30	3
5	April 3rd	Armoured battalion (tanks)	27	3
6	May 15th	Infantry	29	ND
7	June 19th	Infantry	27	4
8	August 14th	Armoured battalion (tanks)	30	4
Total			225	24

ND, Not Done.

approved for food and water contact. The canteen's main body is made of high density polyethylene (HDPE), the cap from low density polyethylene (LDPE) and the sealer of vinyl methyl polysiloxane (VMP). The medical corps of the IDF now recommends that each combat soldier should drink 11l/day of water during heavy training. Consequently, each soldier carries two canteens on his combat harness that ought to be permanently full. Sampling was performed between 7 a.m. and 8 a.m. on the dates presented in Table 1. Sampling and microbial examination were carried out on the same day as outlined below; water was transported to the lab in coolers. Briefly, following soldiers' morning line-up, our laboratory staff collected randomly 25 to 30 canteens and sampled 1 l from each one into sterile collection bottles. Each soldier and his canteens selected for this survey was given a number in order to be followed up later by questionnaires. Canteens' water free residual chlorine was tested with a DR/700 colorimeter (Hach, Germany) according to a previously described method (Anonymous 1998). On every sampling day 5 to 15 random canteens from each unit were tested on site for residual chlorine and excluded from the microbial survey.

### Sampling of canteen water and filling sources

Table 1 sums up the eight sampling days during the whole study period. Time since filling of the different canteens prior to sampling varied from soldier to soldier and ranged

from several hours to several days (based on information obtained from soldiers' questionnaires). The canteens' content of each soldier that participated in the study (total volume of 2l) was transferred to a 2-l sterile polycarbonate sampling bottle (containing 0.1g granular thiosulfate) avoiding direct contact. The water supply of both infantry and armoured units were: lavatory, showers and kitchen (while stationed at permanent base) and outdoor taps and water trailers (while on field training) (Table 2). All filling source types were sampled as follows: metal taps were disinfected with a burner (30 seconds) as a preventive measure against secondary contamination by hands (according to Ministry of Health regulations) (Anonymous 1997b), then running water was allowed to flow for 2 to 3 minutes and finally 1 to 2l was sampled in sterile bottles. Each sampling day, 5 to 10 samples from these sources were tested for the same microbial parameters as canteens. Sampling bottles were kept in a cooler at 4 to 8°C for 2 to 4 hours before laboratory testing.

**Table 2** | Type of water filling source and number of samples used for microbial tests

Water filling source	Number of samples tested	% of samples
Lavatory	85	53.1
Showers	14	8.8
Outdoor taps	55	34.4
Water trailers	5	3.1

### Free residual chlorine concentration of water at filling sources

Israeli disinfection standards require a residual free chlorine concentration of 0.5 mg/l at consumer taps. Chemical analysis of free residual chlorine (as previously described) of showers and lavatory water used by both units (infantry and armoured corps) revealed a concentration range of 0.1 to 0.2 mg/l. Outdoor taps and water trailers free chlorine residual concentration was between 0 and < 0.15 mg/l. No residual free chlorine was detected in canteen water of both units.

### Sampling rules

In an attempt to avoid bias as much as possible, the following rules were implemented before the study:

Canteen water content was directly sampled to test bottle.

1. No disinfection was carried out on the canteen neck and no direct contact between the canteen and sampling bottle was allowed during content transfer.
2. Before sampling, each canteen was gently shaken (for 1 minute) to resuspend any present sediment.
3. The contents of both personal canteens were transferred to one sampling bottle (previously labelled). The final volume was between 1 and 2 l as required for microbial testing. Cases of canteens partial volume (<1 l) were excluded from the study.
4. Water retention in canteens was calculated according to information obtained from questionnaires and was divided into two main groups: 1) 0–1 days (0 representing canteen filling the same day of sampling) and 2) 2–7 days.

