


Comparison of coliform contamination in non-municipal waters consumed by the Mennonite versus the non-Mennonite rural populations

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Abstract

Objectives Mennonites reside in clusters, do not use modern sewage systems and consume water from non-municipal sources. The purpose of this study is to assess risk of *Escherichia coli* exposure via consumption of non-municipal waters in Mennonite versus non-Mennonite rural households.

Methods Results were reviewed for non-municipal water samples collected by the local health department from Mennonite and non-Mennonite lifestyle households from 1998 through 2012. Water contamination was examined with the help of two study variables: water quality (potable, polluted) and gastrointestinal (GI) health risk (none, low, high). These variables were analyzed for association with lifestyle (Mennonite, non-Mennonite) and season (fall, winter, spring, summer) of sample collection. Data were split into two periods to adjust for the ceiling effect of laboratory instrument.

Results From the entire cohort, 82 % samples were polluted and 46 % samples contained *E. coli*, which is consistent with high GI health risk. In recent years (2009 through 2012), the presence of total coliforms was higher in non-Mennonites (39 %, $P = 0.018$) and presence of *E. coli* was higher in Mennonites ($P = 0.012$). Most polluted samples were collected during summer (45 %, $P = 0.019$) and had high GI health risk (51 %, $P = 0.008$) as compared to other seasons.

Conclusions Majority of non-municipal waters in this region are polluted, consuming those poses a high GI health risk and contamination is prevalent in all households consuming these waters. An association of *E. coli* exposure with the Mennonite lifestyle was limited to recent years. Seasons with high heat index and increased surface runoffs were the riskiest to consume non-municipal waters.

Keywords Water quality · Mennonites · Non-municipal waters · Gastrointestinal health risk

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Abbreviations

ACHD	Allen County Health Department, Local Health Department
CFU	Colony-forming units
<i>E. coli</i>	<i>Escherichia coli</i>
GI	Gastrointestinal
GI health risk	Risk of GI illness from <i>E. coli</i> /other coliform drinking water contamination
Lifestyle	Difference in lifestyle between Mennonite and non-Mennonite households
Mennonites	Households following Old Order Anabaptist Mennonite lifestyle
Municipal waters	Treated and regulated water under public health safety requirements

Non-Mennonites	Households not following Old Order Anabaptist Mennonite lifestyle
Non-municipal waters	Untreated and unregulated water from natural sources
Total coliforms	Pathologically significant/non-significant microbial contaminants
Period one	1998 through 2008
Period two	2009 through 2012
US EPA	United States Environmental Protection Agency
Water quality	Potable/polluted for human consumption

Introduction

As developed nations shift the focus of their healthcare efforts towards prioritizing non-communicable over communicable diseases, there are significant sections of their society which stand exposed to high risk of communicable diseases. Many acute and chronic gastrointestinal (GI) diseases, due to consumption of contaminated water, can be attributed to living conditions [1]. There are multiple microorganisms found in drinking water that can cause GI infections [2]. The World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) evaluate the quality of drinking water by testing for the presence of total coliforms, which can contain fecal bacteria such as *Escherichia coli* (Fig. 1) [3–5]. *Escherichia coli*, located in the normal intestinal flora of warm blooded animals, is the most frequent culprit of gastro-enteric bloody diarrhea and resultant fatal

complications [6–8]. Certain *E. coli* strains have been the cause of large-scale acute GI infection outbreaks in developed countries, where approximately 20 % cases developed fatal complications; increasing risk of morbidity and mortality in susceptible populations [8–10]. Presence of *E. coli* in water or food is the best indicator of high risk of severe GI illness and warrants immediate public health intervention [5, 11].

The vast majority of Americans consume municipally provided waters which, as per the United States Safe Drinking Water Act (1974), are strictly regulated by the United States Environmental Protection Agency (US EPA) guidelines and must maintain Maximum Contaminant Level Goal (MCLG) of zero coliform bacteria per 100 ml [12, 13]. Non-municipal water sources are not subject to such regulation, especially sources located on private properties where the federal, state or local laws have no jurisdiction [14, 15]. Therefore, the non-municipal water may contain microorganisms known to cause GI illness.

