ARTICLE IN PRESS

International Journal of Hygiene and Environmental Health xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

On the use of total aerobic spore bacteria to make treatment decisions due to *Cryptosporidium* risk at public water system wells

Philip Berger, Michael J. Messner*, Jake Crosby, Deborah Vacs Renwick, Austin Heinrich

Office of Water, US Environmental Protection Agency, Washington DC, United States

ARTICLE INFO

Keywords:
Total aerobic spores
Alluvial aquifers
Inadequately treated groundwater
Surface water induced recharge
Cryptosporidium

ABSTRACT

Spore reduction can be used as a surrogate measure of *Cryptosporidium* natural filtration efficiency. Estimates of log10 (log) reduction were derived from spore measurements in paired surface and well water samples in Casper Wyoming and Kearney Nebraska. We found that these data were suitable for testing the hypothesis (H_0) that the average reduction at each site was 2 log or less, using a one-sided Student's *t*-test. After establishing data quality objectives for the test (expressed as tolerable Type I and Type II error rates), we evaluated the test's performance as a function of the (a) true log reduction, (b) number of paired samples assayed and (c) variance of observed log reductions. We found that 36 paired spore samples are sufficient to achieve the objectives over a wide range of variance, including the variances observed in the two data sets. We also explored the feasibility of using smaller numbers of paired spore samples to supplement bioparticle counts for screening purposes in alluvial aquifers, to differentiate wells with large volume surface water induced recharge from wells with negligible surface water induced recharge. With key assumptions, we propose a normal statistical test of the same hypothesis (H_0), but with different performance objectives. As few as six paired spore samples appear adequate as a screening metric to supplement bioparticle counts to differentiate wells in alluvial aquifers with large volume surface water induced recharge. For the case when all available information (including failure to reject H_0 based on the limited paired spore data) leads to the conclusion that wells have large surface water induced recharge, we recommend further evaluation using additional paired biweekly spore samples.

1. Introduction

Drinking water providers are often charged with making treatment decisions based on limited information about the microbial pathogen hazard at a well or wellfield (e.g. Hancock et al., 1998). Often, these decisions are primarily based on indicator bacteria rather than pathogen occurrence. In the United States, public water systems (PWSs) are routinely tested for total coliform (TC) and E. coli (EC) under the total coliform and revised total coliform rules (TCR) (USEPA, 2013). In an evaluation of TC and EC data collected by undisinfected PWS wells for the year 2011 (TCR time period), Messner et al. (2017) estimated that about 5% of all undisinfected PWS wells (about several thousand across the United States) have TC detection rates of 20% or greater. They suggested that higher TC detection rates may indicate recent 1) vertical infiltration from the surface or 2) pumping-induced lateral recharge from adjacent surface water.

In the United States, public health authorities have established practices or regulations that govern the public health response when a well water sample is EC positive. Messner et al. (2017) suggest that, for undisinfected PWS wells with high TC detection rates but no EC occurrence, total aerobic spore bacterial samples collected together with TC samples could be used to gain additional information about well vulnerability to pathogens.

Total aerobic spores, like the total coliform, are ubiquitous bacteria, primarily found in soil (Headd and Bradford, 2016; Bradford et al., 2016). Like the total coliform, the total aerobic spores are transported from the soil into surface water by overland flow. Spores may also be entrained within infiltrating ground water and transported to the saturated ground water zone where they may enter wells directly due to pumping. Additionally, spores may enter ground water from surface water by normal surface water/groundwater exchange or be induced into ground water by well pumping. Unlike the total coliforms, spores are resistant to inactivation because the vegetative cell is enclosed by an environmentally protective coat (Headd and Bradford, 2016). Spores

Abbreviations: EC, *E. coli*; L, liter; log, log base 10; H_0, null hypothesis; mL, milliliter; PWS, public water system(s); SFU, spore forming units; TAS, total aerobic spore; TC, total coliform TCR total coliform rule; USEPA, US Environmental Protection Agency

* Corresponding author at: Office of Ground Water and Drinking Water (4607M), 1200 Pennsylvania Ave. NW, Washington DC, 20460, United States.

