Author's response to reviews

Title: High number of diarrhoeal co-infections in travellers to Benin, West Africa

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High number of diarrhoeal co-infections in travellers to Benin, West Africa

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ABSTRACT

BACKGROUND

Travellers’ diarrhoea (TD) is the most frequent health problem among travellers to the tropics. Using routine techniques, the aetiology mostly remains unresolved, whereas modern molecular methods enable reducing the number of equivocal cases considerably. While many studies address the aetiology of TD in Asian, Central American and North African tourist resorts, only few focus on Western Africa.

METHODS

Stool samples from 45 travellers travelling in Benin, West Africa, were analyzed by a new multiplex qPCR assay for Salmonella, Yersinia, Campylobacter, Vibrio cholerae, Shigella or enteroinvasive (EIEC), enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), and enteropathogenic Escherichia coli (EPEC).

RESULTS

Of the 39/45 (87%) travellers having had TD, EPEC was detected in 30 (77%) cases, EAEC in 23 (59%), ETEC in 22 (56%), Shigella or EIEC in 7 (18%), EHEC in two (5%), and Salmonella in one (3%). In 31(79%) of the TD cases two or more bacterial pathogens were identified. Two (8%) samples remained negative: both patients had taken antimicrobials for TD.
CONCLUSIONS

EPEC, EAEC and ETEC were the most common findings. 79% of the cases had a co-infection. As modern diagnostics reveals in most patients a multitude of pathogens, the role of each pathogen should be re-evaluated.

Keywords: traveller’s diarrhoea; TD; EAEC; ETEC; EPEC; West Africa; qPCR; Campylobacter; Salmonella

Words: Abstract 198; Text 2634
BACKGROUND

Travellers’ diarrhoea (TD) has been estimated to be contracted by 80 million individuals every year [1], yet the causative agents often remain poorly characterized. Although TD appears to be of bacterial origin in as many as 75% of the cases [2,3], the causative microorganism remains unidentified in up to half of those reported [2-5]. When using modern PCR-based methods, diarrhoeagenic *E. coli* has been identified in one third of the culture-negative stool samples [6]. Recently, researchers employing more advanced diagnostic techniques have succeeded in decreasing the number of unexplained TD cases to 5-24% [1,7,8].

Enterotoxigenic *Escherichia coli* (ETEC) has been considered the most common causative agent for TD worldwide [2,3]. With improved methodology, however, enteroaggregative *E. coli* (EAEC) has been reported even more often than ETEC [1,8,9]. According to some recent studies [1,7,8], multiple pathogens are detected more frequently (up to 60% of cases) than anticipated before.

In many laboratories the methods available for routine clinical analyses will only identify Salmonella, Campylobacter, Shigella and Yersinia, and *Clostridium difficile* or certain variants of EHEC are investigated on separate request. This approach leaves all patients having the most common TD pathogen, diarrhoeagenic *E. coli*, without accurate bacteriological explanation for their disease. We have recently described a new multiplex qPCR method which allows rapid detection of nine diarrhoeal pathogens at the same time [1]. This method was adopted into routine use at our laboratory in 2012.

While earlier microbial research centred on travellers to Southeast Asia, South Asia, Central America, and Northern Africa [3], other parts of Africa have been given scarce attention.
We know of merely five studies [8,10-13], just two [8,13] of them from this century, describing the aetiology of TD in travellers to Central or Western Africa. In these reports the percentage of unexplained cases ranges between 5 and 60.

