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Human and bovine viruses in the Milwaukee River watershed: Hydrologically relevant representation and relations with environmental variables

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HIGHLIGHTS

- Hydrologic conditions, precipitation, and season explained variability of viruses.
- Human and bovine viruses were more prevalent during runoff periods than during low-flow periods.
- An automated sampling system provided hydrologically relevant samples over long durations.

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ABSTRACT

To examine the occurrence, hydrologic variability, and seasonal variability of human and bovine viruses in surface water, three stream locations were monitored in the Milwaukee River watershed in Wisconsin, USA, from February 2007 through June 2008. Monitoring sites included an urban subwatershed, a rural subwatershed, and the Milwaukee River at the mouth. To collect samples that characterize variability throughout changing hydrologic periods, a process control system was developed for unattended, large-volume (56–2800 L) filtration over extended durations. This system provided flow-weighted mean concentrations during runoff and extended (24-h) low-flow periods. Human viruses and bovine viruses were detected by real-time qPCR in 49% and 41% of samples (n = 63), respectively. All human viruses analyzed were detected at least once including adenovirus (40% of samples), GI norovirus (10%), enterovirus (8%), rotavirus (6%), GII norovirus (1.6%) and hepatitis A virus (1.6%). Three of seven bovine viruses analyzed were detected including bovine polyomavirus (32%), bovine rotavirus (19%), and bovine viral diarrhea virus type 1 (5%). Human viruses were present in 63% of runoff samples resulting from precipitation and snowmelt, and 20% of low-flow samples. Maximum human virus concentrations exceeded 300 genomic copies/L. Bovine viruses were present in 46% of runoff samples resulting from precipitation and snowmelt and 14% of low-flow samples. The maximum bovine virus concentration was 11 genomic copies/L. Statistical modeling indicated that stream flow, precipitation, and season explained the variability of human viruses in the watershed, and hydrologic condition (runoff event or low-flow) and season explained the variability of the sum of human and bovine viruses; however, no model was identified that could explain the variability of bovine viruses alone. Understanding the factors that affect virus fate and transport in rivers will aid watershed management for minimizing human exposure and disease transmission.

1. Introduction

Contamination of environmental waters by human pathogens, including enteric viruses, is recognized as a potential human health hazard to those using recreational waters (Wade et al., 2006, 2008), in drinking water systems (Borchardt et al., 2012), or even via crops contaminated by irrigation (Bosch, 1998). The potential for contamination is large because there are over 100 human-specific viruses present in sewage and viruses are shed in feces of infected humans in concentrations on the order of 10^5 to 10^{11} viruses per gram (Bosch, 1998). Bovine viruses also have been detected in environmental waters and have most commonly been used to trace contamination from cattle farms (Fong et al., 2005; Ahmed et al., 2010), and suggest potential for transmission to cattle exposed to contaminated water sources. Virus contamination can impact groundwater quality (Abbaszadegan et al., 2003; Bradbury et al., 2013) as well as surface water quality (Tani et al., 1995; Jiang and Chu, 2004; Fong and Lipp, 2005).

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Sources of human viruses into environmental waters are limited, although the degree of contamination can be strongly variable in both time and space (Rutsch et al., 2008). Sources include: treated wastewater effluent, partially treated wastewater effluent (from "blending" events), combined sewer overflows (CSO), sanitary sewer overflows (SSO), leaking sanitary and sewer lines, lateral pipes for public and private connections, and misconnected sanitary sewer lines. Septic systems can also introduce viruses to environmental waters when properly functioning (Alhajjar et al., 1988; DeBorde et al., 1998) or during periods of system failure (Borchardt et al., 2011). In addition, authorized application of septic system effluent to the land surface is common for routine septic system maintenance (WDNR, 2001, http://www.legis.state.wi.us/rsb/code/nr/nr113.pdf). Treated wastewater, CSOs, and SSOs are typically discharged directly to surface water systems, while leaking sanitary sewer lines and septic systems discharge to the groundwater system, and ultimately may travel laterally and be transported to surface waters. Bovine viruses are released to the environment in cattle manure in holding ponds, storage areas, or pastures, and are often widely distributed in agricultural areas when manure is land-applied for crop fertilization. Viruses in land-applied septage and manure can move by overland flow or drain tiles to surface waters (Fong and Lipp, 2005) and viruses can infiltrate soil to reach groundwater where they can be pumped back to the surface from wells, become inactivated, or travel through shallow groundwater and discharge as baseflow to surface water systems. In surface water, viruses can remain suspended and be transported with currents or be deposited into sediments which can act as a reservoir from which viruses can persist and be resuspended under certain environmental conditions (Bosch, 1998).