The Old Order Mennonite (Mennonite) families do not utilize electricity or modern plumbing facilities in their houses and also preclude the use of municipally provided waters or the independent operation of systems to purify water, which are often utilized in the non-Mennonite population [16, 17]. Further accentuating the risk of consumption of non-municipal water which can cause GI diseases in the Mennonite population, as opposed to non-Mennonite populations consuming non-municipal water, is the fact that Mennonite traditional practices include living in clusters, with their non-municipal water sources surrounded by animals and farms. In absence of modern sewage or any certifiable sanitary facilities, the ill-disposed feces, garbage and animal dander from these households have a high probability of seepage into nearby water sources [18].

On the other hand, the non-Mennonite rural population living in remote rural locations of the United States, that uses non-municipal waters, also acquires water from nearby natural sources. These households have been often reported to use rural sewage disposal systems and independent water purification systems, reducing the chances of domestic contamination of their non-municipal water sources.

Specific aims of this study

In light of these differences in lifestyle and health habits between Mennonite and non-Mennonite households, it is our hypothesis that the Mennonite community is exposed to higher levels of contaminated water as compared to non-Mennonite households consuming non-municipal water. The first aim of this study is to examine the association of lifestyle variation among Mennonites as compared to non-Mennonites on contamination of non-municipal water

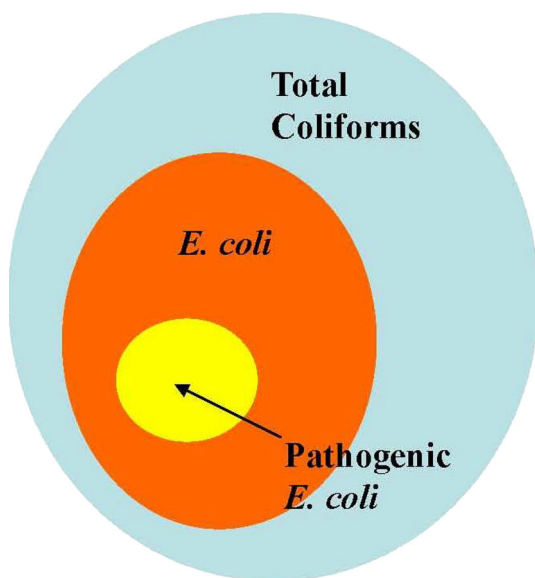


Fig. 1 The spectrum of coliforms [4]

acquired by them from nearby sources. The second aim is to examine the association of seasonal variation on non-municipal water contamination and resulting risk of gastrointestinal diseases via the presence of *E. coli* in these waters. The scheme of this study is presented in Fig. 2.

