Research

Quantitative Microbial Risk Assessment for Contaminated Private Wells in the Fractured Dolomite Aquifer of Kewaunee County, Wisconsin

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BACKGROUND: Private wells are an important source of drinking water in Kewaunee County, Wisconsin. Due to the region's fractured dolomite aquifer, these wells are vulnerable to contamination by human and zoonotic gastrointestinal pathogens originating from land-applied cattle manure and private septic systems.

OBJECTIVE: We determined the magnitude of the health burden associated with contamination of private wells in Kewaunee County by feces-borne gastrointestinal pathogens.

METHODS: This study used data from a year-long countywide pathogen occurrence study as inputs into a quantitative microbial risk assessment (QMRA) to predict the total cases of acute gastrointestinal illness (AGI) caused by private well contamination in the county. Microbial source tracking was used to associate predicted cases of illness with bovine, human, or unknown fecal sources.

RESULTS: Results suggest that private well contamination could be responsible for as many as 301 AGI cases per year in Kewaunee County, and that 230 and 12 cases per year were associated with a bovine and human fecal source, respectively. Furthermore, *Cryptosporidium parvum* was predicted to cause 190 cases per year, the most out of all 8 pathogens included in the QMRA.

DISCUSSION: This study has important implications for land use and water resource management in Kewaunee County and informs the public health impacts of consuming drinking water produced in other similarly vulnerable hydrogeological settings. https://doi.org/10.1289/EHP7815

Introduction

Kewaunee County is a rural county located in northeast Wisconsin (USA), just south of the Door County peninsula and southeast of Green Bay. The principal source of drinking water for approximately 12,000 Kewaunee County residents consists of private wells constructed in the county's fractured Silurian dolomite aquifer (K. Bradbury, Wisconsin State Geologist, personal communication). This aquifer is notoriously vulnerable to contamination from surface and subsurface sources due to its karst features (Bradbury and Muldoon 1992; Erb and Steiglitz 2007; Sherrill 1975, 1978), and the depth of soil overlying the bedrock also influences groundwater vulnerability. In particular, deeper depths to bedrock produce longer transport times and more opportunity for attenuation of microbial contaminants (Erb and Steiglitz 2007; Rasmuson et al. 2020). Exacerbating this problem, the county's rural landscape is home to potential fecal sources in the form of thousands of private septic systems and tens of thousands of cattle (Borchardt et al. 2021). Septic systems are spread ubiquitously throughout the county (Figure 1), and though each is a relatively small source on its own, all are typically located in close proximity to one or more private wells and thus represent a constant hazard of drinking water contamination. In contrast, most of the cattle are housed on one of more than a dozen permitted confined animal feeding operations (CAFOs). These CAFOs tend to store their manure for long periods of time, land-applying large quantities of it to agricultural fields (Figure 1) during brief intervals and only a few times per year (Erb et al. 2015; Janni and Cortus 2020).

Due to its inherent vulnerability and the combination of human and agricultural influences, Kewaunee County's groundwater is in fact regularly contaminated by several different pollutants. Most recently, Borchardt et al. (2021) estimated contamination rates of private wells in the county based on the presence of indicator bacteria and/or nitrate and as a function of depth-to-bedrock, which varies from <1 to >45 m across the county (Clayton 2013). More specifically, Borchardt et al. (2021) found that 39%–43% of wells constructed in depth-to-bedrock of ≤6.1 m (i.e., ≤20 ft) were contaminated with total coliforms and/or $NO_3 - N > 10$ mg/L. This range compared to a 23%–26% contamination rate for wells constructed in depth-to-bedrock of >6.1 m. Furthermore, Borchardt et al. (2021) identified both relevant sources of fecal contamination (i.e., human and bovine) in private well samples along with a number of human and zoonotic gastrointestinal pathogens (e.g., *Cryptosporidium* and *Salmonella* among others). As in many other states, private wells in Wisconsin are not monitored by government agencies, and the testing and treatment of private well water is at the discretion of well owners. Given the prevalence of private wells and the documented groundwater contamination, waterborne infectious disease may be of concern in Kewaunee County, particularly transmission of acute gastrointestinal illness (AGI) via drinking water from private wells.

However, the health risk associated with pathogen levels observed by Borchardt et al. (2021) is currently unknown. Understanding health risk, and not just pathogen occurrence on its own, is crucial in this context because risk (i.e., AGI cases per capita) can be more easily related to the cost–benefit calculus involved with evaluating proposed policy interventions in the county.

Thus, the current study used quantitative microbial risk assessment (QMRA) (Haas et al. 2014) to estimate the risk of waterborne AGI associated with contaminated private wells in Kewaunee

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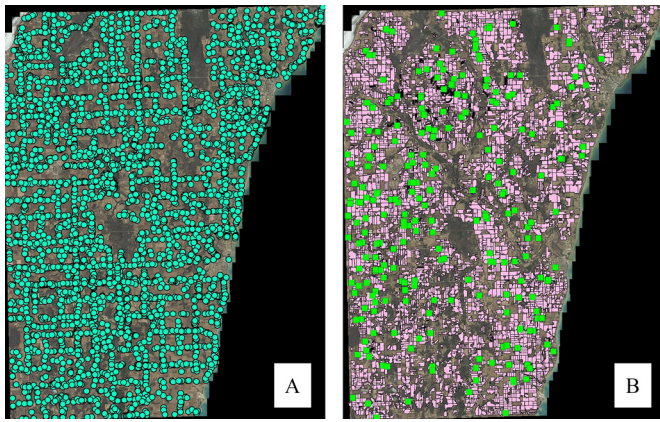
Figure 1. Fecal sources of interest in Kewaunee County including (A) private septic systems, with each green dot representing a single septic system and (B) sources of cattle manure; each green square represents a storage facility (e.g., manure lagoon), and pink shading represents fields with approved nutrient management plans (i.e., those fields on which manure is likely to be spread). Produced using Geographic Information System data layers maintained by the Kewaunee County government and ArcMap software (version 10.3.1, ESRI).

