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High occurrence of hepatitis E virus in samples from wastewater treatment plants in Switzerland and comparison with other enteric viruses

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ABSTRACT

Hepatitis E virus (HEV) is responsible for many enterically transmitted viral hepatitides around the world. It is currently one of the waterborne diseases of global concern. In industrialized countries, HEV appears to be more common than previously thought, even if it is rarely virulent. In Switzerland, seroprevalence studies revealed that HEV is endemic, but no information was available on its environmental spread. The aim of this study was to investigate –using qPCR– the occurrence and concentration of HEV and three other viruses (norovirus genogroup II, human adenovirus-40 and porcine adenovirus) in influents and effluents of 31 wastewater treatment plants (WWTPs) in Switzerland. Low concentrations of HEV were detected in 40 out of 124 WWTP influent samples, showing that HEV is commonly present in this region. The frequency of HEV occurrence was higher in summer than in winter. No HEV was detected in WWTP effluent samples, which indicates a low risk of environmental contamination. HEV occurrence and concentrations were lower than those of norovirus and adenovirus. The autochthonous HEV genotype 3 was found in all positive samples, but a strain of the non-endemic and highly pathogenic HEV genotype I was isolated in one sample, highlighting the possibility of environmental circulation of this genotype. A porcine fecal marker (porcine adenovirus) was not detected in HEV positive samples, indicating that swine are not the direct source of HEV present in wastewater. Further investigations will be necessary to determine the reservoirs and the routes of dissemination of HEV.

1. Introduction

Many waterborne diseases, like gastroenteritis or hepatitis, are caused by viruses and are a major threat to public health (Bosch et al., 2008). Human viruses such as adenovirus type 40 (HAdV-40) and noroviruses (NoV) genogroup I (GGI) and genogroup II (GGII) are commonly found in wastewater due to fecal excretion. Wastewater is treated physically, chemically,

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and biologically in wastewater treatment plants (WWTPs) in order to eliminate or reduce contaminants before the release of environmentally safe water. Fecal pollution of environmental water is a major health concern since environmental waters are used for drinking water supply and food production. Moreover, released viruses might reach diverse food items such as vegetables, fruits and raw shellfish (Bosch et al., 2008). Some viruses, like HAdV-40 and NoV, are good fecal indicators for evaluating the microbiological quality of environmental water, since they are excreted in high concentrations and are persistent in environmental water (Roslev and Bukh, 2011). Furthermore, viruses can be used to track the sources of fecal contamination (Roslev and Bukh, 2011). It is possible to distinguish between human and animal sources of pollution, since many human and animal viruses have a very narrow host spectrum. For example, HAdV-40, bovine adenovirus (BAdV) and porcine adenovirus (PAdV) are good indicators for determining the source of fecal contamination (Hundesa et al., 2006).

Hepatitis E is a waterborne disease responsible for over 50% of acute viral hepatitis cases in endemic countries (Dalton et al., 2008; Meng, 2010). The disease is caused by the hepatitis E virus (HEV), which is a non-enveloped positive-strand RNA virus (Dalton et al., 2008; Meng, 2010). HEV infection in humans can be caused by 4 genotypes (GI, GII, GIII and GIV) resulting in a single serotype (Dalton et al., 2008; Meng, 2010). Epidemics occur in countries with poor sanitation systems (Asia, Africa, Middle East and Mexico) and are due to GI and GII (Dalton et al., 2008; Meng, 2010). GI is a hyper-virulent genotype, responsible for most of the large outbreaks (Dalton et al., 2008; Meng, 2010; Bose et al., 2011). Furthermore, GI strongly affects pregnant woman by causing fulminant hepatic failure, which can lead to the death of both mother and child (Bose et al., 2011). For a long time, HEV was considered non-endemic in industrialized countries as only sporadic travel-associated cases were reported (Purcell and Emerson, 2008). However, the increasing number of autochthonous cases and the high seroprevalence reported in certain countries indicated that HEV is actually endemic to these countries (Purcell and Emerson, 2008). These autochthonous cases are due to GIII in most industrialized countries and to GIV in Eastern Asia (Purcell and Emerson, 2008; Lewis et al., 2010; Colson et al., 2012). Whereas GI and GII are restricted to humans, GIII and GIV have a wider host range within mammals and their main reservoir is suspected to be pigs and wild boar (Lewis et al., 2010; Meng, 2010; Rose et al., 2011; Wacheck et al., 2012). Hepatitis E has received ever more attention in recent years and is now considered an emerging problem. Its success in spreading may illustrate weaknesses in water management systems or food processes related to pork.