### Microbial water tests

Water collected from canteens and filling sources was tested for the following microbial parameters: total coliforms (TC), faecal coliforms (FC), total heterotrophic plate count (HPC), *Aeromonas* spp. and bacteriophages. Bacterial enumeration was performed by the membrane filtration method as already described on the following media: m-ENDO-LES for coliforms, m-TEC for faecal coliforms,

*R<sub>2</sub>A* for total count (Anonymous 1998) and m-Ryan's *Aeromonas* for *Aeromonas* spp (Atlas 1993). A 100 ml canteen or source water sample was filtered through 0.45-µm membrane filters (Gelman Sciences, USA) and placed on the specific medium followed by incubation at 36 ± 1°C for the requested time interval as already described (Anonymous 1998). All tests were performed in triplicate and numbers were plotted as average. Somatic coliphages as indicators of faecal viral contamination were monitored according to the MPN method using *E. coli* CN<sub>13</sub> as host for presence/absence (Armon & Kott 1993).

### Questionnaires

Soldiers whose canteens were sampled for the survey (from both units) were also asked to fill in a questionnaire (two weeks prior and subsequent to sampling) with the following queries:

1. Name, personal number and unit;
2. Last date and source of canteen filling and timing of the most recent use of the canteen;
3. Gastrointestinal symptoms as mentioned in Table 6 and dates of the symptoms.

### Statistical analysis

Student's *t*- and chi-squared tests were applied where appropriate to compare coliforms, *Aeromonas* spp. and HPC among the two independent groups (infantry and tanks). The *p*-value was considered statistically significant at  $p \leq 0.001$ . The *Z*-test was performed for statistical significance of proportions in two independent groups: faecal coliforms and coliphages. Odds ratios were calculated for all exposure-illness (symptoms in our case) possibilities based on the equation:

$$OR = \frac{A \times D}{B \times C}$$

where:

*A* = soldiers with GI symptoms (sick) that drank poor-quality water

*B* = soldiers without GI symptoms (healthy) that drank poor-quality water

*C* = soldiers with GI symptoms (sick) that drank good-quality water

*D* = soldiers without GI symptoms (healthy) that drank good-quality water

Poor-quality water was defined as canteen water exceeding the Israeli drinking water standard (see above) and good-quality water as canteen water meeting the standards.

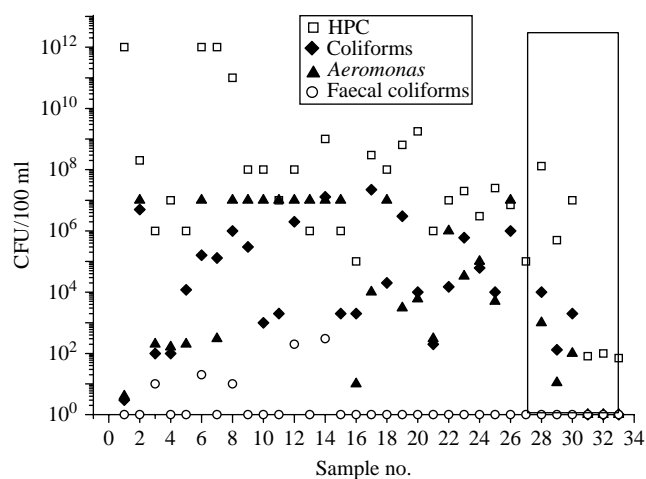
The OR tests were performed for 45 possibilities of exposure-disease as follows:

- *GI disease symptoms*: diarrhoea, vomiting, diarrhoea + vomiting;
- *Exposure to*: total coliforms, *Aeromonas* spp., HPC, faecal coliforms and coliphages;
- *Soldiers sub-groups*: infantry, armoured and infantry + armoured corps.

