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Classical and molecular detection methods in aquatic environmental virology for waterborne diseases outbreaks

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Abstract

Waterborne diseases commonly addresses the Human adenoviruses (HAdVs), Noroviruses (NoVs), Hepatitis A virus (HAV), Hepatitis E virus (HEV), Parvoviruses, Rotaviruses (RVs), Caliciviruses, and Enteroviruses including Coxsackieviruses and Polioviruses. Waterborne virus-based illness may be transmitted through drinking water contamination linked diseases transmission sources as seawater, freshwater and also sewage. This paper described the classical methods of detection in aquatic environmental virology as cell culture-based, immunoassay and molecular-based technologies. The implementation of molecular-based tools and wastewater-based epidemiology studies to high-throughput for aquatic biomonitoring should be up to date to mitigating the risk and impact on aquatic environments.

Keywords: Aquatic environments; Molecular-based technologies; Waterborne diseases; Virology

1 Introduction

Epidemiological studies have been proposed some pathogens which may be transmitted by ingestion of contaminated water such as Hepatitis A (HAV), Hepatitis E (HEV) viruses and enteric viruses including adenovirus, astrovirus, rotavirus, calicivirus, Norwalk and other Small round structured viruses (SRSV) [1,2]. In terms of the treatment of water supply two disinfectants such as free chlorine - a stronger disinfectant to pathogen inactivation, and monochloramine have been applied to avoid the cross-contamination in the water distribution system [1].

In the past, there were some protocols that has been established by the International Organization for Standardization (ISO) for detecting three bacteriophage groups in water (ISO 10705-1, ISO 1075-2, ISO 10705-4) [2].

Enteric virus groups may be considered emerging waterborne pathogens, based on their biochemical properties and viral structural belong to the families *Picornaviridae* (polioviruses, enteroviruses, coxsakieviruses, hepatitis A virus, and echoviruses), *Adenoviridae* (adenoviruses), *Caliciviridae* (noroviruses, caliciviruses, astroviruses, and small round-structured viruses), and *Reoviridae* (reoviruses and rotaviruses) [3]. Faecal contamination is a vehicle for pathogenic viruses spread through water environments [4].

Moreover, there are another group of viruses (enveloped virus) have been detected in water environments such as *Orthomyxoviridae* (Influenza virus), *Paramyxoviridae* (measles virus, mumps virus, respiratory syncytial virus),

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Herpesviridae, *Coronaviridae* and others [4]. These viruses are more resistant to environmental conditions, water treatments and disinfectants than enveloped viruses like coronavirus [4].

In environmental samples of seawater, freshwater and also sewage have been reported the presence of infectious virus for up to 120 days in these aquatic environments. Several routes may be associated with poor hygiene conditions as described in figure 1 [5]. Moreover, the virus survival had also been detected for up to 100 days in soil samples at temperature 30°C [3]. The prevalence of animal-specific enteroviruses and adenoviruses in hosts had been reported in aquatic environments and may be used as potential tools in water quality management in the waterborne diseases studies [3].

Infectious viruses in upon exposure recreational waters constitutes a public health risk which adenoviruses as an indicator of bathing water quality [5]. Waterborne disease commonly include the Human adenoviruses (HAdVs), Noroviruses (NoVs), Hepatitis A (HAV), Hepatitis E (HEV), parvoviruses, enteroviruses and Rotaviruses (RVs) [5]. SARS-CoV-2 RNA particles had been detected into fresh water line and faecal waste contaminated. This is a highlighted issue environmental impact in the public health [6].