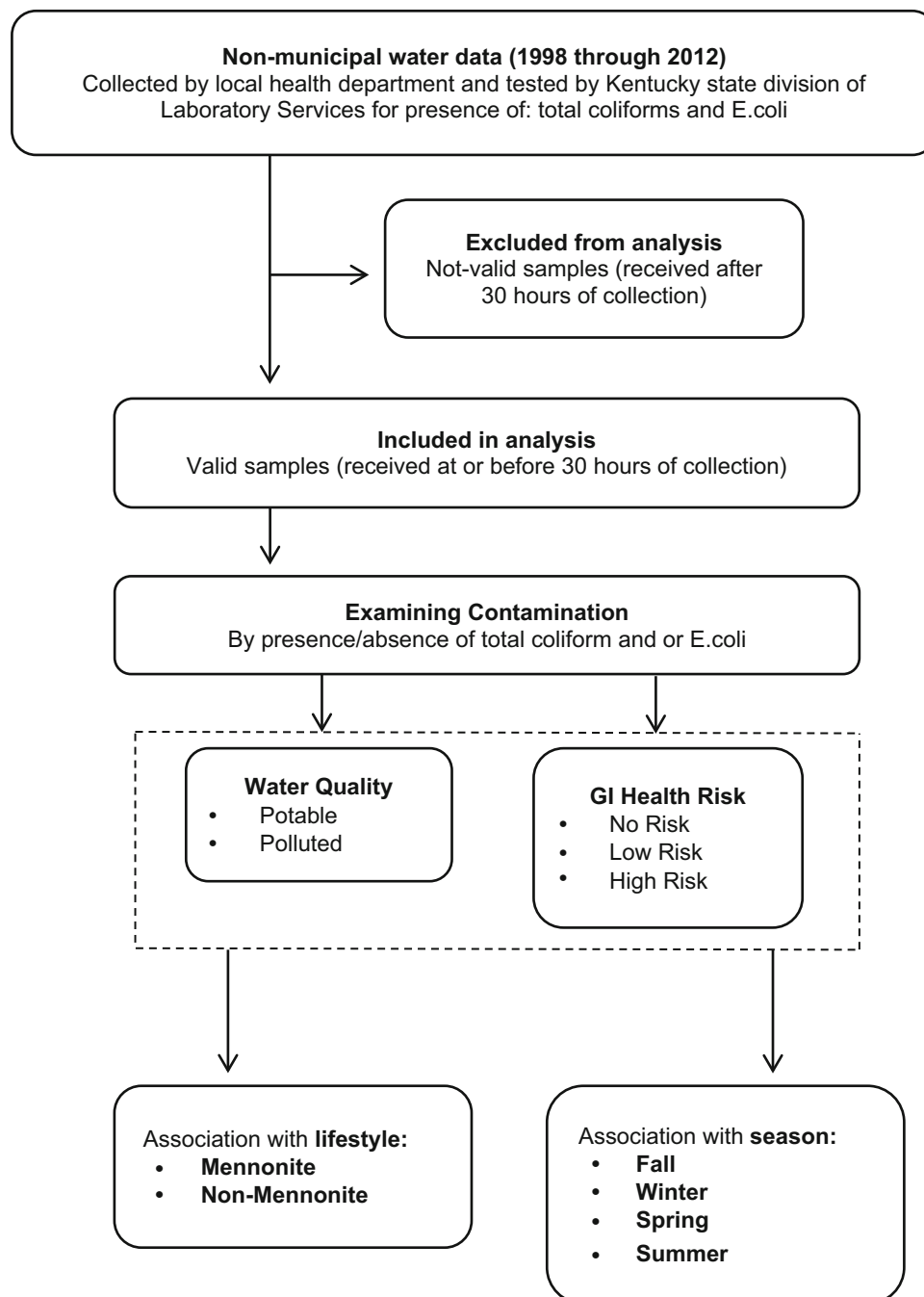
Any epidemiological evidence about the Mennonite community uncovered in this study will play an important role in shaping public health for a sizeable population worldwide, who utilize non-municipal waters for daily activities in absence of universally accepted safety protocols [19, 20]. To the best of our knowledge, this is the first

study to probe drinking water contamination in the Mennonite community.

Methods

This is a cross-sectional study of quality of non-municipal water samples collected by the local health department from August 1998 through October 2012. This study was approved by the Institutional Review Board and written consents were not required.

Fig. 2 Scheme of research



Water sample source

All samples were collected from non-municipal water sources, defined as: springs, wells, ponds and other sources not governed by the US EPA drinking water quality requirements.

Study population

All samples were acquired from households in Allen County, Kentucky, USA, which is home to a sizeable population of the Mennonite community and other non-Mennonite rural households [17, 19].

Data collection and validity

All samples were collected as per an US EPA directed protocol from non-municipal water sources by the Allen County Health Department, Scottsville, Kentucky and analyzed by the Division of Laboratory Services, Department of Public Health, Frankfort, Kentucky [16, 21]. Samples were collected in a standard 100 ml cup. Water collected from a running water line was initially allowed to run for approximately 2 min before sample collection. In the case of a natural spring, collection cups were held face down and water from the deepest possible layer was collected.

Difference between collection time and laboratory receiving time was calculated as holding time. As per the US EPA guidelines for drinking water compliance, samples received by the laboratory within or at 30 h of collection were deemed valid and those received later were excluded from our study [22].

Independent variables

Lifestyle To address the effect of lifestyle differences, all samples were identified as collected from either a Mennonite or a non-Mennonite household.

Seasons To address the effect of seasonal variation, date of sample collection was used to identify the season: fall was September through November; winter was December through February; spring was March and April and summer was May through August. Approximately 2–4 samples were collected each month.

Laboratory analysis

Samples were analyzed for the presence of total coliforms and *E. coli* as colony-forming units (CFUs) per 100 ml. Colilert reagent was used to detect the presence of total coliform and *E. coli* (“Appendix” Fig. 3) [23]. Quanti-Tray and Quanti-Tray/2000 filled with a mixture of Colilert reagent and water sample were used to calculate the presence of Most Probable Number (MPN) of coliforms (“Appendix” Fig. 4) [24].