## RESULTS

Microbial results of water sampled from soldiers' canteens indicate that the quality of water was below the Israel Ministry of Health standards for drinking water quality ( $>3$  coliforms and 0 fecal coliforms/100 ml). Seventy-five percent of the canteens contained total coliforms level in the range of 1 to  $>10^8$  CFU/100 ml, and 8.6% of canteens contained faecal coliforms (range: 1 to  $2 \times 10^4$  CFU/100 ml) (data not shown). In addition, *Aeromonas* spp. varied from  $10^2$  to  $10^7$  CFU/100 ml, heterotrophic plate count was in the geometric average of  $\sim 10^8$  CFU/ml and coliphages positive for presence in 3.1 to 10% of the canteens.

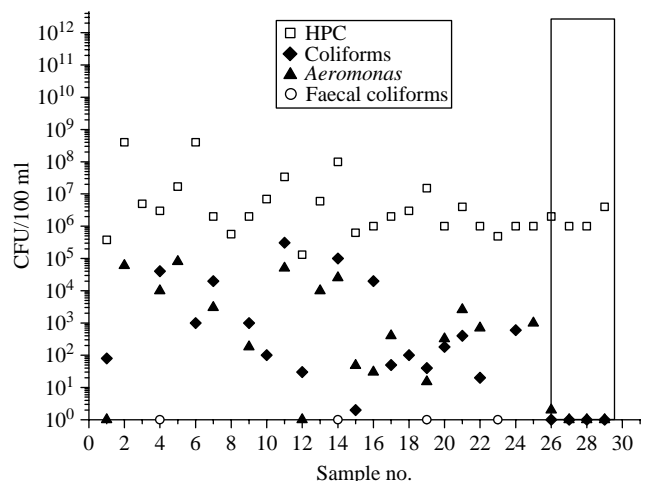
Each unit was tested four times for canteen water microbiological parameters. Figures 1 and 2 represent elevated microbial results of soldiers from armoured battalion (tanks) and infantry battalion canteens' water compared with sources water (sources data included in the black rectangle frame). Figures 1 and 2 descriptive presentation represents the general trend of all sampling days, revealing significantly higher counts of microbial parameters (HPC, coliforms and faecal coliforms) of canteens' water in comparison to filling sources of both units (*t*-test,  $p < 0.001$ ).



**Figure 1** | Bacteriological parameters of canteen water content from armoured battalion (tanks) soldiers, during January sampling tour. Note: the black rectangle frame comprises microbial values obtained from water filling sources of this unit.

Comparison of microbial quality of canteen water between infantry and armoured units in the course of a survey period of almost 7 months revealed that armoured corps' canteen water quality was significantly inferior compared to infantry (chi-squared analysis,  $p \leq 0.001$ ).

Our previous experience with field soldiers and knowledge of their canteen filling practices enabled calculation of the time elapsed since the last filling, thus the storage time of canteens' water (Table 3). The first group comparison was between canteen filling the same day of sampling (labelled 0) vs. 1 day prior to sampling and the second group



**Figure 2** | Bacteriological parameters of canteen water content from infantry battalion soldiers, during January sampling tour. Note: the black rectangle frame comprises microbial values obtained from water filling sources of this unit.



**Table 3** | Canteen storage time vs. numbers of certain microorganisms: total coliforms, *Aeromonas* spp. and HPC (both infantry and armoured corps)

Time since last canteen filling (days) comparison	Total coliforms	<i>Aeromonas</i> spp.	HPC*
0:1	$p < 0.001^\dagger$	$p < 0.001$	$p < 0.001$
1:(2-7)	$p < 0.001$	$p < 0.001$	$p < 0.001$

\*Heterotrophic plate count.