County. Waterborne pathogen concentrations were measured by Borchardt et al. (2021), and other QMRA inputs (i.e., daily drinking water consumption and dose–response parameters) were extrapolated from the published literature. Furthermore, in addition to estimating cases for the county overall, cases were also estimated by depth-to-bedrock and fecal source as determined by microbial source tracking. Results of this study have important implications for land use and water resource management in Kewaunee County and may also be of interest to the broader scientific community, because they provide valuable information about the health risks associated with fecal contamination in other, similar vulnerable groundwater settings.

Methods

General Approach

QMRA was conducted in four steps: *a*) hazard identification; *b*) exposure assessment; *c*) dose–response assessment; and *d*) risk characterization (Haas et al. 2014). The primary inputs in these steps were daily drinking water consumption, pathogen concentrations in contaminated well water, and dose–response parameters that describe the infectivity of individual pathogens. An important aspect is that this study took advantage of measured site-specific pathogen concentrations obtained from a year-long, county-wide pathogen occurrence study conducted in Kewaunee County from 2016–2017 ($n = 138$ private well samples) (Borchardt et al. 2021).

The mathematical calculations involved in the QMRA were stratified based on two critical factors from the associated pathogen occurrence study, depth-to-bedrock and contamination source. Values for depth-to-bedrock were extracted from publicly available well construction reports (WGNHS 2019) and stratified as ≤6.1 m or >6.1 m. For depth-to-bedrock, wells were initially placed in three strata (<1.5 m, 1.5 to 6.1 m, and >6.1 m) (Table S1). Given the low number of wells in the lowest stratum (<1.5 m to bedrock), it was combined with the middle stratum to create the two strata used for analysis here (i.e., ≤6.1 and >6.1 m) (Table S1). Moreover, the 6.1 m cutoff is important in terms of well contamination and relevant to Kewaunee County code. Analyses from Borchardt et al. (2021) showed little difference in well contamination when depth-to-bedrock was shallow

(e.g., 1.5 m vs. 6.1 m), but a greater effect of depth-to-bedrock was observed when comparing shallow depths to greater depths (e.g., ≤6.1 m vs. >6.1 m). In addition, current county code includes restrictions on manure application when depth-to-bedrock is <20 ft (6.1 m) (Kewaunee County Department of Land & Water Conservation, 2021).

Contamination source was defined by the co-occurrence of pathogens with source-specific microbial source tracking (MST) targets in individual well samples (see "Exposure Assessment" section for further details). For example, when *Salmonella* was detected with the human fecal marker HF183 *Bacteroides*, it was assumed that the *Salmonella* originated from a human fecal source. More generally, because the pathogens considered were all feces-borne, it was assumed that co-occurring MST targets and pathogens originated from the same fecal source. Because MST markers included two fecal sources (human and bovine), this assumption produced three contamination source categories: human feces, bovine feces, and unknown; the final category arises when pathogens are detected with no MST markers or with both human and bovine markers simultaneously.

Hazard Identification

The hazard identified was gastrointestinal illness due to waterborne pathogens consumed in drinking water drawn from private wells in Kewaunee County. Pathogens and MST markers were identified in large-volume (522–1,517 L) private well samples ($n = 138$) collected by dead-end ultrafiltration in Kewaunee County during five seasonal sampling events from April 2016 to March 2017 as described in Borchardt et al. (2021). All samples were taken from flame-sterilized taps prior to water softening or any other treatment systems, then processed and analyzed for 33 gene targets by quantitative polymerase chain reaction (qPCR), including 15 gastrointestinal pathogens known to infect human hosts. Laboratory methods and quality control are described in Borchardt et al. (2021).

The QMRA included risk estimates for eight human and zoonotic gastrointestinal bacteria, parasites, and viruses detected at least once: adenovirus group A, *Campylobacter jejuni*, *Cryptosporidium* (divided into three categories, including *C. parvum*, *C. hominis*, and ungenotyped *Cryptosporidium* spp.), enteropathogenic *Escherichia coli* (EPEC), *Giardia duodenalis*, and nontyphoidal *Salmonella*. Norovirus (genogroups I and II), other adenoviruses (groups B, C, D, and F), and enterovirus were not detected and were therefore not considered in the QMRA. Rotavirus group A was detected, but risk was not estimated for this pathogen because the qPCR assays involved cannot distinguish wild-type rotavirus from vaccine (though group A rotavirus was used as an MST marker, see "Exposure Assessment" section). Somewhat similarly, rotavirus C was also detected, but risk estimates for this pathogen were not performed because no dose–response model is available.

Exposure Assessment

The goal of exposure assessment was two-fold. First, it estimated the number of Kewaunee residents exposed to contamination (per day) for each stratum of the data set (i.e., for each combination of depth-to-bedrock and contamination source). Then, for each of these populations, it estimated the mean daily dose of each pathogen to which that population was exposed. It was assumed that Kewaunee residents unexposed to contamination were at zero risk for exposure to feces-borne pathogens in private wells. The size of exposed populations for each depth-to-bedrock stratum i and contamination source j (N_{ij}) was estimated using the following expression:

$$N_{ij} = W_i \times F_i \times S_{ij} \times T \qquad (1)$$

where W_i is the number of wells in depth-to-bedrock stratum i (i equals 1 or 2 for <6.1 m and ≥ 6.1 m, respectively); F_i is the stratumwide private well contamination rate as determined by coliforms and nitrate; S_{ij} is the proportion of contaminated wells in depth-to-bedrock stratum i that is associated with source j (j ranges from 1 to 3 for human feces, bovine feces, and unknown, respectively); and T is the number of people served per well per day (see Table 1 for quantitative summaries of inputs to Equation 1). Note that F_i is assumed herein to represent presumptive fecal contamination, a prerequisite for contamination by feces-borne gastrointestinal pathogens, though due to the inclusive indicators used in its definition (see below), it may also represent contamination via other sources (e.g., chemical fertilizers). Note also that F_i and S_i were assumed to be constant each day throughout the year, a simplifying assumption necessitated by the extent of available data.