Water contamination scoring

Microbial aspects quantified by the WHO guidelines for drinking water (2011) and the US EPA revised Total Coliform Rule (2013) were used to define two categorical outcomes for measurement of water contamination in our study (Table 1) [5, 13]: “water quality” and “gastrointestinal (GI) health risk”.

Water quality Sample quality was potable when both, total coliform and *E. coli*, were absent. Sample quality was polluted when at least one total coliform was detected.

GI health risk Sample posed no risk when total coliform and *E. coli* were both absent. Sample posed low risk when total coliforms were present, but *E. coli* was absent. Sample posed high risk when at least one *E. coli* was present.

Limitation of instrument precision

The Quanti-Tray instrument, which can calculate maximum 200.5 CFUs/100 ml, was used from August 1998 through December 2008 [21, 24]. The Quanti-Tray/2000 instrument, which can calculate maximum 2419.6 CFUs/100 ml, was used from January 2009 through October 2012 [21, 24]. This was a form of truncation arising due to limitation of instrument precision. To account for these changes in upper limit of instrument precision, data were split into two time periods [25]. This limitation did not affect the specific aims of our study.

Period one Period one was from August 1998 to January 2009, when Quanti-Tray was used. Measurements exceeding 200.5 CFU/100 ml in this period were reported at their truncated value of 200.5 CFU/100 ml. 180 out of 240 valid samples were included in this period.

Period two Period two was from January 2009 through October 2012, when Quanti-Tray/2000 was used. Measurements exceeding 2419.6 CFU/100 ml in this period were reported at their truncated value of 2419.6 CFU/100 ml. 60 out of 240 valid samples were included in this period.

Table 1 Outcomes to measure water contamination

Outcome measures	Score	Total coliforms (CFUs/100 ml)	<i>E. coli</i> (CFUs/100 ml)
Water quality [5, 13]	Potable	Zero	Zero
	Polluted	≥1	Zero or ≥1
GI health risk [5, 13]	No risk	Zero	Zero
	Low risk	≥1	Zero
	High risk	≥1	≥1

CFUs colony forming units, GI gastrointestinal

Table 2 Association of coliform contamination with lifestyle in two time periods

Characteristics	Total valid samples (N=240)	Lifestyle		
		Mennonite (N = 62)	Non-Mennonite (N = 178)	P value
Period #1 (N)	180	41	139	
Water quality				0.8
Potable (%)	33 (18.3)	7 (17.1)	26 (18.7)	
Polluted (%)	147 (81.7)	34 (82.9)	113 (81.3)	
GI health risk				0.2
No risk (%)	33 (18.3)	7 (17.1)	26 (18.7)	
Low risk (%)	70 (38.9)	21 (51.2)	49 (35.3)	
High risk (%)	77 (42.8)	13 (31.7)	64 (46.0)	
Mean total coliform CFUs/100 ml (95 % CI)	119.9 (108.1–131.6)	98 (75.2–120.9)	126.3 (110.9–141.7)	0.1
Mean <i>E. coli</i> CFUs/100 ml (95 % CI)	30.8 (22.7–38.9)	15.2 (3.4–27.0)	35.4 (25.4–45.3)	0.052
Period #2 (N)	60	21	39	
Water quality				0.2
Potable (%)	7 (11.7)	1 (4.8)	6 (15.4)	
Polluted (%)	53 (88.3)	20 (95.2)	33 (84.6)	
GI health risk				0.012
No risk (%)	7 (11.7)	1 (4.8)	6 (15.4)	0.2
Low risk (%)	17 (28.3)	2 (9.5)	15 (38.5)	0.018
High risk (%)	36 (60.0)	18 (85.7)	18 (46.2)	0.012
Mean total coliform CFUs/100 ml (95 % CI)	1053.6 (915.8–1191.4)	1426 (1154.9–1697.0)	853.1 (698.9–1007.2)	0.020
Mean <i>E. coli</i> CFUs/100 ml (95 % CI)	184.8 (119.7–249.9)	241.4(106.1–376.6)	154.3 (80.5–228.2)	0.014

Period #1—1998 through 2008

Period #2—2009 through 2012

CI confidence interval, GI gastrointestinal

Statistical analysis

Data were entered using Microsoft Access and analyzed with SAS [26, 27]. Analyses for total coliform and *E. coli* counts along with proportion of truncated and non-truncated values were conducted separately for each period. In addition, associations of water quality and GI health risk with lifestyle variation were conducted for each period.