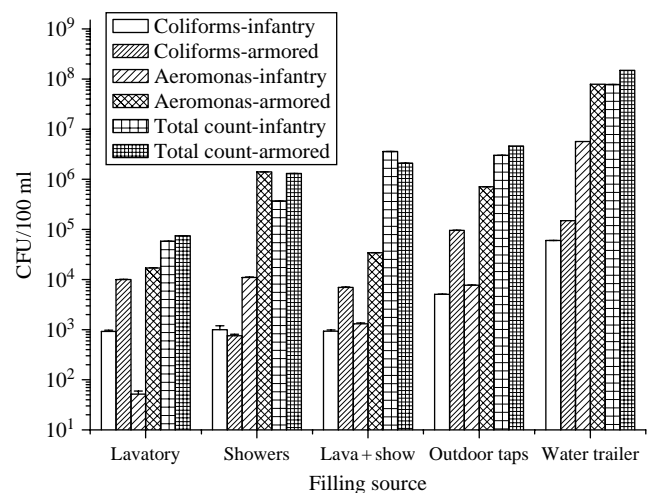
†t-test,  $p$ -value of two-sided t-test.

1 day vs. 2 to 7 days range prior to our sampling day. According to the obtained results  $\sim 2/3$  of canteens were filled on days 0 and 1 and  $1/3$  between days 2 and 7 (data not shown). The results showed significant differences between days 0 and 1, and 1 and (2-7) groups for HPC, total coliforms and *Aeromonas* spp. bacterial parameters. These significant results provided statistical evidence to other study results showing increasing numbers of microbial parameters (except coliphages) as a function of retention time (data not shown). Faecal coliforms and coliphages that are more adequate indicators of water pollution with enteric pathogens (bacterial and viral) (Armon & Kott 1993; Anonymous 1998) were also analysed statistically (Table 4). In this case, only first group statistical comparison (0:1) and related to coliphages revealed a significant difference ( $p \leq 0.001$ , Z-test), while the others were not significantly different.

As already mentioned, source water quality was found to be far better than that of the canteen water and in most cases bacteria isolated from canteen water were absent at source. However, there were enough positive samples from the various water sources that harboured test bacteria, making a comparison between the various sources of water supplies feasible (Figure 3). Our findings indicate that both trailer and outdoor tap sources revealed higher concentrations of tested bacteria in comparison with lavatory and shower sources.

**Table 4** | Canteen storage time vs. faecal coliforms and coliphages (both infantry and armoured corps)

Time since last canteen filling (days)	Fecal coliforms	Coliphages
0:1	$p \leq 0.074$	$p \leq 0.001^*$
1:(2-7)	$p \leq 0.092$	$p \leq 0.984$

\*Z-test, significantly at  $p \leq 0.001$ .**Figure 3** | Microbial quality of canteen water as a function of filling sources for both tested units (infantry and armoured corps) (error bars represent standard deviation).

Comparison of the two water supply source groups revealed that outdoor taps/trailers were significantly more contaminated with total coliforms, *Aeromonas* spp. and HPC compared to lavatory/shower sources ( $t$ -test). No significant statistical variance was found between the two sources as related to faecal coliforms and coliphages ( $Z$ -test) (Table 5).

From the epidemiological point of view it was interesting to compare the reported symptoms of individuals from the two units and to verify if there is any correlation related to their canteen water quality. Reported GI symptoms such as cramps, diarrhoea, nausea and vomiting were analysed by the chi-square test based on reported information from the original administered questionnaires (Table 6). This analysis revealed no significant difference among the symptoms percentage between infantry and armoured units ( $p \geq 0.05$ ).

Finally, in order to evaluate the health risk of drinking canteen water, an odds ratio test was conducted, investigating 45 possibilities of exposure-illness (as previously described in Methods chapter) (Table 7).

Table 7 summarizes 14 scenarios with a calculated odds ratio of  $> 1$ . In these cases it is possible to assume that there is a linkage between some exposures and illness (in our case based on reported symptoms). Examination of these 14 cases indicates two main trends based on elevated odds ratio:

**Table 5** | Statistical analysis of total coliforms, *Aeromonas*, HPC (t-test) faecal coliforms and coliphages (Z-test) as a function of water supply sources lavatory/shower versus outdoor taps/trailers for both units (infantry and armoured corps)

Water source type comparison	Total coliforms*	<i>Aeromonas</i> spp.*	HPC*	Faecal coliforms†	Coliphages‡
Lavatory/shower: outdoor taps/trailers	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.998$	$p \leq 0.343$

Note: The  $N_0$  hypothesis assumption for both tests was: there is no significant difference between base (lavatory/shower) and field (outdoor taps/trailers) water sources.