The present study looks into the aetiology of TD using modern methods and focusing on a group travelling together under similar conditions in a non-touristic destination in Sub-Saharan Africa.
METHODS

Study population/recruitment

The participants were recruited from among 96 travellers (Figure 1) destined for Grand Popo, Benin, West Africa in November 2009. They were each given questionnaires and test tubes for stool samples either at an information session or a health care appointment before the journey; brochures on pertinent health issues were also handed out. The participants were asked to provide stool samples prior to departure and on return, and to fill in the questionnaires before (Q1) and immediately after (Q2) the trip, and a third one (Q3) three weeks later. As the initial number of volunteers providing Q1 and pre-travel stool samples proved quite low (20/96), the researchers went to meet the returning travellers at the airport not only to remind the volunteers of the study but also to ask for new ones to participate. On that occasion 44 new subjects were included in the research despite lacking pre-travel samples, 17 of whom failed to return both stool sample and questionnaire. Of the initial 20 participants who had provided the first faecal sample before the journey, two (10%) did not give the second one after it. The 19 subjects (2 recruited at start, 17 at airport) failing to provide post-travel samples were considered dropouts. Of the 45 who gave a second stool sample and answered Q2, 27 also returned Q3. Thus, 18 pre-travel and 45 post-travel samples were analyzed for pathogens with qPCR.

The journey in brief

The group travelled together for 9 days before returning to Finland. They were accommodated at a residence and three nearby hotels. Meals were eaten in smaller groups at local restaurants.
Definition and classification of travellers’ diarrhoea

Travellers’ diarrhoea was defined according to the WHO criteria [14] as the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual. Severe TD was defined as six or more unformed diarrhoeal stools per 24 hours or diarrhoea accompanied by fever or containing blood, or requiring hospitalization, and mild TD as 1-2 unformed daily stools. Cases not fulfilling these criteria were classified as moderate. Diarrhoea was defined as travel-related had it begun abroad or within seven days after returning home.

The questionnaires

The subjects filled in three questionnaires: pre-travel questionnaire (Q1) covered demographic particulars, current medical conditions, knowledge about travel-related risks, vaccination status and possible diarrhoeal symptoms at the time of first (pre-travel) faecal sample. Questionnaire 2 (Q2) was collected on return to Finland. The questions in Q2 concerned diarrhoeal and other symptoms during and just after the trip, medications taken over its course, risk behaviour, and contacts with local health care. The third questionnaire (Q3) collected three weeks after return was a follow-up focusing on symptoms over the first three weeks at home, possible medications, and contacts with health care. Adherence to TD prevention advice was measured in Q2 by the following questions: “Did you drink tap water, purified water or bottled water?”, “Did you eat cold salads”, “Did you eat uncooked meat or fish?”, “Did you always/often/seldom wash hands before eating?” and “Did you use utensils/bare fingers?”

Collection of stool samples
The volunteers were asked to give a pre-travel stool sample within one week before departure and a post-travel sample from the first (or second) stool passed after returning home. The samples were collected as swabs in Copan M40 Transystem tubes (Copan Diagnostics, Brescia, Italy) and sealed in special mailing envelopes. As the group returned to Finland on a Saturday evening, they were advised to refrigerate their post-travel samples and mail them only on Monday morning; the samples thus reached the laboratory in 1-3 days. There the swabs were frozen in -20°C for later analysis.

**Nucleid acid extraction**

The extraction process and qPCR have been recently described in detail [1]. Briefly, total nucleic acids were extracted using the standard semi-automated protocol of easyMAG (bioMérieux, Marcy-l'Etoile, France). The swap was thawed and directly inoculated into 2mL of lysis buffer, and processed according to manufacturer’s instructions. A general extraction protocol with 25μL elution volume was performed. A 0.5μL of eluted template was utilized per each qPCR reaction in 20μL final volume.

**qPCR analysis of bacterial pathogens**

The analyses were carried out with a multiplex qPCR method [1] which covers the following pathogens: diarrhoeagenic *E. coli* including EPEC, ETEC, EAEC, EHEC and EIEC or *Shigella* as well as *Salmonella*, *Yersinia*, *Vibrio cholerae* and *Campylobacter spp.* This assay described in detail recently [1] allows a rapid and simultaneous analysis of all these pathogens, providing results in just four hours.

**Statistical analysis**
Differences between the various traveller groups were examined by Chi-square tests using SPSS 19.0.0.1 (SPSS Inc., Chicago, IL, USA).

