Water Research 113 (2017) 11–21

Contents lists available at ScienceDirect

Water Research

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

Hydrologic, land cover, and seasonal patterns of waterborne pathogens in Great Lakes tributaries

P.L. Lenaker [a, *], S.R. Corsi [a], M.A. Borchardt [b], S.K. Spencer [b], A.K. Baldwin [c], M.A. Lutz [a]

[a] U.S. Geological Survey, Wisconsin Water Science Center, 8505 Research Way, Middleton, WI 53562, USA
[b] U.S. Department of Agriculture, Agricultural Research Service, 2615 Yellowstone Dr. Marshfield, WI 54449, USA
[c] U.S. Geological Survey, Idaho Water Science Center, 230 Collins Road, Office 145, Boise, ID 83702, USA

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form
11 January 2017
Accepted 29 January 2017

Keywords:
Human virus
Bovine virus
Land cover
Hydrology
Seasonality
Great Lakes tributaries

ABSTRACT

Great Lakes tributaries are known to deliver waterborne pathogens from a host of sources. To examine the hydrologic, land cover, and seasonal patterns of waterborne pathogens (i.e. protozoa (2), pathogenic bacteria (4) human viruses, (8) and bovine viruses (8)) eight rivers were monitored in the Great Lakes Basin over 29 months from February 2011 to June 2013. Sampling locations represented a wide variety of land cover classes from urban to agriculture to forest. A custom automated pathogen sampler was deployed at eight sampling locations which provided unattended, flow-weighted, large-volume (120 −1630 L) sampling. Human and bovine viruses and pathogenic bacteria were detected by real-time qPCR in 16%, 14%, and 1.4% of 290 samples collected while protozoa were never detected. The most frequently detected pathogens were: bovine polyomavirus (11%), and human adenovirus C, D, F (9%). Human and bovine viruses were present in 16.9% and 14.8% of runoff-event samples (n = 189) resulting from precipitation and snowmelt, and 13.9% and 12.9% of low-flow samples (n = 101), respectively, indicating multiple delivery mechanisms could be influential. Data indicated human and bovine virus prevalence was different depending on land cover within the watershed. Occurrence, concentration, and flux of human viruses were greatest in samples from the three sampling locations with greater than 25% urban influence than those with less than 25% urban influence. Similarly, occurrence, concentration, and flux of bovine viruses were greatest in samples from the two sampling locations with greater than 50 cattle/km^2 than those with less than 50 cattle/km^2. In seasonal analysis, human and bovine viruses occurred more frequently in spring and winter seasons than during the fall and summer. Concentration, occurrence, and flux in the context of hydrologic condition, seasonality, and land use must be considered for each watershed individually to develop effective watershed management strategies for pathogen reduction.
Published by Elsevier Ltd.

1. Introduction

Human and bovine pathogens are disease-causing microorganisms which can deteriorate groundwater and surface water resources. Fecal contamination by human and bovine pathogens, including viruses, bacteria, and protozoa, is a potential human health hazard when exposed to contaminated recreational waters (Sinclair et al., 2009), drinking water sources (Borchardt et al., 2012), wildlife (i.e. shellfish, white-tailed deer, geese, rodents, etc.) (Ley et al., 2002), crop irrigation (Bosch, 1998), and dairy production (de Oliveira et al., 2012). Various environmental factors (i.e. pH, temperature, salinity, UV light exposure, etc.) influence the fate, transport, and occurrence of human and bovine pathogens in surface water as well as other watershed-specific factors such as, land cover composition, hydrologic condition, and season.

Aquatic contamination from pathogens can vary considerably in space and time (Rutsch et al., 2008) similar to many non-point source contaminants in urban and rural runoff. To that effect, non-point sources of pathogens from human waste include leaking sanitary sewer infrastructure, landfills, degraded public and private sanitary lateral line connections or misconnections, improper sanitary sewer line connections, properly functioning and defective septic systems, land application of septic and municipal waste effluent, and stormwater drainage systems. Point sources of pathogens from human waste include municipal sanitary sewer overflows (SSO), combined sewer overflows (CSO), and treated as well

* Corresponding author.
E-mail address: plenaker@usgs.gov (P.L. Lenaker).

http://dx.doi.org/10.1016/j.watres.2017.01.060
0043-1354/Published by Elsevier Ltd.

as partially treated wastewater effluent, and industrial effluent (Ahmed et al., 2010; Corsi et al., 2014). Point and non-point sources of pathogens from agricultural sources related to bovine production include cattle manure in holding ponds, grazing pastures, barnyards, and agricultural practices that apply cattle manure to agricultural croplands.