Studying the occurrence of enteric pathogens in influents at WWTP provides an efficient overview of the presence of these pathogens in the population. HEV has been detected in WWTPs in France (Clemente-Casares et al., 2003), Italy (La Rosa et al., 2010) and Spain (Clemente-Casares et al., 2009; Rodriguez-Manzano et al., 2010). The presence of the non-endemic GI in wastewater was recently reported in Spain and Italy (Clemente-Casares et al., 2009; La Rosa et al., 2010). HEV seroprevalence rates in populations from industrialized countries are usually relatively low (i.e. ranging from 1% to 5%) in comparison to those in developing countries, where rates from 15% to 60% have been reported (Dalton et al., 2008). Seroprevalence rates exceeded 20% in some regions within the USA (Thomas et al., 1997; Meng et al., 2002) and Japan (Li et al., 2000), showing that seroprevalence rates can reach locally unexpected higher values. However, comparison of seroprevalence between regions is problematic due to a lack of standardised serological tests (Bendall et al., 2010). In Switzerland, two blood donor studies reported HEV seroprevalence of 3.2% and 4.9% respectively (Lavanchy et al., 1994; Kaufmann et al., 2011). Furthermore, 26 cases of asymptomatic HEV seroconversion were recorded in a cohort of 667 workers including 332 WWTP workers in 5 years (Tschopp et al., 2009). Since these infections were asymptomatic, it was hypothesized that the workers were infected by the low pathogenic HEV GIII. However, neither the genotype involved in these seroconversions, nor the source of infection, could be determined accurately.

The present study investigated the occurrence and the concentration of HEV in the influents and effluents of 31 WWTPs located in the same area as the above mentioned cohort study (Jeggli et al., 2004; Tschopp et al., 2009). The objectives were to assess the environmental circulation of HEV in Switzerland and to determine whether HEV GI is present in wastewater. As points of comparison, the occurrence and concentration of two human viruses, HAdV-40 and NoV-GGII, were assessed. PAdV, a porcine fecal marker, was searched in order to evaluate whether any detected HEV might be of porcine origin.

2. Materials and methods

2.1. Sampling site selection

Thirty-one municipal WWTPs were selected within the Canton of Zurich in Switzerland (about 1.39 million inhabitants; 1729 km^2). All WWTPs comprise a cleaning and an activated sludge step (Zurich WWTP website, 2013). The selection was made using the following criteria. First, WWTPs where a seroconversion in workers had been ascertained in the recent cohort study on hepatitis E incidence (Tschopp et al., 2009) were included. Second, the WWTP servicing Zurich's international airport was included because international traveling increases the probability of the occurrence of genotypes GI and GII. Third, WWTPs where occupational hygiene measurements had been taken in a previous study (Oppliger et al., 2005; Daneshzadeh Tabrizi et al., 2010) were included. Finally, further WWTPs were selected to represent a well-balanced sample of the whole canton. The final sample included 6 very large (>50,000 inhabitants and inhabitant-equivalents), 12 large (10,000–50,000 inhabitants and inhabitant-equivalents) and 13 small WWTPS (2000–10,000 inhabitants and inhabitant-equivalents). Very small WWTPs (<2000 inhabitants and inhabitant-equivalents) were not included, but there was always a larger WWTP in the same area. A total of 247 pig farms housing about 43,000 pigs were recorded in the Canton of Zurich (Federal Office of Statistics, 2013). These WWTPs treat only household sewage and farmers are not allowed to use these sewer systems to