Inputs for Equation 1 were based on a variety of sources. W_i was determined based on analysis of county and state records as described in Borchardt et al. (2019). F_i was determined based on the results of two synoptic sampling events (i.e., 2-d "snapshot" events) conducted as part of the related pathogen occurrence study (Borchardt et al. 2021). More specifically, F_i ($i = 1$ for ≤ 6.1 m and $i = 2$ for >6.1 m) was developed based on data presented in Table 2 of Borchardt et al. (2019). These data represent contamination rates defined by total coliforms, *E. coli*, and $NO_3 - N > 10$ mg/L for two county-wide synoptic sampling events. Borchardt et al.'s (2019) data are summarized by event (fall of 2015, summer of 2016) and three depth-to-bedrock strata (<1.5 m, $1.5–6.1$ m, and >6.1 m), whereas the QMRA is concerned with annual risk (i.e., all season) and only two depth-to-bedrock strata (≤ 6.1 m and >6.1 m). Thus, some additional calculations and assumptions were required to incorporate Borchardt et al.'s (2019) data into the current analysis.

The simplest case was for $i = 2$ (>6.1 m depth-to-bedrock), because this stratum is the same in both the current study and Borchardt et al. (2019). Here, F_2 was calculated as the average of $F_{2,\text{fall}}$ and $F_{2,\text{summer}}$ (Table S1). That is, the two seasons were weighted equally, and it was assumed that spring and winter could be characterized by that same average. The parameters $F_{2,\text{fall}}$ and $F_{2,\text{summer}}$ were each modeled as uniform distributions with (min, max) equal to the lower and upper limits, respectively, of corresponding 95% confidence intervals (CIs) from Table 2 of Borchardt et al. (2019) (Table S1).

For $i = 1$, additional steps had to be taken to combine Borchardt et al.'s (2019) two strata (<1.5 m and $1.5–6.1$ m) into the single ≤ 6.1 m strata used in the current study. For each season (fall and summer), the contamination rates for each of Borchardt et al.'s (2019) two strata were simulated as uniform distributions using their reported 95% CIs, as described above (Table S1). Stratum-specific contamination rates were then averaged within seasons, weighting by the number of wells sampled in each stratum during each season such that depth-to-bedrock strata were represented correctly and in proportion to their actual frequency in the county (Table S1). This method produced the parameters $F_{1,\text{fall}}$ and $F_{1,\text{summer}}$, which were averaged to calculate F_1 and assumed to apply year-round as described above for the corresponding $i = 2$ parameters (Table S1).

For S_{ij}, contaminated wells were randomly sampled ($n = 138$) during five seasonal sampling events, and qPCR analyses were used to identify pathogens and MST targets. Human-specific MST targets included adenovirus group A, *Bacteroidales*-like Hum M2, human *Bacteroides* (HF183), *Cryptosporidium hominis*, and group A rotavirus G1 P[8]. Bovine-specific MST targets included *Bacteroidales*-like Cow M2, *Bacteroidales*-like Cow M3, ruminant *Bacteroides*, bovine enterovirus, bovine polyomavirus, and group A rotavirus G10 P[11]. If neither or both types of MST target were present in a well sample, then the contamination source was considered unknown.

Finally, T was determined based on combining 2017 U.S. Census Bureau data for Kewaunee County (U.S. Census Bureau 2017) with additional data presented in Kewaunee County's 20-y plan (Bay-Lake Regional Planning Commission 2016). More specifically, Bay-Lake Regional Planning Commission data indicate that households in Kewaunee County are made up of 2.48 residents on average (based on 2010 population and housing data), whereas the U.S. Census Bureau estimates 2.6 residents per household for the year 2017 (U.S. Census Bureau 2017). Assuming one private well per household, these values were used to construct a uniform distribution (min = 2.2, max = 2.6) of residents per private well (Table S1).

Mean daily pathogen doses (D_{ijk}) were calculated for each depth-to-bedrock stratum i, source j, and pathogen k, where k ranges from 1 to 8 for each pathogen included in the QMRA. D_{ijk} was estimated as the product of mean pathogen concentrations (C_{ijk}) and per capita daily water consumption (V), i.e., $D_{ijk} = C_{ijk} \times V$. Daily water consumption was extrapolated from the empirical distribution for all ages listed in Table 3-23 of U.S. Environmental Protection Agency's *Exposure Factors Handbook* (U.S. EPA 2011) (see R Script S1 in Supplemental Material), and mean pathogen concentrations were determined based on results of the countywide pathogen occurrence study (Borchardt et al. 2021).

Furthermore, because pathogen concentrations in the occurrence study were measured using qPCR, dose harmonization was required to make them compatible with the dose units used for published dose–response studies. Thus, the quantity C_{ijk} (in units of infectious organisms per liter) is equal to $C_{\text{qPCR},ijk}/H$, where $C_{\text{qPCR},ijk}$ is in units of genomic copies per liter, and H is a dose harmonization factor with units of genomic copies per infectious organism (Table S2). Values of H for bacterial pathogens (*C. jejuni*, *Salmonella*, and EPEC) were extrapolated from published comparisons of qPCR and culture measurements collected

Table 1. Intermediate QMRA results for calculating exposure of Kewaunee residents to contaminated private wells (n, people per day), stratified by depth-to-bedrock and contamination source.

Depth to bedrock	Contamination source	Number of wells, W	Contaminated proportion, F	Source-associated proportion, S	People per well, T	People per day, n
≤ 6.1 m	Bovine feces	680 (510, 850)	0.41 (0.33, 0.50)	0.21 (0.13, 0.32)	2.4 (2.2, 2.6)	140 (70, 250)
	Human feces			0.26 (0.16, 0.36)		160 (90, 270)
	Unknown			0.53 (0.41, 0.64)		350 (220, 510)
>6.1 m	Bovine feces	4,170 (3,980, 4,330)	0.24 (0.19, 0.30)	0.29 (0.19, 0.41)		690 (420, 1,070)
	Human feces			0.098 (0.044, 0.18)		230 (100, 450)
	Unknown			0.60 (0.49, 0.71)		1,440 (1,010, 1,950)

Note: Point estimates are expected values based on 2DMC simulations; values in parentheses indicate 95% CIs determined during exposure assessment (see "Exposure Assessment" section for further details). As described by Equation 1, $N_{ij} = W_i \times F_i \times S_{ij} \times T$. Note also that the three depth-to-bedrock strata considered by Borchardt et al. (2021) were combined into two for the current analysis, as described in "General Approach" section (see also Table S1 for further details). 2DMC, 2-dimensional Monte Carlo; CI, confidence interval.

in surface water samples, with the value for EPEC extrapolated from that of *Salmonella* based on physiological similarity (i.e., both organisms are Gram-negative bacteria) (Corsi et al. 2016). Similarly, values for protozoan pathogens (all *Cryptosporidium* types and *G. duodenalis*) were extrapolated from published comparisons of qPCR and immunofluorescent antibody (IFA) measurements collected in groundwater from Minnesota (Stokdyk et al. 2019, 2020). Finally, the value of H for adenovirus was based on Kundu et al. (2013) because it is the most widely used value in the QMRA literature.