**Ethical clearance and informed patient consent**

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the protocol approved by the Ethics Committee of the Department of Medicine in Helsinki University Central Hospital. Written informed consent was obtained before enrolment from all patients and volunteers, and legal caretakers of minors.
RESULTS

Demographics of the study population

Background information on the volunteers is provided in Table 1. Of the 45 study subjects, 27 (60%) were female. The average age was 46 years (range 15 – 68 years). Seven had medication for an underlying chronic illness (hypertension, diabetes, hypercholesterolaemia).

GI and other symptoms in the study population

Of the 45 subjects, 39 (87%) had contracted TD over the journey (Figure 1). 14 (36%) cases proved severe, 19 (49%) moderate, and 6 (15%) mild. Severe TD was accompanied by fever in 11/39 (28%) cases. Only two reported having contacted local healthcare, and none was hospitalized. TD started on average on the 6th day of travel, and in 50% of the cases the symptoms lasted for over three days. Of the 18 initially recruited who had returned both pre- and post-travel samples, 12 (67%) had developed TD and six (33%) had remained asymptomatic, whereas all the 27 recruited at the airport had had TD over the journey. The third questionnaire (Q3) was returned by 27 study subjects: two with TD (7%) still had slightly loose stools, 22 (81%) no longer had any symptoms, and three (11%) initially asymptomatic had not developed symptoms over the follow-up either.

Microbiological findings

All 18 pre-travel samples were tested negative for the nine bacterial pathogens. The results of post-travel sample analyses are provided in Table 2. Only 3/45 post-travel stools proved negative. 35/45 (78%) subjects were identified to have contracted two or more pathogens;
31/39 (79%) of those with TD had multiple pathogens. EPEC (82%) was the most common finding followed by EAEC (60%) and ETEC (56%). No differences were found between symptomatic and asymptomatic subjects in the relative proportions of the various pathogens.

**Antimicrobial medications**

Data on the use of antimicrobials are provided in Table 3. Consistent with the Finnish recommendations [15], none of the travellers had used antimicrobials for prophylaxis of TD.; 22 had taken doxycycline as antimalarial chemoprophylaxis. Six (15%) subjects had taken antimicrobials against TD during the trip: 4 had used ciprofloxacin for 1–3 days, one azithromycin for 2 days and one penicillin (sic!), but did not report the number of days. After returning home, 8 more took a course of antimicrobial medication. Two were prescribed ciprofloxacin at the travel clinic 3-10 days after the journey, as their symptoms had not resolved; four with ongoing symptoms had EIEC/Shigella in their stool samples, and were treated with ciprofloxacin, adhering to the Finnish recommendation that all Shigella cases need to be treated because of its low infectious dose; one took ciprofloxacin for a urinary tract infection; one already asymptomatic self-administered ciprofloxacin despite being told that no medication was needed for the EAEC detected in her post-travel stool sample.

**Risk behaviour among the subjects**

The travellers considered themselves aware of all the health-related risks e.g. malaria and TD, and most of them had been vaccinated according to national recommendations [16]. Despite this, not more than 31% of the subjects with TD and 33% of the asymptomatic actually followed all TD prevention advice (see table 4).
DISCUSSION

In addition to providing a wide-range analysis of bacterial pathogens in TD, the present study contributes to the limited knowledge of diarrhoeal pathogens in travellers to West Africa.

There are no previous reports of TD in tourists to Benin, and very few of travellers to West Africa [8,10-13] One of the noteworthy findings was the exceptionally high incidence of TD among our study subjects. Although Sub-Saharan Africa is generally presented as an area with a 20 – 60% risk for TD [17-20], 87% of our travellers reported TD. However, part of our group were recruited at the airport only after the journey; all these volunteers had TD, which apparently encouraged them to participate in this study. The actual proportion of patients with TD should, therefore, be evaluated from those recruited prospectively: 60% (12/20) of them had TD.