For categorical characteristics, frequency of the characteristics between groups was conducted and tested using Pearson's Chi-squared test [28]. Fisher's exact test was used when any cell counts were less than 5 [28]. For continuous characteristics, the Wilcoxon rank sum test was used [29]. Splitting into separate periods resulted in insufficient amount of data; hence, regression models were not used to estimate relationships [30]. Statistical significance was defined as $P < 0.05$.

Results

A total of 303 samples were collected from Mennonite (24 %) and non-Mennonite (76 %) households, out of which 63 were excluded due to holding time of greater than 30 h and 240 were

retained as valid samples. General associations of lifestyle with seasons and contamination, without splitting data into separate time periods, are presented in “Appendix” Table 4. There were no significant associations between lifestyle and water contamination scores (water quality, GI health risk) when data were not split into separate time periods.

Associations of coliform contamination during two separate time periods are presented in Table 2. During period one (1998 through 2008), the mean *E. coli* count was borderline higher in non-Mennonites (35 CFUs) as compared to Mennonites (15 CFUs, $P = 0.052$). During period two (2009 through 2012), majority of low-risk samples were collected from non-Mennonites (39 %) as compared to Mennonites (10 %, $P = 0.018$). However, during period two, Mennonite samples (86 %) had high GI health risk (one or more *E. coli* CFUs) as compared to non-Mennonites (46 %, $P = 0.012$). This period also showed high mean count of total coliforms from Mennonite samples (1426 CFUs) as compared to non-Mennonite samples (853 CFUs, $P = 0.02$). Similarly, high count of *E. coli* was found in Mennonite samples (241 CFUs) as compared to non-Mennonites (154 CFUs, $P = 0.014$).

Associations of seasons with water quality and GI health risk, without splitting data into separate periods, are

Table 3 Association of seasons with water quality and GI health risk

Seasons	Total valid samples (N = 240)	Water quality			GI health risk			
		Potable (N = 40)	Polluted (N = 200)	P value	No risk (N = 40)	Low risk (N = 87)	High risk (N = 113)	P Value
Seasons				0.055*				0.053*
Fall (%)	60 (25)	16 (40)	44 (22)	0.016**	16 (40)	24 (27.6)	20 (17.7)	0.016***
Winter (%)	42 (17.5)	7 (17.5)	35 (17.5)	1.0**	7 (17.5)	17 (19.5)	18 (15.9)	0.8***
Spring (%)	38 (15.8)	7 (17.5)	31 (15.5)	0.8**	7 (17.5)	14 (16.1)	17 (15)	0.9***
Summer (%)	100 (41.7)	10 (25)	90 (45)	0.019**	10 (25)	32 (36.8)	58 (51.3)	0.008***

Fall september–november, Winter december–february, Spring march–april, Summer may–august, GI gastrointestinal

*P value for overall effect of season in model

**P value comparing percentages of potable vs. polluted samples collected in a specific season against all other seasons

***P value comparing percentages of no risk, low risk and high risk samples collected in a specific season against all other seasons

presented in Table 3. Analyzing the water quality during different seasons, we found that fall season had the highest number of potable samples ($n = 16$), and the proportion of potable samples collected in the fall season (40 %) was also significantly higher than the proportion of polluted samples collected in that season (22 %, $P = 0.016$). Summer season had the highest number of polluted samples ($n = 90$), and the proportion of polluted samples collected in that season (45 %) was also significantly higher than the proportion of potable samples collected in that season (25 %, $P = 0.019$). Analyzing the GI health risk during different seasons, we found that safest samples (no risk, zero total coliform CFUs) were mostly collected in fall season (40 %), as compared to winter (18 %), spring (18 %) or summer (25 %, $P = 0.0016$). In contrast, worst samples (high GI risk—one or more *E. coli* CFUs) were mostly collected in summer (51 %), as compared to fall (18 %), winter (16 %) and spring (15 %, $P = 0.008$).

Discussion

Upon examining the water quality and GI health risk from consumption of non-municipal waters among Mennonite and non-Mennonite households of Allen County, Kentucky, we found that a majority of water from these sources was polluted (total coliform contamination) and almost half of the samples of non-municipal waters were exposed to high GI health risk (*E. coli* contamination). An association of *E. coli* exposure with the Mennonite lifestyle was limited to recent years. However, a concrete association between non-municipal water contamination and Mennonite or non-Mennonite lifestyle was not found in our dataset.