Watersheds have various complex sources of human and bovine pathogens, and once released into the environment, they can be transported to surface water by way of various pathways depending on the hydrologic conditions at the time. For example, human virus occurrence during low-flow periods suggests a continuous source of sewage contamination to the watershed such as wastewater treatment effluent, exfiltration from failing wastewater infrastructure, or illicit connections of sanitary sewers and/or septic systems (Rutsch et al., 2006). In addition to the continuous sources of human viruses described above, sources during runoff periods include periods of high-flow stress on the sanitary sewage system due to increased flow volumes. These high-flow-induced sources can include sanitary sewer overflows, combined sewer overflows, and leaks in sewage conveyance infrastructure. Bovine virus occurrence during low-flow periods suggests direct cattle access to streams as a continuous source, while overland flow from barnyards, pastures, and manure application, in addition to subsurface drain tiles would be high-flow-induced sources. Watershed transport mechanisms will influence the fate of the aforementioned sources and can determine the resulting survival, occurrence, and magnitude of waterborne pathogens present in surface waters (Ferguson et al., 2003).

Given all of the potential sources, the influences on survival, and the fate and transport mechanisms within a watershed, it is challenging to properly represent variability and magnitude of pathogens in streams. Previous research has begun to address some of these challenges. For example, a diverse group of human and bovine viruses have been detected previously in watersheds with varying land use (Corsi et al., 2014). A study in Michigan sampled nine rivers for enterovirus and rotavirus, providing insight into spatial variability (Jenkins et al., 2005). Another study in California reported on human adenoviruses in urban runoff, emphasizing the influence that specific land cover class can have on pathogen presence in surface waters (Jiang, 2001).

These studies and more have made progress on understanding some of the factors that impact waterborne pathogen dynamics in streams. The challenge moving forward is to implement a study designed to address a larger proportion of these influential factors that also adequately represents pathogen occurrence and variability. Such a comprehensive monitoring program would need to include consideration of short-term (inter-event) and long-term (intra-event) hydrologic variability, seasonal and annual temporal variability, land cover, source-specific discharges (i.e. municipal wastewater effluent, CSO, etc.), and a comprehensive suite of target pathogens.

The objectives of this study were to 1) quantify multiple pathogens within four microbial categories (human viruses, bovine viruses, pathogenic bacteria and protozoa), 2) compare pathogen variability in streams due to hydrologic condition including low-flow periods, and periods of increased runoff due to rainfall and snowmelt, 3) implement a hydrologically appropriate sampling strategy that represents water from all portions of run-off event hydrographs (initial flush, rising flow, peak flow, and receding flow periods), 4) examine seasonal patterns in pathogen prevalence in streams, and 5) describe variability in pathogen prevalence in streams in relation to land cover composition. Results provide further understanding of environmental factors and inherent watershed properties which influence pathogen presence in Great Lakes tributaries and could help improve watershed management decisions aimed at minimizing human exposure to waterborne pathogens.

2. Methods

2.1. Sampling locations

Eight Great Lakes tributaries were selected as sampling locations that represent watersheds with diverse land cover compositions from high to low urban and agricultural land cover (Fig. 1; Table 1). All land cover categories were defined by the 2011 National Land Cover Database products.

2.2. Sample collection

Sampling locations were monitored for waterborne pathogens over a 29 month period from February 2011 to June 2013. Flow-weighted composite samples were collected using a custom-designed automated large-volume virus sample collection and filtration system (modified from Corsi et al., 2014) (Fig. 2). Specific details regarding flow-weighted composite sampling using this system were previously described (Corsi et al., 2014) and details describing modifications for the current study are presented in supporting information (Text S1). Briefly, the automated subsample sequence utilized five ball valves to direct water flow between a flow sensor, two whole-water collection bottles, and two filtration cartridges (Fig. 2). The automated subsample sequence is described further in supporting information (Text S2).

Samples were collected during low-flow and runoff-event periods with “runoff-event periods” defined as periods of increased runoff due to rainfall and snowmelt. Thresholds for runoff-event samples were set at individual sampling locations to trigger sampling when water levels increased over the most recent low-flow levels which varied temporally. Three runoff-event period samples were targeted on a quarterly/seasonal basis, and low-flow period samples were collected every other month over the 29 month sample period. Further details on field replicate and blank sample collection and results are presented in supporting information (Text S2).

Recovery controls for glass wool filtration were performed as previously (Lambertini et al., 2008). Details describing recovery control sampling and results are in supporting information (Text S3).

2.3. Laboratory methods

Pre-filters and glass wool filters were eluted immediately in the laboratory upon receipt and the eluates concentrated by polyethylene glycol following standard elution and secondary concentration procedures (Lambertini et al., 2008; Millen et al., 2012). Eluates or final concentrated sample volumes (FCSV) from a sample-paired pre-filter and glass wool filter were combined for qPCR analysis. FCSV volumes were between 0.9 mL and 57 mL (mean = 7.4 mL) which were archived at −80 °C until nucleic acid extraction. Extraction procedures were the same as those described previously (Corsi et al., 2014) except for the addition of an initial freeze-thaw step for extracting *Cryptosporidium* oocyst DNA (Giovanni and LeChevallier, 2005).

Real-time qPCR was performed for genes specific to eight human viruses, eight bovine viruses, four bacteria and two protozoa and is further described in supporting information (Text S4). All gene targets and references for primers and hydrolysis probes and standard curve performance parameters are listed in supporting information (Table S2).