In addition to the incidence of TD being high among our travellers, one third of the cases proved severe. Their proportion appears higher than reported elsewhere, yet the criteria for severe disease vary between studies [18,19]. The severity of the clinical picture presumably explains the comparatively high (15%) use of antimicrobials for TD over the journey and also afterwards (18% for TD). In two recent investigations conducted among Dutch [18] and Swiss [19] travellers, only 5 and 7% used antimicrobials for TD, yet, in these studies only 4 and 8% of the TD cases were considered severe.

Even though the travelers considered themselves well-informed of TD as a health risk, barely one third of them followed the TD prevention advice given. The high number of TD cases among these travellers could be ascribed to this, yet poor adherence is very common (60 – 95%) among tourists in general [18,20-22]. Moreover, even strictly followed preventive measures appear not to succeed in preventing TD [18-22].
While traditional routine diagnostics in most countries only covers a few bacterial pathogens, modern PCR methods have become increasingly applicable alongside [1]. Indeed, our assay has been successfully used in routine diagnostics of TD in the Helsinki capital area since 2012. As obvious, even these approaches cannot be designed to cover more than a limited number of pathogens. Some authors [7] have suggested that the unresolved TD cases could be ascribed to microbes earlier not recognized as causing TD. In our study using modern diagnostic methods, the proportion of unexplained TD was as low as 8%. Furthermore, the two TD patients with negative stool samples had taken a course of ciprofloxacin and had their symptoms go away by the time of the stool sampling. We thus identified a pathogen in all patients with symptoms at the time of sampling. The data are consistent with earlier studies where PCR-based methods have substantially decreased the number of unexplained cases [1,6,8].

The selection of TD pathogens contracted in Benin was of special interest. A large variety of pathogens was detected with the various diarrhoeagenic E. coli as the most prevalent findings. Among the nine bacteria studied, EPEC, EAEC and ETEC proved most frequent, followed by EIEC/Shigella and Salmonella. No cases with Vibrio cholerae, Yersinia or Campylobacter were found. Campylobacter, although common in Southeast Asia and Nepal [3,5,23,24], has not been considered a major pathogen in West Africa in previous reports either [8,10-13,24]. Vibrio cholerae and Yersinia are rare in TD everywhere. It is noteworthy that, while EPEC is one of the most frequent findings in childhood diarrhoea in developing countries [25], its significance as a TD pathogen among adults has not been confirmed [5,26]. Despite the high number of EPEC cases in our study, there was only one case of TD with EPEC as the sole pathogen.

An impressive finding was the multitude and variety of pathogens in the faecal samples: in 78% of the travellers’ samples two or more bacterial pathogens were identified, four of them
with up to four pathogens. These findings can evidently be ascribed both to a substantial exposure to contaminated foods and the effective diagnostic methods used. The results are also consistent with some previous reports [7,8,26] as well as our recent research [1], where 20-60% of travellers to varying destinations have proved to carry multiple pathogens.

Indeed, we also isolated bacterial pathogens in the stools of the small group of asymptomatic travellers: 67% had two or more bacterial pathogens, one had even four. The low number of asymptomatic travellers (merely six) does not allow any definite conclusions from these data. An abundance of bacterial pathogens in asymptomatic travellers has, however, recently been reported in two other studies [5,8].

The results of this investigation further attest to the need to improve routine diagnostics of TD. While merely 26% of our travellers having the disease (assuming that all EIEC/Shigella had been Shigella spp.) would have obtained a diagnosis using methods prevalent in routine diagnostics in most countries, the current technique revealed a pathogen in 92% of those with ongoing (n=37) or recently subsided (n=2) symptoms. In numerous earlier investigations [3] detecting just one pathogen in most TD patients, diarrhoeagenic *E. coli* (e.g. EAEC or EPEC) were not all examined, and even half of the stool samples proved negative for bacterial pathogens[3]. With these results in hand, we face the dilemma of how to determine the actual role of the various pathogens. The clinical characteristics of diseases caused by the various pathogens should each be re-examined in larger studies using modern methods.