Dose–Response Assessment

Dose–response assessment was based on published methods for all pathogens (Table S3). Model selection for each pathogen was based on multiple criteria. Models validated against (or developed with) observational epidemiological data were selected when available, because these models would be expected to represent wild-type pathogens and natural host populations (i.e., including a mix of "normal," immune, and highly susceptible hosts). Dose–response models selected for this reason included the models for all three *Cryptosporidium* categories and *G. duodenalis* (Burch 2019, 2020; DuPont et al. 1995; Messner et al. 2001) as well as Teunis et al.'s (2008) EPEC model and the World Health Organization's *Salmonella* model (WHO 2002). In the absence of validated models, and in the presence of multiple alternative models, an attempt was made to select a model that predicted a moderate level of infectivity (i.e., for *C. jejuni*) (Medema et al. 1996; Schmidt et al. 2013). Finally, Crabtree et al.'s (1997) exponential dose–response model for adenovirus (and Haas et al.'s 1993 morbidity ratio) was selected because it is the most widely cited in the literature.

Dose–response models varied in terms of their underlying assumptions about the exposure and infection processes. The simplest model used was the exponential with morbidity:

$$P_{ill} = P_{ill|inf} \times [1 - \exp(-r_{inf}D)], \tag{2}$$

where P_{ill} is the probability of illness, $P_{ill|inf}$ is the morbidity ratio, r_{inf} is the exponential dose–response parameter (i.e., the exponential model's single-hit probability of infection), and D is mean daily pathogen dose. This model assumes that the number of pathogens in the exposure media are Poisson-distributed (with mean D), that a single infectious unit is capable of initiating infection, that the parameter r_{inf} is constant among hosts and/or exposures, and that some proportion of hosts (in the range $0 < P_{ill|inf} < 1$) will become ill once infected (Haas et al. 2014).

The next most complex model was the exact beta-Poisson (for illness outcomes):

$$P_{ill} = 1 - {}_1F_1(\alpha_{ill}, \alpha_{ill} + \beta_{ill}, -D), \tag{3}$$

where ${}_1F_1(...)$ is Kummer's confluent hypergeometric function, α_{ill} and β_{ill} are shape parameters for an assumed underlying beta distribution of the single-hit probability of illness (r_{ill}), and other parameters are as defined previously. This model also assumes that the number of pathogens in the exposure media are Poisson-distributed (with mean D) and that a single infectious unit is capable of initiating illness (Haas et al. 2014).

In some cases, the approximate beta-Poisson model (for illness outcomes) was also used:

$$P_{ill} = 1 - \left(1 + \frac{D}{\beta_{ill}}\right)^{-\alpha_{ill}}, \tag{4}$$

where α_{ill} and β_{ill} are the shape and rate parameters for an assumed underlying gamma distribution of r_{ill}, and other

parameters are as defined previously. This model also assumes that the number of pathogens in the exposure media are Poisson-distributed (with mean D) (Haas et al. 2014).

The exact beta-Poisson with morbidity model was defined as:

$$P_{ill} = P_{ill|inf} \times [1 - {}_1F_1(\alpha_{inf}, \alpha_{inf} + \beta_{inf}, -D)], \tag{5}$$

where α_{inf} and β_{inf} are shape parameters for an assumed underlying beta distribution of r_{inf}, and other parameters are as defined previously. This model also assumes that the number of pathogens in the exposure media are Poisson-distributed (with mean D), that a single infectious unit is capable of initiating infection, and that some proportion of hosts (in the range $0 < P_{ill|inf} < 1$) will become ill once infected (Haas et al. 2014).

Finally, for the logistic-normal Poisson with morbidity model, the basic underlying dose–response relationship is assumed to be exponential (with a morbidity ratio), as in Equation 2. However, to incorporate variability in natural populations, the parameters r_{inf} and $P_{ill|inf}$ are also assumed to follow logistic-normal distributions (Burch 2020):

$$logit_{10}(r_{inf}) \sim Normal(\mu_r, \sigma_r), \tag{6}$$

$$logit_{10}(P_{ill|inf}) \sim Normal(\mu_{Pill|inf}, \sigma_{Pill|inf}), \tag{7}$$

where the function $logit_{10}$ is a log_{10}-based logistic function, e.g., $logit_{10}(r_{inf}) = log_{10}[r_{inf}/(1-r_{inf})]$. Furthermore, the parameters μ_r and $\mu_{Pill|inf}$ are mean values (in logistic space) of r and $P_{ill|inf}$, and the parameters σ_r and $\sigma_{Pill|inf}$ are corresponding standard deviations. This model also assumes that the number of pathogens in the exposure media are Poisson-distributed (with mean D), that a single infectious unit is capable of initiating infection, and that some proportion of hosts (in the range $0 < P_{ill|inf} < 1$) will become ill once infected.

Risk Characterization

The primary output of interest was predicted total AGI cases per year. This value was calculated by summing predicted AGI cases across all pathogens and across all strata in the data set. Individual summands were equal to $N_{ij} \times P_{ill,ijk} \times 365$ d/y, where N_{ij} represents exposed residents (people per day) in depth-to-bedrock stratum i for fecal source j (see Equation 1) and $P_{ill,ijk}$ is mean daily risk of illness for pathogen k within depth-to-bedrock stratum i for fecal source j. Values of N_{ij} and $P_{ill,ijk}$ were calculated using 2-dimensional Monte Carlo (2DMC) simulations in R [version 3.5.1 (R Development Core Team) with the package mc2d (Pouillot and Delignette-Muller 2010)]. This approach allows independent inclusion of the effects of variability and uncertainty on risk estimates, where variability refers to heterogeneity in natural exposure conditions, and uncertainty refers to heterogeneity in model parameters.