E-mail addresses: berger.philip@epa.gov (P. Berger), messner.michael@epa.gov (M.J. Messner), crosby.jake@epa.gov (J. Crosby), vacsrenwick.deborah@epa.gov (D. Vacs Renwick), heinrich.austin@epa.gov (A. Heinrich).

https://doi.org/10.1016/j.ijheh.2018.03.001
Received 16 November 2017; Received in revised form 28 February 2018; Accepted 7 March 2018
1438-4639/ Published by Elsevier GmbH.

may survive for years in the subsurface (Meschke, 2001; Setlow, 2007), as compared with total coliforms, which may survive only weeks (Sidhu et al., 2015). Spores are found at high concentrations in surface water (Weiss et al., 2005).

Given pathogen sources at close proximity to surface locations of wells, water recharge, infiltrating vertically from the ground surface could entrain viral, bacterial and parasitic protozoan pathogens *(e.g., Cryptosporidium*). Messner et al. (2017) suggest three metrics for using spores to evaluate well vulnerability to pathogens: 1) spore presence/absence in well water, 2) spore concentration in well water or 3) spore log10 (log) reduction, based on paired surface and well water samples in sandy alluvial aquifers. Wells believed to be receiving high volumes of recent surface water would be evaluated using paired samples. Wells likely receiving only infiltrating recharge or a mixture of infiltration and induced surface water could be evaluated by any of the three metrics. However, paired spore samples probably are most informative for wells located in alluvial aquifers (stream deposits).

In this paper, we focus only on the relative vulnerability of wells in sandy alluvial aquifers inducing large volumes of recent surface water as shown by spore log reduction values. Wells with paired spore samples showing large reductions are less likely to be producing well water with a large volume of recent surface water. Measured spore reduction in sandy alluvial aquifers by subsurface passage (natural filtration) is recommended in USEPA guidance (USEPA, 2010) as an alternative treatment.

We use statistical analysis to evaluate some of the available total aerobic spore log reduction data. We evaluate biweekly spore data from two sites: 1) Casper Wyoming (data available from 2002 to 2017, e.g. Gollnitz et al., 2005), and 2) Kearney Nebraska (data available for 2011–2012, Miller and Miller and Associates, 2013), (referred to as “Casper” and “Kearney,” respectively). At each site, “demonstration of performance” studies were conducted using methods consistent with those recommended in EPA’s Long Term 2 Enhanced Surface Water Treatment Rule Toolbox Guidance Manual (USEPA, 2010). One site (Kearney) has the minimum number of spore samples recommended by the document (36 paired samples collected over 18 months); the other site (Casper) has a large number of supplemental spore samples.

We establish quantitative objectives for demonstrating log reduction, then use Casper and Kearney data to: 1) assess statistical variability for the two data sets, 2) evaluate the statistical power associated with different numbers of paired samples and 3) assess the impact of differing levels of variability on the statistical power of a fixed number of paired samples. Finally, we consider the use of paired spore samples as part of a screening decision, i.e. to decide whether a well is receiving large volumes of recent surface water, based on a much smaller number of samples.

2. Total aerobic spores as surrogates for *Cryptosporidium*

Due to their environmental resistance, physical properties, and ubiquity in ambient waters, total aerobic spores are used to indicate the vulnerability of wells inducing large volumes of recent surface water into wells located in sandy alluvial aquifers. Paired surface water and ground water spore measurements are recommended by USEPA guidance (USEPA, 2010) as surrogate measures of *Cryptosporidium* removal (reduction) via subsurface passage (natural filtration). Headd and Bradford (2016) and Bradford et al. (2016) reviewed and analyzed the suitability of total aerobic spores as *Cryptosporidium* surrogates in ground water. They conclude; *“spores and oocysts share many commonalities with regard to biology and survivability, and the environmental prevalence and ease of detection make aerobic spores a promising surrogate for Cryptosporidium oocysts in surface and groundwater.”* However, the authors suggest that more research is needed. For example, as reported by Bradford et al. (2016), enhanced *Cryptosporidium* mobility in sand aquifers occurs at a range of physicochemical conditions.