**CONCLUSIONS**

In the group of travellers to Benin, West Africa, 67% of contracted TD. EPEC, EAEC and ETEC were the most common findings with 79% of the cases having a co-infection. As
modern diagnostics reveals in most patients a multitude of pathogens, the role of each pathogen should be re-evaluated.
List of abbreviations:

EAEC  enteroaggregative *Escherichia coli*
EHEC  enterohaemorrhagic *Escherichia coli*
EIEC  enteroinvasive *Escherichia coli*
EPEC  enteropathogenic *Escherichia coli*
ETEC  enterotoxigenic *Escherichia coli*

qPCR – quantitative PCR

TD  traveller’s diarrhoea

Competing interests: JA and JK have filed a patent application related to the qPCR method described in the paper. TL, SHP, JR and AK declare that they have no competing interests.

Involvement of the authors: Study concept and design: JR, JK, AK; acquisition of data TL, SHP, JA, JR, JK, AK; analysis and interpretation of data TL, SHP, JA, JK, AK; drafting of the manuscript TL, JK, AK; statistical analysis TL; final approval of the version published TL, SHP, JA, JR, JK, AK

Authors’ information: MD TL is a specialist in internal medicine specialising in infectious diseases at the Helsinki University Central Hospital; MSc SHP is a researcher at the University of Helsinki; PhD JA is a clinical microbiologist at Helsinki University Hospital Laboratory; MD JR is a specialist in travel medicine in the Travel Clinic of Aava Medical centre; MD PhD JK is an specialist in clinical microbiology at Helsinki University Hospital
Laboratory; Associate Professor AK is a specialist in internal medicine and in infectious diseases at the Helsinki University Central Hospital, and the Head of the Travel Clinic of Aava Medical Centre.

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REFERENCES


FIGURE LEGENDS AND TABLES

Figure 1. The study protocol.
Table 1. Background data of 45 travellers to Benin, West Africa

<table>
<thead>
<tr>
<th></th>
<th>Yes n (%)</th>
<th>No n (%)</th>
<th>Data not available n (% of all)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: female (%)</td>
<td>27 (60)</td>
<td>18 (40)</td>
<td>0</td>
</tr>
<tr>
<td>Self-reported good knowledge about travel-related risks (Q1)</td>
<td>34 (77)</td>
<td>0</td>
<td>11 (23)</td>
</tr>
<tr>
<td>Underlying chronic disease (Q1)</td>
<td>7 (16)</td>
<td>38 (84)</td>
<td>0</td>
</tr>
<tr>
<td>Appropriate antimalarial prophylaxis* (Q2)</td>
<td>42 (93)</td>
<td>2 (4)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

Pre-travel vaccination status (Q1)

<table>
<thead>
<tr>
<th></th>
<th>Yes n (%)</th>
<th>No n (%)</th>
<th>Data not available n (% of all)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow fever *</td>
<td>40 (89)</td>
<td>0</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Tetanus + diphtheria *</td>
<td>36 (80)</td>
<td>0</td>
<td>9 (20)</td>
</tr>
<tr>
<td>Polio *</td>
<td>21 (64)</td>
<td>12 (36)</td>
<td>12 (27)</td>
</tr>
<tr>
<td>MMR *, **</td>
<td>19 (42)</td>
<td>1 (2)</td>
<td>15 (33)</td>
</tr>
<tr>
<td>Hepatitis A *</td>
<td>39 (87)</td>
<td>0</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Typhoid fever*** (either peroral or injectable)</td>
<td>4 (9)</td>
<td>10 (22)</td>
<td>31 (69)</td>
</tr>
<tr>
<td>Meningococcal*** (type not specified)</td>
<td>2 (4)</td>
<td>9 (20)</td>
<td>34 (76)</td>
</tr>
<tr>
<td>Cholera***</td>
<td>3 (7)</td>
<td>10 (22)</td>
<td>32 (71)</td>
</tr>
</tbody>
</table>

* National guidelines recommend to all travellers to Benin. [16]

** Either has a history of mumps, measles and rubella or has received two doses of MMR vaccine.

*** Vaccinations according to risk assessment. [16]
Table 2. Bacteriological findings related to TD symptoms over the course of the journey in 45 travellers.