A tabulation of all QMRA inputs and their treatment with respect to 2DMC simulations is available in Table S1. QMRA inputs with variability components included mean pathogen concentrations and per capita daily water consumption. QMRA inputs with uncertainty components also included mean pathogen concentrations, along with dose harmonization factors, dose–response model parameters, and inputs used to calculate N_{ij}. The variability and uncertainty of most QMRA inputs was estimated based on published literature. Variability and uncertainty in pathogen concentrations were estimated by bootstrap sampling the original data set of Borchardt et al. (2021) for each depth-to-bedrock stratum i, fecal source j, and pathogen k. As part of this procedure, individual concentration measurements were assigned season-specific weights to ensure equal representation of fall,

winter, spring, and summer in annual risk estimates. These weights were based on the total number of observations per season for each *ijkm* subset of Borchardt et al.'s (2021) data (see Table S1 for further details).

Each 2DMC simulation consisted of 4 million iterations, with 2,000 iterations in the variability dimension and 2,000 iterations in the uncertainty dimension. Simulation size was optimized by examining the stability of total predicted cases as a function of the number of iterations at intervals between 2.5×10^2 and 6×10^6 iterations. Uncertainty in QMRA outputs was summarized using 95% CIs, which were calculated using the 2.5th and 97.5th percentiles of output distributions.

Results

Exposure Assessment

The two major quantities of interest during exposure assessment were the number of Kewaunee residents exposed to contamination (per day) and the daily exposure doses (infectious organisms) of each pathogen. These two quantities, each stratified by depth-to-bedrock and contamination source, are presented in this section.

Countywide (i.e., across all strata in the data set), approximately 3,010 Kewaunee County residents were estimated to be exposed to contaminated private well water each day. Totals for individual strata ranged from as few as 140 people per day to as many as 1,440 people per day (Table 1). Exposures associated with bovine contamination accounted for nearly 30% of total exposures (830 people per day), whereas exposures associated with human contamination accounted for 13% (390 people per day); almost 60% of daily exposures (1,790 people per day) could not be associated with a single fecal source based on MST. By depth-to-bedrock, more than 75% of daily exposures (2,360 people per day) occurred in the >6.1 m category, reflecting the fact that few wells are constructed in the ≤6.1 m category (680 vs. 4,170 wells; Table 1).

Mean doses for predicted exposure varied by strata and pathogen (Table 2). The highest exposure levels were for nontyphoidal *Salmonella* and *C. parvum*. Mean daily doses for *Salmonella* were predicted to exceed 10^{-2} CFU per person per day in ≤6.1 m depth-to-bedrock when associated with either bovine or human fecal contamination. Mean daily doses of *C. parvum* were predicted to exceed 10^{-2} oocysts per person per day when associated with bovine fecal contamination. In contrast, mean daily doses were generally ≤10^{-3} infectious organisms per person per day for all other pathogens, including many pathogens with predicted mean daily doses equal to zero in most strata.

Predicted Cases of AGI

Countywide, the QMRA predicted a total of 301 AGI cases per year (95% CI: 80, 2,200) due to contaminated private wells (Table 3). By individual pathogen, the largest number was associated with *C. parvum*, which was predicted to cause 190 AGI cases per year (95% CI: 2, 1,380); other pathogens associated with abundant case numbers included ungenotyped *Cryptosporidium* spp. and *Salmonella*, each of which was predicted to cause ≥40 AGI cases per year (Table 3). Five gastrointestinal pathogens known to infect humans were not detected in study wells (adenovirus group B, adenovirus groups CDF, enterovirus, norovirus genogroup I, norovirus genogroup II; Borchardt et al. 2021) and therefore did not contribute to the predicted number of waterborne AGI cases from private wells. Rotavirus groups A and C were detected in study wells and would be expected to increase the estimated number of cases, but they were not included in the QMRA due to

Table 2. Predicted daily exposure doses for all modeled pathogens, stratified by depth-to-bedrock and contamination source.

Pathogen	≤6.1 m depth to bedrock			>6.1 m depth to bedrock		
	Bovine feces	Human feces	Unknown	Bovine feces	Human feces	Unknown
Adenovirus A (TCID$_{50}$)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	1.4×10^{-4} (0.0, 4.3×10^{-4})	0.0 (0.0, 0.0)
Campylobacter jejuni (CFU)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	5.7×10^{-3} (0.0, 1.5×10^{-2})	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
EPEC (CFU)	0.0 (0.0, 1.4×10^{-1})	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	4.8×10^{-3} (0.0, 1.8×10^{-2})
Salmonella (CFU)	3.0×10^{-1} (0.0, 8.3×10^{-1})	6.9×10^{-2} (0.0, 6.6×10^{-1})	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 2.1×10^{-2})
Cryptosporidium hominis (oocysts)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 2.7×10^{-4})	0.0 (0.0, 0.0)
Cryptosporidium parvum (oocysts)	1.9×10^{-2} (2.3×10^{-4}, 4.8×10^{-2})	7.4×10^{-5} (0.0, 1.2×10^{-3})	8.3×10^{-5} (0.0, 2.3×10^{-4})	5.4×10^{-2} (9.4×10^{-3}, 1.2×10^{-1})	0.0 (0.0, 0.0)	1.9×10^{-4} (0.0, 4.8×10^{-2})
Cryptosporidium spp. (oocysts)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	3.9×10^{-3} (0.0, 1.3×10^{-2})	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Giardia duodenalis (cysts)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 4.3×10^{-3})	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 3.9×10^{-3})

Note: Point estimates are mean daily doses derived from 2DMC simulations (see "Risk Characterization" section); bracketed values indicate 95% CIs reflecting uncertainty in model inputs. 2DMC, 2-dimensional Monte Carlo; CI, confidence interval.

Table 3. Predicted AGI cases per year among private well users in Kewaunee County, stratified by depth-to-bedrock and contamination source.