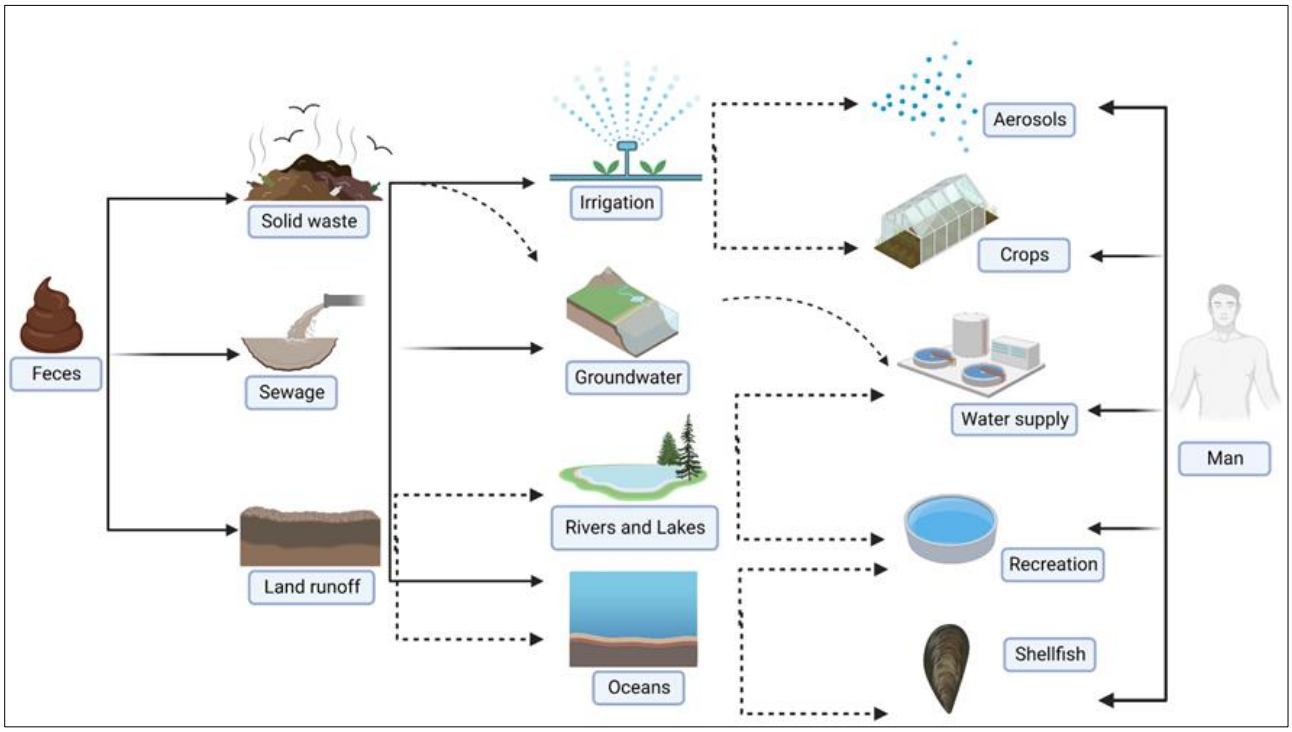

Figure 1 Waterborne transmission: Transmission routes for enteric viruses like for example: (i) shellfish contaminated by marine waters polluted by fecal materials, (ii) contamination of drinking and irrigation water with human sewage or sewage-polluted recreational waters; (iii) contamination of ready-to-eat or prepared-for-eat foods as a result of poor personal hygiene by food handlers who are infected; (iv) production of aerosols from vomit and (v) through contact with contaminated surfaces. This figure was created and designed by the author using scientific image and illustration software with publication license into journals.

2 Methods of detection in waterborne diseases

2.1 Classical Methods in Aquatic Environmental Virology

2.1.1 Cell culture-based

Cell culture-based methods like monolayer plaque assays may be able to estimate the levels of concentrations of some viruses in surface waters but for viral detection it had been showed some disadvantages. Concentration of samples type like freshwater and marine water have been performed by glass wool filtration and by nitrocellulose membrane filtration, respectively [5]. Adenovirus serotypes 40 and 41 and human norovirus, hepatitis A virus cannot be grown easily in cell culture [1].

Classical viral growth assays such as plaque assays are not the predilection for water treatment infrastructure. Notably, about a 10-day incubation period is required to detect replicating adenoviruses via plaque assays [1]. Although integrated cell culture-PCR (ICC-PCR) is more effective than traditional plaque assays unfortunately it has not been indicated at water treatment utilities [1,7].