All subjects with fever also had TD. The figures are given separately for each traveller, and for the groups with or without TD. Differences between the two groups were analyzed with Chi-square tests and indicated with asterisks when proved significant.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>All n=45 (% of all)</th>
<th>Asymptomatic n=6 (% of these)</th>
<th>TD n=39 (% of these)</th>
<th>Fever n=11 (% of these)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>34 (82)</td>
<td>4(67)</td>
<td>30(77)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>EAEC</td>
<td>27 (60)</td>
<td>4(67)</td>
<td>23 (59)</td>
<td>7 (64)</td>
</tr>
<tr>
<td>ETEC</td>
<td>25 (56)</td>
<td>3(50)</td>
<td>22 (56)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>EIEC/Shigella</td>
<td>7 (16)</td>
<td>0</td>
<td>7(18)</td>
<td>5 (45)*</td>
</tr>
<tr>
<td>EHEC</td>
<td>2 (4)</td>
<td>0</td>
<td>2(5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>1 (2)</td>
<td>0</td>
<td>1(3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Yersinia spp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No pathogen</td>
<td>3 (7)</td>
<td>0</td>
<td>3(8)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Single pathogen</td>
<td>7 (16)</td>
<td>2 (33)</td>
<td>5 (13)</td>
<td>2(18)</td>
</tr>
<tr>
<td>Multiple pathogens</td>
<td>35 (78)</td>
<td>4(67)</td>
<td>31(79)</td>
<td>8 (73)</td>
</tr>
<tr>
<td>3 or more pathogens</td>
<td>15 (33)</td>
<td>1(17)</td>
<td>14 (36)</td>
<td>4 (36)</td>
</tr>
</tbody>
</table>

All differences were statistically insignificant except *p=0.006.
**Table 3.** Medication used by travellers during the journey.

The figures are given separately for those with or without TD. Differences between these two groups were statistically insignificant when analyzed with chi-square tests.

<table>
<thead>
<tr>
<th>Medication</th>
<th>TD group n=39 (% of these)</th>
<th>Asymptomatic group n=6 (% of these)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loperamide</td>
<td>19 (49)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Probiotics* (p=0.632)</td>
<td>18 (69)</td>
<td>4 (66)</td>
</tr>
<tr>
<td>Proton Pump Inhibitors (p=0.292)</td>
<td>2 (5)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Anticrobial medication during travel (excluding doxycycline)</td>
<td>6**(15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Anticrobial medication after travel (excluding doxycycline)</td>
<td>8*** (21)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Doxycycline as antimalarial (p=0.349)</td>
<td>18 (46)</td>
<td>4 (67)</td>
</tr>
</tbody>
</table>

*Information missing from 13 travellers; % calculated on the basis of data from others.

** 4 took ciprofloxacin, 1 azithromycin and 1 penicillin (sic!).

*** all took ciprofloxacin, one for UTI, 7 for TD.
Table 4. Adherence to TD prevention advice.

The figures are given separately for those with and without TD. Differences between the two groups were statistically insignificant when analyzed with Chi-square tests.

<table>
<thead>
<tr>
<th></th>
<th>TD group n=positive/information available (% of these)</th>
<th>Asymptomatic group n=positive/information available (% of these)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ignored any &quot;TD prevention advice&quot;</td>
<td>28/38 (69)</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>Drank only bottled water</td>
<td>38/39 (97)</td>
<td>6/6 (100)</td>
</tr>
<tr>
<td>Ate cold salads</td>
<td>24/38 (63)</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>Ate uncooked meat or fish</td>
<td>3/15 (20)</td>
<td>½ (50)</td>
</tr>
<tr>
<td>Consumed alcohol 3 or more units/day</td>
<td>19/33 (58)</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>Ate without utensils (bare fingers)</td>
<td>10/39 (26)</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>Washed hands only seldom</td>
<td>3/39 (8)</td>
<td>1/6 (17)</td>
</tr>
</tbody>
</table>