Wells located near surface water can receive substantial recent recharge from that surface water, either ponded water or from flowing streams, enhancing the likelihood of *Cryptosporidium* occurrence in well water (Gallas-Lindemann et al., 2013). In the United States and elsewhere, water from many wells located on lake or river banks receive chlorination plus full conventional treatment, including coagulation, settling and rapid sand filtration (Hunt et al., 2003). As was demonstrated in Davenport, Iowa (Colford et al., 2005), optimized conventional surface water treatment plants using engineered filtration can provide adequate treatment of poor quality surface water thereby minimizing the *Cryptosporidium* hazard. However, wells located adjacent to surface water and producing water from sand and gravel (alluvial) aquifers rely on bioparticle reduction by subsurface passage and disinfection (Ray et al., 2002; Abbaszadegan et al., 2011). If the disinfection process includes treatment by ultraviolet (UV) light, then the *Cryptosporidium* hazard is minimized (USEPA, 2006). However, if the PWS relies only on subsurface passage and chlorination, then an unknown magnitude *Cryptosporidium* hazard remains because *Cryptosporidium* is resistant to inactivation by chlorination and may be incompletely removed by subsurface passage (e.g. Hancock et al., 1998).

Gallas-Lindemann et al. (2013) collected samples from “radial and vertical wells” from 2009 to 2010 and report 5 (of 66) well samples positive for *Cryptosporidium*. “Radial wells” are horizontal collector wells and are always installed in alluvial or other sand aquifer types. *Cryptosporidium* measurements from the adjacent Rhine river were 66–250 oocysts/1000 Liter (L) while *Cryptosporidium* measurements from the wells were 4–66 oocysts/1000 L, when detected. Based on these data, Gallas-Lindemann et al. (2013) suggest that the Rhine bank-filtered wells exhibited a *Cryptosporidium* reduction of “1-2 orders of magnitude.” *Cryptosporidium* occurrence in well water is also reported in bedrock aquifers such as karst limestone (Füchslin et al., 2012; Khaldi et al., 2011; Schijven et al., 2003).

Total aerobic spore removal to monitor drinking water treatment plant bioparticle removal performance is well established (Brown and Cornwell, 2007; Nieminski et al., 2000; Mazoua and Chauveheid, 2005; Huertas et al., 2003). Others have used spores of sulfite-reducing clostridia (anaerobic bacteria) to monitor treatment plant performance (e.g. Hijnen et al., 2000) or to evaluate log reduction by subsurface passage (e.g. Schijven et al., 1998).

Although there is no US EPA-approved standard method for total aerobic spore assay in drinking water samples, the laboratories performing the sample assays for Casper and Kearney used a protocol similar to that described in Rice et al. (2012).

Assuming spores are *Cryptosporidium* surrogates, we evaluate the reduction of total aerobic spores by subsurface passage from river to well water in sandy alluvial aquifers. We assume that high spore reduction is equivalent to high *Cryptosporidium* reduction and therefore lower *Cryptosporidium* risk. We focus on spore reduction and its uncertainty because 1) spore reduction is adequately measured in multiple locations (Weiss et al., 2005; Ray et al., 2002; Gollnitz et al., 2005, Miller and Miller and Associates, 2013) and 2) Cryptosporidium hazard decision-making may benefit from more insight into the uncertainty associated with average spore reduction values. When evaluating reduction data, it is difficult to directly compare spore reduction by subsurface passage with *Cryptosporidium* reduction by engineered conventional filtration. Conventional filtration includes adding a coagulant which together with the settling step enhances *Cryptosporidium* reduction during engineered rapid sand filtration.

3. Total aerobic spore reduction

Total aerobic spores were successfully used to demonstrate at least 2.0 log *Cryptosporidium* removal at the Casper alluvial aquifer adjacent to the North Platte river (Gollnitz et al., 2005). As part of the *Cryptosporidium* “demonstration of performance” decision, total aerobic spore removal is periodically monitored at select wells (Gollnitz et al., 2005) (Wyoming is the only US state that does not have state authority to