2.2 Molecular Methods in Aquatic Environmental Virology

2.2.1 Immunoassay and molecular-based technologies

Immunoassay (ELISA) can be used to detect viral proteins and molecular methods (qPCR-based technologies) also had been applied to detect viral genomes [1]. UV light treatment of viruses as the mechanism for disinfection can be inactivates bacteriophage MS2 generating genomic replication inhibition by several key-role mechanisms such cleavage of the major capsid protein for example [1]. Furthermore, another assay like immunocapture qPCR (IC-qPCR) technique is another promising option to detect infectious viral particles [1,7].

2.2.2 Molecular methods

In the past, waterborne pathogens were measured by detection of faecal bacterial indicator organisms (FiOS). Furthermore, several studies showed there was no correlation with levels of human enteric viruses [5]. Nowadays, the detection of enteric viruses in waters bathing is by molecular methods such as Reverse Transcription RT-PCR or nucleic acid sequence-based amplification (NASBA) which amplify RNA/DNA for monitoring and/or surveillance of enteric viruses in recreational waters [5,7].

Waterborne outbreaks have been reported mainly by noroviruses which are the most important cause of viral gastroenteritis disease [5]. Bioluminescent enzyme immunoassay (BLEIA) was introduced to detect norovirus capsid ant [7].

Virus detection in water environment has been described as different methods individually or combined with others techniques: Polymerase chain reaction (PCR), Nucleic Acid Sequence Base Amplification (NASBA), loop-mediated isothermal amplification (LAMP), single primer isothermal amplification (SPIA), and recombinase polymerase amplification (RPA), Microarray, Atomic Force Microscope (AFM), Fluorescent microscopy, biosensors, Enzyme Linked Immunosorbent Assay (ELISA), Flow cytometry [2, 7]. Biosensors-based tools has been implemented to rapidly detect viral disease such as Lab-On-Chip (LOC) or Point-of-Care Testing (POCT) used recently in routine laboratory [7].

Next Generation Sequencing (NGS) platforms such as pyrosequencing, Ion Torrent, Illumina, MinION, and ABI/Solid and viral metagenomic to investigate emerging novel viral pathogens and unknown enteric viruses in environmental samples [7]. DNA viruses as *Papillomaviridae* had been detected in sewage samples by NGS-based metagenomic technologies [7,8,9,10]. Recently NGS sequencing (ChIP-Seq) analysis applied for detection methods of waterborne viruses [7,9,10,11].

For simultaneous detection of viruses in water (poliovirus, coxsackievirus, Echovirus and Hepatitis virus A) was developed the combining assays to well-optimized molecular-based assays for example PCR reaction combined with plaque forming (traditional indirect methods for detection of viruses); Multiplex PCR and Multiplex RT-qPCR assay (e.g., astroviruses, adenoviruses, rotaviruses, sapoviruses, and enteroviruses) [2,7]. Nested and Semi-Nested PCR [2,7], digital PCR (dPCR), and droplet digital PCR (ddPCR) were introduced [7]. NASBA methods is used for RNA detection using three enzymes: T7 RNA polymerase, Reverse transcriptase and RNase H [2,9,10].

Another series of methods which include: (i) concentration by absorption (elution technique) used in ELISA, (ii) positively charged filter (used in molecular techniques), (iii) Hydro-Extraction Method, (iv) Freeze-Drying, (v) precipitation of viruses from solution with 8% polyethylenoglycol 600 (used in NASBA) [7]. Moreover, molecular methods based combined cell cultures by plaque forming tests with molecular biology techniques (PCR and biosensors) [2,7]. Methods for waterborne diseases as described in figure 2.