\*t-test,  $p \leq 0.001$  significantly different.

‡Z-test.

- (1) Relationship between diarrhoea or diarrhoea/vomiting and the presence of coliphages in canteen water of infantry. According to these results, it can be assumed that the presence of coliphages in canteen water indicates the presence of pathogens that cause diarrhoea and vomiting if consumed.
- (2) Relationship between vomiting, presence of faecal coliforms and armoured corp. Presence of faecal coliforms in canteen water may indicate the presence of pathogens that cause vomiting if consumed.

## DISCUSSION

Epidemiological studies carried out on drinking water quality impact on human health are not an easy task due to multiple factors involved. For example, food intake is a major factor that may screen the real infection source on one side and may be the major factor on the other side, without any possible exclusion from such studies. Canadian researchers that measured empirically the level of gastrointestinal (GI) illness related to the consumption of tap water prepared from sewage-contaminated surface waters that

met the corresponding water quality criteria used an ingenious approach in order to minimize the nutrition bias (Payment *et al.* 1991a,b). In their study, the investigated population was selected based on comparable households with similar diet and cultural behaviour in order to diminish these effects. In the present study, two military units were selected based on similar age group, combat fitness, training stage, nutrition (IDF field units have uniform food supply) and similar cleaning practices.

The main goal of the present study was to find out whether canteen microbial water quality differs among infantry and armoured soldiers corps, and if so does it have an impact on the epidemiology of gastrointestinal diseases. The microbial parameters were selected according to their indicative potential of microbial pollution, as follows: total and faecal coliforms as universal indicators of health related water microbiology (Edberg *et al.* 1997), HPC and *Aeromonas* spp. as indicators of water organic load that support potential regrowth of opportunistic pathogens (Rusin *et al.* 1997; Momba & Notshe 2003; Pryor *et al.* 2004), and finally coliphages as adequate indicators of possible viral water contamination (Pryor *et al.* 2004; Gino *et al.* 2007; Costan-Longares *et al.* 2008). Enumeration

**Table 6** | Distribution of GI symptoms data among soldiers of the two units tested, whose canteen water was sampled for microbial quality (only individuals that replied to the specific questionnaire were included)

GI symptoms	No. of soldiers answering the questionnaires	No. of soldiers that reported symptoms/total [armoured battalion]	No. of soldiers that reported symptoms/total [infantry battalion]	Chi-squared test value
Diarrhoea	213	17/98	8/115	3.49
Cramps	212	23/92	17/120	2.159
Vomiting	210	5/98	4/112	0.042
Nausea	209	11/102	10/107	0.013
Cramps with nausea and/or vomiting	217	8/105	9/112	0.019

Note: Chi-squared test analysis at  $p \geq 0.05$  showed that there was no significant difference between the two groups (infantry and armoured corps) as related to various symptoms.

**Table 7** | The odds ratio of various combinations of exposure-illness (present study-symptoms) exceeding calculated value of > 1