Addressing the first aim of this study, we found that during period one (1998 through 2008), water pollution and GI health risk levels were substantially high and there

was no difference in these outcomes between the Mennonite or non-Mennonite lifestyles. However, during period two (2009 through 2012), samples from Mennonite households had significantly higher *E. coli* contamination, which exposed them to a high GI health risk. There was a similar relationship for high volume of total coliforms and *E. coli* microorganisms in samples collected from Mennonite households during period two. This increase in health risk among Mennonites could be due to the relatively steady rise in septic tank installations and adaptation of other safe waste disposal techniques by the non-Mennonite households during recent years [16]. However, results from period two should be interpreted with caution due to the small sample size. It is important to note that the water samples from non-Mennonite households had considerable contaminations of total coliforms and *E. coli*, which indicate that the non-municipal waters consumed by non-Mennonites are also substantially polluted and carry a considerable GI health risk.

The second aim of this study found that summer was the riskiest season to consume non-municipal waters. As is evident in the existing literature, higher *E. coli* contamination in non-municipal water sources was found to be associated with summer months [31–33]. Surface runoffs during rainfall and high heat indices during summer are well-known facilitating factors for distribution of fecal coliforms, i.e., *E. coli* [33–35]. Runoffs, which contain animal fecal material, in this hilly rural farming region, increased the chances of *E. coli* contamination in water sources and thus increased the burden of GI health risk in the surrounding population [31, 32].

Presence of naturally occurring total coliforms is not classified as a direct health risk, but rather, indicates presence of multiple unwanted microorganisms and increases the potential risk of exposure to *E. coli* (Fig. 2)

[2, 4, 5]. On the other hand, *E. coli* contamination of one or more CFUs per 100 ml is directly associated with serious GI illness and has been established as a triggering point for public health safety measures [5, 12, 13, 36].

During period two, majority samples collected from non-Mennonite households, as compared to Mennonites, had significant presence of low GI health risk (zero *E. coli*, but one or more total coliform CFUs), but the average count of total coliform CFUs was higher among Mennonites. Irrespective of the mixed indications of health risk between the two lifestyles, there was substantial low GI health risk during period two in the entire region, along with a high mean of total coliform CFUs. Evident from these associations, both, Mennonites and non-Mennonites consuming non-municipal waters have a significantly increased potential of exposure to high GI health risk factors (fecal bacteria like *E. coli*). Incidences of Hepatitis A infections have been widely observed after consumption of water with high total coliform count, which can be reiterated by the fact that heavy presence of total coliforms increases chances of presence of viruses and other pathogenic contaminants [13, 37]. Bacterial load of as low as 23 total coliforms per 100 ml is capable of causing an acute outbreak of Hepatitis A in healthy children and other susceptible populations [38]. Similarly, a low infectious dose of 10–100 organisms per liter can cause Shigellosis, which is responsible for over one million deaths worldwide [39]. Hence, even in absence of detectable *E. coli* and in presence of high counts of total coliform, non-municipal waters could still carry a substantial potential of high GI health risk.

This study was successful in engaging the conservative Mennonite community; an important breakthrough for future public health policies. A top health priority in this region should be safeguarding drinking water sources and an immediate intervention strategy for all users of non-municipal waters.

Limitations

The main limitation of our study was low power of analysis, which further decreased due to exclusion of samples violating the holding time criteria. The maximum detectable limit of Quanti-Trays may have masked the true value of contamination in many samples; however, this did not affect the specific aims of our study. Data were not split into separate time periods while analyzing associations with seasonal variation as that would have critically reduced the sample size.

Although there is a statistical difference in sample collection from Mennonite versus non-Mennonite households during different seasons (“Appendix” Table 4), this may be due to logistic reasons. The distribution of main outcome variables, i.e., lifestyle, water quality and GI health risk, was not known prior to data collection and the selection of samples did not depend on these outcome factors. Thus, associating any predictor variable with these outcome variables is valid with limitation to only sample size. To validate these results further, a larger study is recommended in which similar number of samples should be collected during each season from same point sources.

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Conflict of interest No conflict of interest to disclose.

Appendix

See Figs. 3 and 4 and Table 4.