Pathogen	≤6.1 m depth to bedrock			>6.1 m depth to bedrock			Total by pathogen
	Bovine feces	Human feces	Unknown	Bovine feces	Human feces	Unknown	
Adenovirus A	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (0, 10)	0 (0, 0)	2 (0, 10)
Campylobacter jejuni	0 (0, 0)	0 (0, 0)	2 (0, 20)	0 (0, 0)	0 (0, 0)	0 (0, 0)	2 (0, 20)
EPEC	0 (0, 270)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	7 (0, 500)	7 (0, 770)
Salmonella	30 (0, 120)	10 (0, 100)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 30)	40 (0, 180)
Cryptosporidium hominis	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 7)	0 (0, 0)	0 (0, 7)
Cryptosporidium parvum	10 (0.005, 90)	0.2 (0, 1)	0.1 (0, 1)	130 (0.2, 1,080)	0 (0, 0)	50 (0, 540)	190 (2, 1,380)
Cryptosporidium spp.	0 (0, 0)	0 (0, 0)	0 (0, 0)	60 (0, 470)	0 (0, 0)	0 (0, 0)	60 (0, 470)
Giardia duodenalis	0 (0, 0)	0 (0, 0)	0 (0, 10)	0 (0, 0)	0 (0, 0)	0 (0, 40)	0 (0, 40)
Total by contamination source (within depth-to-bedrock)	40 (0.5, 410)	10 (0, 110)	2 (0.006, 30)	190 (2, 1,200)	2 (0, 10)	57 (0.009, 910)	301 (80, 2,200)
Total by depth to bedrock	52 (10, 450)			249 (30, 1,800)			

Note: Point estimates are expected number of cases derived from 2DMC simulations (see "Risk Characterization" section); values in parentheses indicate 95% CIs reflecting uncertainty in model inputs. 2DMC, 2-dimensional Monte Carlo; CI, confidence interval.

limitations in available qPCR assays and dose–response models, respectively (see "Hazard Identification" section).

More cases were predicted to occur in >6.1 m depth-to-bedrock (249 cases per year) than in ≤6.1 m depth-to-bedrock (52 cases per year), and more cases were associated with a bovine fecal source (230 cases per year) than with a human fecal source (12 cases per year). However, a relatively large number of predicted cases (59 cases per year) could not be successfully associated with either fecal source, because pathogens often co-occurred with either both source-specific MST markers or neither.

The predicted etiology of AGI cases differed between the two depth-to-bedrock strata (Table 3). Within the ≤6.1 m depth-to-bedrock stratum, the largest number of cases were associated with *Salmonella*, which was predicted to cause 40 cases per year in this stratum. In contrast, *Salmonella* was associated with few cases in the >6.1 m depth-to-bedrock stratum, where the largest number of cases were associated with *C. parvum* and ungenotyped *Cryptosporidium* spp. (predicted to cause 180 and 60 cases per year, respectively, in the >6.1 m depth-to-bedrock stratum).

Similarly, the predicted etiology of AGI cases also differed among the three contamination sources (Table 3). Within the human source stratum, *Salmonella* was associated with the largest number of cases (10 cases per year), whereas in the bovine source stratum, *C. parvum* (140 cases per year), ungenotyped *Cryptosporidium* spp. (60 cases per year), and *Salmonella* (30 cases per year) were associated with the largest number of cases. A large number of predicted *C. parvum* cases (50 cases per year) were also associated with an unknown contamination source.

Finally, the numbers of annual AGI cases predicted by QMRA were subject to a high degree of uncertainty (Table 3). The upper and lower boundaries of 95% CIs for most pathogens typically varied by an order of magnitude or more from their corresponding point estimates, and within individual strata, many 95% CIs for individual pathogens could not exclude the value zero. Nonetheless, when predicted cases were summed across pathogens and strata for the county as a whole, many 95% CIs did exclude zero, which provides a high degree of confidence that the pathogen contamination observed by Borchardt et al. (2021) are responsible for some level of waterborne AGI in the county.

Discussion

The current study used QMRA coupled with pathogen measurements from Borchardt et al. (2021) to predict the total AGI cases per year in Kewaunee County due to contamination of private wells by bovine and human fecal material. The estimated number of cases was stratified by two factors that affect groundwater contamination in Kewaunee County, depth-to-bedrock and fecal source, to show the influence of these factors and inform the prioritization of proposed policy interventions in the county.

Risk of Waterborne AGI Due to Private Wells

The risk of waterborne AGI due to private wells has only been quantified in one prior study. Murphy et al. (2016a) estimated the risk of waterborne AGI due to five index pathogens for private wells throughout Canada using QMRA and extrapolated pathogen levels from the published literature. Murphy et al.'s (2016a) overall annual risk estimate (2.7×10^{-2} illnesses per person per year) is similar to that of the current study, which can be calculated as $301/11,510 = 2.6 \times 10^{-2}$ illnesses per person per year. The denominator in this calculation is total people per year at risk of exposure, or $(W_1 + W_2) \times T$ based on the current study's defined terms (see Equation 1, Table 1, and R Script S2 in online Supplemental Material). Although the overall risk estimate reported by Murphy et al. (2016a) was similar to our estimate, the predicted etiologies were different.

The hazard identification and exposure assessment steps of our QMRA produced site-specific etiologies for AGI associated with private well water. Using empirical pathogen measurements, the current study predicts the majority of AGI cases for private wells in Kewaunee County to be caused by *Cryptosporidium parvum* (63% of 301 total cases per year), followed by ungenotyped *Cryptosporidium* spp. (20%), and *Salmonella* (13%) (with other pathogens each contributing <3% of total cases). Using published values for pathogen occurrence and concentration, Murphy et al. (2016a) predicted the majority of AGI cases for private wells in Canada to be caused by norovirus (71% of 78,073 total cases per year), followed by *Cryptosporidium* (15%), and *Campylobacter* (12%) (with other pathogens each contributing <2% of total cases). Because dose–response models used by Murphy et al. (2016a) were similar to those used in the current study, the discrepancy in etiologies seems largely attributable to the identity and quantity of pathogens considered.