Possible combinations of exposure-illness that strongly suggest a significant link	A	B	C	D	Odds ratio*	Confidence interval (95%)
Diarrhoea + TC <sup>†</sup> + (Infantry + Armour)	19	42	6	47	1.04	0.3–3.1
Diarrhoea + TC + Infantry	15	82	3	35	2.13	0.5–9.94
Diarrhoea + <i>Aeromonas</i> + Armour	2	21	3	46	1.46	0.1–12
Diarrhoea + Coliphages + (Infantry + Armour)	4	21	13	135	1.97	0.5–7.4
Diarrhoea + Coliphages + Infantry	1	1	9	101	<b>11.2</b>	0.3–459
Diarrhoea + Coliphages + Armour	3	20	4	34	1.275	0.2–7.8
Vomiting + FC <sup>‡</sup> + (Infantry + Armour)	1	14	6	162	1.92	0.08–18.5
Vomiting + FC + Armour	1	9	1	67	<b>7.44</b>	0.2–305
Vomiting + <i>Aeromonas</i> + Armour	1	22	1	47	2.13	0.06–82
Diarr./Vomit. + TC + Infantry	18	79	6	32	1.21	0.4–3.8
Diarr./Vomit. + <i>Aeromonas</i> + Infantry	2	21	4	45	1.07	0.1–7.7
Diarr./Vomit. + Coliphages + (Infantry + Armour)	4	21	17	131	1.46	0.4–5.3
Diarr./Vomit. + Coliphages + Infantry	1	1	13	97	<b>7.46</b>	0.2–295
Diarr./Vomit. + Coliphages + Armour	3	20	4	34	1.275	0.2–7.8

A—soldiers with GI symptoms (sick) that drank poor-quality water, B—soldiers without GI symptoms (healthy) that drank poor-quality water, C—soldiers with GI symptoms (sick) that drank good-quality water, D—soldiers without GI symptoms (healthy) that drank good-quality water.

\* $p < 0.05$  was obtained for the odds ratios in bold.

<sup>†</sup>TC-Total Coliforms.

<sup>‡</sup>FC-Fecal Coliforms.

of the selected microbial indicators and statistical analysis revealed that canteen water of armoured corps was significantly more contaminated compared to infantry (chi-squared analysis,  $p \leq 0.001$ ). As the cohort of both units is highly similar (age, training stage, nutrition and activities) the difference may be based on several other possible factors such as: supply sources, diversity in military equipment, canteen usage frequency and discipline.

Microbiological examination of drinking water supply sources such as showers and lavatories of both units revealed only background microbial pollution very similar to the one commonly reported by Public Health laboratories in Israel on urban water supply (Elkana *et al.* 1996). Nevertheless, these sources contained residual chlorine according to Ministry of Health requirements and regulations. Remarkably, residual chlorine was never detected in personal canteens of both units, therefore no chemical disinfectant barrier was available (data not shown). However, statistical analysis comparison performed between shower, lavatory (in use as base training water supply), outdoor taps and water trailers (in use as field

water supply) revealed that total coliforms, HPC and *Aeromonas* spp. numbers were significantly higher ( $t$ -test,  $p \leq 0.001$ ) in field devices' water. Such a significant difference was not found with faecal coliforms and coliphages numbers in these two water supply sources (FC  $p \leq 0.998$  and coliphages  $p \leq 0.343$ , Z-test).

The explanations for these results can only be speculated based on poor disinfection of outdoor taps and water trailers (free residual chlorine 0 to < 0.15 mg/l) and harsh field conditions (dust, hot weather and long retention time), however no further detailed evaluation was performed. Growth of TC, HPC (that can originate from soil too) (Manios *et al.* 2006) and *Aeromonas* spp. in the absence of chlorine are well documented and this may explain the field water devices contamination (Volk & Chauret 2002). Faecal coliforms (mainly *E. coli* and some thermotolerant *K. pneumoniae*) as sanitary indicator microorganisms and a sub-group of the TC group can grow as well in the absence of chlorine, but their presence is strong evidence of direct faecal pollution that was not conceived to occur (otherwise the numbers were expected to be much higher,



and this was not the case). Coliphages that are obligatory parasites will not propagate without the specific host, therefore their presence is proof of random contamination, mainly hand contact with taps.