The survival, fate, and transport properties of viruses in the environment vary depending on virus type as well as the environmental conditions to which they are exposed (Schijven and Hassanizadeh, 2000; Rzezutka and Cook, 2004; John and Rose, 2005; Bosch, 1998). Potentially influential factors include temperature, desiccation, UV light exposure, inactivation by other microorganisms, hydrologic flow conditions, filtration or adsorption in porous media, adsorption to sediments, and deposition and resuspension in sediments. Human and bovine viruses do not replicate outside of their host, so once in the environment, consideration of survival and inactivation is important, but not growth. Due to the small size of viruses and the potentially long survival time in the environment, travel times in groundwater of months to years are relevant for delivery of viruses to drinking water wells or surface water resources. Survival in surface water is likely shorter than that in groundwater because of UV exposure, higher temperatures (depending on the time of year and location), and the opportunity for more interactions with other organisms that can inactivate viruses (Meixell et al., 2013).

Virus contamination has been documented in rivers under different conditions and settings. For example, human virus input to coastal areas from urban rivers in southern California was greatest during the rainy season (Jiang and Chu, 2004). Nine rivers with wastewater effluent influence and a wide range of land cover in the lower peninsula of Michigan were sampled one time during summer low-flow conditions and three rivers were positive for viable human enteric viruses (Jenkins et al., 2005). Bovine viruses were detected in wet- and dry-weather conditions in the Maroochy Coastal River in Australia (Ahmed et al., 2010) and were more prevalent during cool water temperatures than warm water temperatures in a study of the lower Altamaha River in Georgia, USA (Fong et al., 2005).

A key challenge in studying virus contamination of riverine ecosystems is collecting hydrologically relevant samples. With changes in flow from rainfall or snowmelt, contamination levels of many constituents will also change. In addition, diel changes in UV light exposure and temperature in a river likely result in diel variability in virus survival. This suggests that proper characterization of viruses must be accomplished by sampling in a hydrologically and temporally relevant manner over extended periods of time, but this can be difficult. Large volumes of water (typically > 100 L) must be filtered and some filtration methods require pH adjustment of sample water before filtration. Because of these technical details, previous river sampling for viruses has commonly been limited to collection of large volume grab samples over relatively short periods of time (Noble and Fuhrman, 2001; Jiang and Chu, 2004; Fong et al., 2005; Jenkins et al., 2005; Aslan et al., 2011). Hydrologically relevant samples require sampling through low flow periods as well as entire runoff periods to capture all components of the hydrograph including the first flush, rising flow, peak flow, and receding flow periods. Virus inactivation likely differs between daylight and non-daylight periods, suggesting that 24 h would be a reasonable sampling duration during low-flow periods.

The objectives of the present study were to develop sampling techniques for hydrologically and temporally relevant virus sampling and to characterize virus occurrence and variability in three locations within the Milwaukee River watershed, Wisconsin: 1) an urban subwatershed where wastewater is municipally collected but the treated effluent is not discharged to the river; 2) a rural subwatershed where wastewater is treated primarily with septic systems; and 3) the Milwaukee River at the mouth into Lake Michigan, which represents combined urban and rural watershed inputs. A third objective was to relate virus occurrence to hydrologic and climatic conditions. Results provide further understanding of primary factors that influence virus presence in rivers and could lead to improved watershed management decisions for minimizing human exposure to waterborne viruses.

2. Methods

2.1. Monitored sites

Three streams within the Milwaukee River watershed in Wisconsin, USA were monitored for human and bovine viruses over a 17 month period, February 2007 to June 2008 (Table 1, Fig. 1). One site was composed mainly of rural land use (Cedar Creek) and the other was mainly urban land use (Underwood Creek). The third site was at the mouth of the Milwaukee River which includes a mix of different land uses. The Milwaukee River monitoring site was located downstream of input from Cedar and Underwood Creeks.

Flow-weighted composite samples were collected during low-flow periods and during periods of increased runoff due to rainfall and snowmelt (hereafter referred to as "runoff events"), resulting in event-mean virus concentrations. These sampling techniques require instantaneous flow measurements that are used to compute the volume of streamflow over time. Flow-weighted samples were collected by specifying the volume of streamflow between subsamples. The volume between subsamples varied by sampling period based on anticipated streamflow levels. With these methods, subsample collection frequency increases as streamflow increases. Runoff samples consisted of numerous 5 L subsamples to cover the entire event hydrograph (between 7 and 206 h sampling duration). Runoff-event sampling was initiated when water level became elevated above low flow, and sampling was ended after flow returned to near baseflow levels. Low-flow samples consisted of numerous 5 L subsamples collected over approximately 24 h. Exact sample volumes varied by sampling event (Table 1). Flow-weighted sampling allowed for straightforward total virus loading and unit-area loading computation as well as valid comparison among sampling locations.

2.2. Sample collection

Samples were collected using custom-designed automated large-volume virus sample collection and filtration systems that were housed at each monitoring site (Fig. 2). Remote telemetry allowed unattended operation for initiating and monitoring sampling. This allowed sample coverage of entire runoff events and extended low-flow periods without deploying field personnel. A variable-speed peristaltic pump was used to pump water from the stream into the sampling system with a