The empirical exposure assessment of the current study produced site-specific predictions for the etiologies of AGI, so pathogens absent from the published literature were included, whereas others common in the literature were excluded. For example, the current study predicted a relatively large number of cases due to *Salmonella*, but Murphy et al. (2016a) did not include *Salmonella* among their index pathogens. Similarly, Murphy et al. (2016a) predicted a large number of norovirus cases, but Borchardt et al. (2021) never detected norovirus in groundwater samples from Kewaunee County ($n = 138$ private well samples). Thus, norovirus was excluded from further

consideration in the current QMRA. QMRAs that rely on published pathogen measurements may misrepresent risk or etiologies because some pathogens are underrepresented in the groundwater literature (Stokdyk et al. 2019, 2020). Because the expected etiologies for waterborne AGI have implications for water management, like treatment and mitigation strategies, site-specific exposure assessments can contribute to efficient and effective groundwater management.

Finally, although Murphy et al.'s (2016a) approach and etiological results differed from ours, it is notable that their overall risk estimate was virtually identical. This is particularly interesting given the fact that their study examined all private wells in Canada, whereas ours was conducted for private wells influenced by major documented fecal sources. The explanation here is likely related to the microbiological methods underlying many QMRAs. More specifically, given the power of most environmental pathogen occurrence studies, very low pathogen concentrations consistent with frequently cited U.S. and international acceptable risk thresholds are exceedingly difficult to detect in practice (WHO 2017). Thus, most occurrence studies published in the peer-reviewed scientific literature (i.e., those reporting positive pathogen detections) tend to find the same relatively high concentrations, which are consistent with higher risk values (Owens et al. 2020). Because Murphy et al. (2016a) extrapolated data from previously published occurrence studies to all private wells in Canada, it is perhaps unsurprising in retrospect that the pathogen concentrations they used produced relatively high risk estimates.

Waterborne AGI Rates and All-Cause AGI

For context, it is informative to compare the current study's results to estimated incidence rates of waterborne AGI in the United States and other developed countries. Unlike estimates in the current study, which is focused on private wells, the majority of comparable estimates are for public drinking water systems. Such systems typically serve multiple households, often treat their source water (e.g., using filtration and/or disinfection), and sometimes rely on surface water instead of (or in addition to) groundwater. For example, waterborne AGI has been estimated to occur at a rate of 1.5×10^{-2} to 6.0×10^{-2} illnesses per person per year for public systems in the United States, with slight variation due to source (i.e., surface water vs. groundwater) (Colford et al. 2006; Messner et al. 2006). Similarly, waterborne AGI has been estimated to occur at a rate of 7×10^{-3} to 4.7×10^{-2} illnesses per person per year for municipal drinking water systems in Canada, with variation across source, system size, and extent of water treatment (Murphy et al. 2016a, 2016b). The current study's overall annual risk estimate (2.6×10^{-2} illnesses per person per year) for private well users in Kewaunee County falls within both ranges. However, the degree of uncertainty associated with estimated rates of waterborne AGI (including in the current study) precludes more detailed evaluation of potential sources of variability across studies.

Additional perspective can be gained by comparing the current study's results to estimated incidence rates of all-cause AGI (i.e., those including foodborne transmission, person-to-person transmission, and other exposure routes). In the United States, the rate of all-cause AGI has been estimated at 6.5×10^{-1} illnesses per person per year, with substantial uncertainty due to study design and case definitions (range: 3×10^{-1} to 3.48×10^{0} illnesses per person per year) (Roy et al. 2006). Extrapolating this value to Kewaunee County, waterborne AGI probably accounts for approximately 4% of all AGI in the county ($2.6 \times 10^{-2}/6.5 \times 10^{-1} = 4 \times 10^{-2}$). This proportion appears to be relatively high when compared to analogous values for private wells and municipal drinking water systems in Canada (0.5% and 1.7%, respectively) (Murphy et al. 2016a, 2016b), though it is also somewhat low when compared to the estimated proportion of all-cause AGI associated with public water systems in the United States (8.5%) (Messner et al. 2006). Furthermore, and as already referred to, the level of uncertainty in all of these estimates may obscure such comparisons.

Comparison to U.S. EPA's Acceptable Risk Benchmark

The current study's results suggest that drinking water from private wells in Kewaunee County exceeds the U.S. EPA's defined acceptable risk threshold for public water systems, which is 1×10^{-4} infections per person per year (U.S. EPA 1989). This conclusion is based on the overall annual risk estimate of 2.6×10^{-2} illnesses per person per year and the biological principle that illness is conditional on infection (i.e., that the risk of infection would be expected to be even higher than the estimated risk of illness) (Haas et al. 2014). Moreover, it is noteworthy that the threshold of 1×10^{-4} infections per person per year is exceeded in both depth-to-bedrock categories ($52/1,620 = 3.2 \times 10^{-2}$ and $249/9,890 = 2.5 \times 10^{-2}$ illnesses per person per year for ≤6.1 m and >6.1 m, respectively) and for nearly all individual pathogens, including *C. parvum* ($190/11,510 = 1.7 \times 10^{-2}$ illnesses per person per year), ungenotyped *Cryptosporidium* spp. ($60/11,510 = 5.2 \times 10^{-3}$ illnesses per person per year), *Salmonella* ($40/11,510 = 3.5 \times 10^{-3}$ illnesses per person per year), EPEC ($7/11,510 = 6.1 \times 10^{-4}$ illnesses per person per year), adenovirus A ($2/11,510 = 1.7 \times 10^{-4}$ illnesses per person per year), and *C. jejuni* ($2/11,510 = 1.7 \times 10^{-4}$ illnesses per person per year). Of note, the U.S. EPA's acceptable risk threshold of 1×10^{-4} infections per person per year is specifically applicable to public water systems (U.S. EPA 1989), so private wells are not necessarily required to meet this same standard. Nonetheless, acceptable risk thresholds have never been established specifically for private wells, so the public water system standard represents the only available acceptable risk threshold with which to compare.