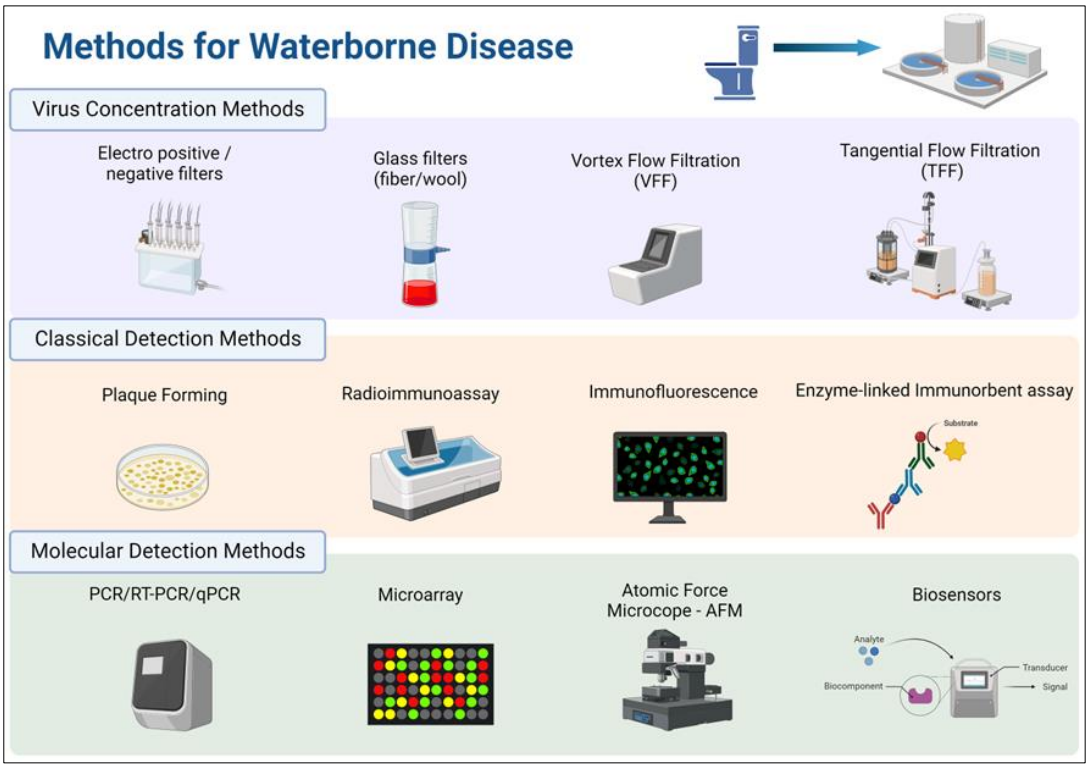

Figure 2 Methods for waterborne diseases: Virus Concentration as (i) electro positive/negative filters; (ii) glass filters; (iii) vortex flow filtration – VFF; (iv) tangential flow filtration – TFF. Classical Detection as (i) Plaque forming; (ii) Radioimmunoassay; (iii) Immunofluorescence; (iv) Enzyme-linked immunosorbent assay – ELISA. Molecular Detection as (i) PCR/RT-PCR/qPCR; (ii) Microarray; (iii) Atomic Force Microscope – AFM; (iv) Biosensors. This figure was created and designed by the author using scientific image and illustration software with publication license into journals.

3 Discussion

This overview supports the importance of water-based epidemiology monitoring of viral contamination to prevention of waterborne diseases and adopted prophylactic measures in order to mitigating the environmental risks [2]. The implementation molecular-based tools and wastewater-based epidemiology studies to high-throughput for aquatic biomonitoring should be up to date to avoid the risk and Impact on public health and aquatic environments. Novel perspectives to control of waterborne viruses has been introduced in continuous surveillance programs for ecosystems monitoring. Guidelines for investigation, control and prevention emerging waterborne diseases may be useful tool for immediate responses to waterborne outbreaks [10,11].

4 Conclusion

Waterborne virus-based illness may be transmitted through drinking water contamination linked diseases transmission sources as seawater, freshwater and sewage. The implementation of molecular-based tools and wastewater-based epidemiology studies to high-throughput for aquatic biomonitoring and surveillance should be up to date to mitigating the risk and impact on aquatic environments. This short overview described the classical methods of detection in waterborne diseases and molecular methods enriching and clarifying for a better understanding in aquatic environmental virology with the purpose of preventing the spread from possible sources of viral contamination as well as various state-of-the-art methodologies for detecting numerous infectious viruses.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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