Consequently, the water quality at the source was found to be far better than that of the canteen water as in many cases indicator bacteria were present in canteens but were absent at source. Sanitary research regarding canteen drinking water significantly points toward the potential cause of high concentrations of bacteria in canteen water to be related to secondary contamination and not to filling sources (Figures 1 and 2). Based on these findings, it can be assumed that contamination of canteen water is mainly caused by secondary contamination, derived from soldiers' use of the canteen (dirty hands with lubrication materials, grease and oils that may enhance bacterial regrowth, more often used by armoured corps) and environmental factors (dust and sand produced in larger quantities during tanks training). Another important aspect that was analysed in the present study that may impact canteen water quality in both units was the water retention time or as previously described the time since the last canteen filling. In general, the results clearly show that from one day since the last canteen filling time, experimental indicators (TC, HPC and *Aeromonas* spp.) numbers increased significantly, worsening canteen water quality. The significant difference between the same day filling and sampling (labelled 0) and 1 day prior to sampling (labelled 1) versus the group 2–7 days prior to sampling strengthen this observation, based on potential regrowth of vegetative bacteria in organically polluted drinking water (Evison & Sunna 2001). A well-known practice (among field soldiers) is the occasional use of canteens to hold other sweet beverages that without good disinfection may significantly increase the organic load and enhance microbial regrowth in a very short time period (hours). Faecal coliform and coliphage numbers as a function of canteen retention time did not differ significantly (except coliphages between days 0 and 1) and the previous explanation for water sources can be applied in this case. Due to insufficient positive numbers obtained with coliphages the significant difference between times 0 and 1 days since last filling could not be explained.

The most difficult part of the present study was to find out if different microbial water qualities of canteens showed

an increase in gastrointestinal symptoms in the two units tested. The chi-squared test applied on diarrhoea, cramps, vomiting and nausea and a combination of all among the two units (as reported by distributed questionnaires) did not result in any significant difference (Table 6). It should be mentioned that these findings may be biased by another parameter that was not actually tested and could not be excluded from the study, e.g. food. However, besides gastrointestinal symptoms (analysed in this study) there are other possible clinical problems such as skin, eye and ear infections (Bernard 1989; Griffin *et al.* 2003; Yu *et al.* 2007) that were not surveyed and still may impair military activity.

Finally, an odds ratio estimate was performed between the various parameters as a measure of the association between exposure and symptoms. Among the selected 45 exposure-illness (symptoms) possibilities, 14 had a value of  $> 1$  revealing a potential apparent association and 3 had strong association (7.44, 7.46 and 11.2). All three cases were related to the actual best sanitary indicator organisms: faecal coliforms and coliphages.

In summary, canteen water of both units contained high numbers of microbial parameters, including sanitary indicators such as faecal coliforms and coliphages, whereas the armoured unit revealed statistical significance due to possible secondary contamination with bacterial growth supporting lubricants (frequently used in these units). Canteen water retention time of  $> 1$  day increased bacterial contaminants' regrowth ability and possibly increased the occurrence of illness for the users. Water filling supply sources were reasonably clean, meeting national drinking water standards; however, water trailers and outdoor taps presented a contamination hazard due to poor maintenance. Without excluding food contamination, it was very difficult to point towards canteen contamination as a major source of GI illness in combat units; however, other clinical outcomes can derive from such a situation such as mini-outbreaks that are not always reported. It should be mentioned that field solutions for untreated water sources were already suggested for canteen water purification in the 1990s through use of Chlor-Floc (CF) as emergency water purification tablets, but not for everyday use with treated water (Powers *et al.* 1994).

## CONCLUSIONS

The present study shows that sanitary field conditions are still a matter of improvement in order to diminish the illness risk among combat soldiers. From a canteen drinking water quality aspect, the following measures should be employed: a) canteens should be rinsed with running water, disinfected by chlorination, and if not available by boiling water, before each filling from a safe water supply source; b) every day water refilling to prevent regrowth of bacterial contaminants; c) allocate a sanitary verified tap for one task, in order to fill water tanks/canteens, and not to be used for other technical purposes (especially in armoured corps); d) water trailers and outdoor taps should be tested weekly for sanitary quality and disinfected by professionally trained personnel. In hot climates where drinking water contamination by sand, dust and organics presents a serious threat to combat troops, such simple and costless means are very much needed in order to prevent waterborne diseases.

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