For international context, it may also be helpful to describe results in relation to the WHO's acceptable risk threshold of 10^{-6} disability adjusted life-years (DALYs) per person per year (WHO 2017). Strictly speaking, calculation of DALYs was outside the scope of the current study due to our study site's location in the United States and the precedent there of using an acceptable risk threshold based on incidence rates. Nonetheless, because the U.S. EPA and WHO thresholds are roughly equivalent and because our overall risk estimate very clearly does not adhere to the U.S. EPA threshold (Owens et al. 2020), it can be presumed that it also does not adhere to the WHO threshold. For example, assuming a conversion of 1.5×10^{-3} DALYs illness^{-1} for *Cryptosporidium* (WHO 2017), our *C. parvum* risk estimate alone is 2.6×10^{-5} in the current study, more than an order of magnitude greater than the WHO threshold.

Comparison to Notifiable Disease Data in Kewaunee County

Several of the illnesses caused by pathogens in the current study are notifiable diseases, and comparison of predicted case counts from this study to corresponding reported case counts from public health authorities is helpful for putting results in context. For example, the Wisconsin Department of Health Services (WDHS) reported fewer than five cases of cryptosporidiosis and six cases of salmonellosis for Kewaunee County in 2015 (the most recent year for which data are available) (WDHS 2017). Accounting for known biases in these numbers, specifically combined underreporting and underdiagnosis factors of 100 for cryptosporidiosis and 30 for nontyphoidal salmonellosis (Scallan et al. 2011), leads to estimated "true" values of fewer than 500 cryptosporidiosis

cases and approximately 180 nontyphoidal salmonellosis cases in the county per year. Two conclusions can be drawn from this analysis. First, the predicted case counts for *Cryptosporidium* (250 total when summing both ungenotyped *Cryptosporidium* spp. and *C. parvum*) and *Salmonella* (40 total) in Table 3 are reasonable given reported public health data, providing some assurance that this QMRA is producing realistic results. Second, it seems as if substantial proportions of cases for both pathogens in Kewaunee County may be caused by drinking contaminated private well water.

Strengths and Limitations

The current study relied on a large site-specific pathogen occurrence study. This occurrence study included 138 private well samples collected during five countywide sampling events from April of 2016 to March of 2017, with at least one sampling event per season (two were in spring). Furthermore, the occurrence study provided data on 15 waterborne pathogens for the current study's QMRA (8 of which were detected and used to produce risk estimates), a relatively broad scope largely enabled by Borchardt et al.'s (2021) use of qPCR (as opposed to traditional culture and/or microscopy techniques). Although it is unlikely that all organisms identified via qPCR are viable (Haas 2020), our use of dose–harmonization (see "Exposure Assessment" section) helps mitigate this concern. The data we used for dose–harmonization are the most up-to-date data available, and because most of them are based on *in situ* environmental measurements, they adjust for viability while simultaneously providing unit conversions from qPCR units to units used in dose–response models.

The combination of site-specific measurements and a broad suite of pathogens produced risk estimates and etiologies that represent the study area. In addition, our evaluation of depth-to-bedrock and the fecal sources associated with pathogen presence can inform public policy to mitigate illness from private well water. Finally, the dose–response assessment for *C. parvum*, which was associated with the largest number of cases out of all pathogens in the current study, has been previously validated against retrospective epidemiological data from outbreaks of waterborne cryptosporidiosis (Burch 2019), adding confidence in the current study's AGI estimates.

The current study's limitations are largely related to its basis in predictive mathematical modeling (i.e., QMRA). Ideally, health risk would have been estimated using epidemiological methods; that is, the distribution of AGI in Kewaunee County would have been enumerated empirically in a randomized sample of its residents, after which the occurrence of cases could have been related to the exposure of interest (i.e., waterborne pathogens in private wells). As in many other settings, however, this approach is currently infeasible for Kewaunee County due to a combination of financial, logistical, and ethical constraints, and QMRA was pursued as a feasible alternative. Importantly, the quality of risk estimates is limited by the collective quality of QMRA inputs, and correspondingly, the uncertainty of risk estimates is considerable due to uncertainty in underlying inputs (e.g., see 95% CIs on predicted cases in Table 3). Nonetheless, this level of uncertainty is typical of similar QMRAs (e.g., see Murphy et al. 2016a), and in the absence of an empirical epidemiological study, the current study's results represent the best available data with which to interpret the public health impacts of Borchardt et al.'s (2021) empirical results.

Related to its predictive nature, the current study is also limited by the major assumptions used to produce risk estimates. In particular, these include assumptions that *a*) all private well users in Kewaunee County drink their tap water untreated; *b*) that private well users who are not exposed to "contamination" (i.e., to >0 total coliforms, >0 *E. coli*, and/or $NO_3 - N > 10 \text{ mg/L}$) are not exposed to waterborne gastrointestinal pathogens; *c*) that well sampling in Borchardt et al.'s (2021) occurrence study was representative with respect to temporal variations within seasons; and *d*) that MST markers indicate the source of fecal-borne pathogens. The first assumption likely increases risk estimates relative to their "true" values, whereas the second likely decreases them (because pathogen occurrence tends not to be perfectly correlated with indicators of fecal contamination, and so some pathogens are likely present in private wells without measurable fecal indicators). The direction of bias potentially introduced by the third major assumption is unknown but could be addressed by conducting sampling events even more frequently within seasons during future studies. Likewise, the direction of potential bias due to the fourth assumption is also unknown, because multiple fecal sources can affect a single well, including fecal sources not tested, and MST analysis is subject to false-negative results when concentrations in groundwater are low.

Policy Implications and Future Research Needs

The current study's predictions suggest several potentially effective strategies for risk mitigation. For example, interventions focused on reducing the impact of cattle manure (e.g., improved treatment technologies and/or changes in land application practices) seem to hold the greatest overall potential for reducing risk, as 230 of 301 cases per year were associated with a bovine fecal source (Table 3). Similarly, farm management practices focused on reducing the impact of *C. parvum* appear to hold promise, as *C. parvum* was predicted to cause 190 cases per year (140 of which were associated with a bovine fecal source; Table 3). Finally, further research in the county would also be valuable, particularly an observational epidemiological study. Such a study could validate (or refute) the current study's risk estimates, especially in light of the current study's uncertainty for predicted case numbers and provide additional data with which to evaluate proposed policy interventions. More generally, and perhaps most importantly, multiple policy options are likely available and multiple interventions may be required to substantially reduce risk.

Acknowledgments

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