Fig. 3 Colilert reagent for detection of total coliforms and *E. coli* [21, 23]. The mixture of Colilert reagent and water sample turns yellow if any coliform bacteria are present and yellow plus fluorescent if specifically *E. coli* is present (color figure online)

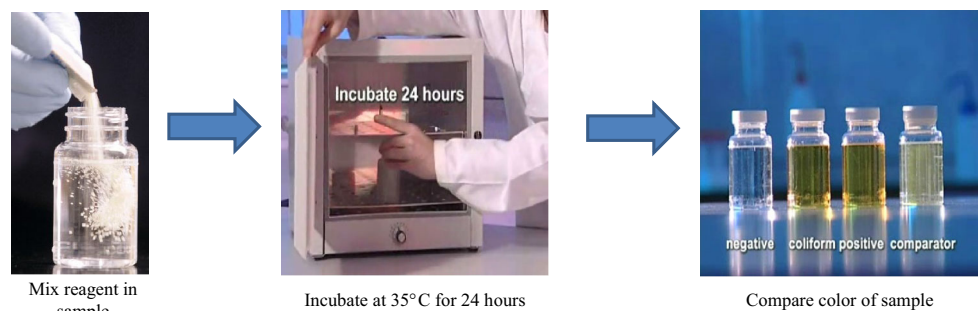


Fig. 4 Quanti-Trays for calculating most probable numbers of total coliforms and *E. coli* [21, 24]. A table or software is used to calculate the Most Probable Number of total coliforms and *E. coli* in Quanti-Trays (or Quanti-Tray/2000) with changed colors (color figure online)

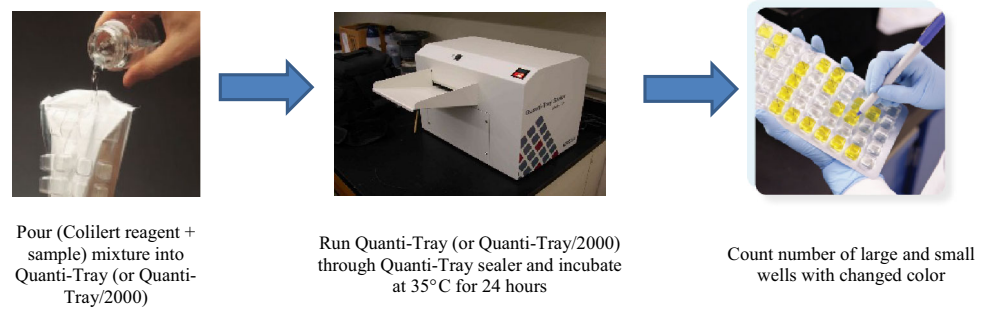


Table 4 General association of lifestyle with season and contamination without splitting into separate time periods

Characteristics	Total	Lifestyle		
		Mennonite	Non-Mennonite	P Value
Total water samples (%)	303	74 (24.4)	229 (75.6)	0.3
Not-valid (%)	63 (20.8)	12 (16.2)	51 (22.3)	
Valid (%)	240 (79.2)	62 (25.8)	178 (74.2)	
Mean holding time in hours (95 % CI)	30 (23.1–36.9)	32.6 (13.9–51.3)	29.2 (22.2–36.2)	0.7
Seasons				0.007
Fall (%)	82 (27.1)	13 (17.6)	69 (30.1)	0.034
Winter (%)	52 (17.2)	18 (24.3)	34 (14.8)	0.060
Spring (%)	47 (15.5)	18 (24.3)	29 (12.7)	0.016
Summer (%)	122 (40.3)	25 (33.8)	97 (42.4)	0.2
Contamination scores in valid samples (N = 240)				
Water Quality				0.4
Potable (%)	40 (16.7)	8 (12.9)	32 (18)	
Polluted (%)	200 (83.3)	54 (87.1)	146 (82)	
GI health risk				0.7
No risk (%)	40 (16.7)	8 (12.9)	32 (18)	
Low risk (%)	87 (36.3)	23 (37.1)	64 (36)	
High risk (%)	113 (47.1)	31 (50)	82 (46.1)	
Contaminant levels				
Mean total coliform CFUs/100 ml (95 % CI)	353.3 (267.2–439.4)	285.5 (200.7–370.4)	547.8 (325.8–769.9)	0.3
Mean <i>E. coli</i> CFUs/100 ml (95 % CI)	69.3 (35.1–103.4)	91.8 (9.3–174.3)	61.4 (25.4–97.5)	0.8

CI confidence interval, GI gastrointestinal, Fall september–november, Winter december–february, Spring march–april, Summer may–august

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