Occurrence of Human Enteric Viruses in Water Sources and Shellfish: A Focus on Africa

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Abstract
Enteric viruses are a diverse group of human pathogens which are primarily transmitted by the faecal–oral route and are a major cause of non-bacterial diarrhoeal disease in both developed and developing countries. Because they are shed in high numbers by infected individuals and can persist for a long time in the environment, they pose a serious threat to human health globally. Enteric viruses end up in the environment mainly through discharge or leakage of raw or inadequately treated sewage into water sources such as springs, rivers, dams, or marine estuaries. Human exposure then follows when contaminated water is used for drinking, cooking, or recreation and, importantly, when filter-feeding bivalve shellfish are consumed. The human health hazard posed by enteric viruses is particularly serious in Africa where rapid urbanisation in a relatively short period of time has led to the expansion of informal settlements with poor sanitation and failing or non-existent wastewater treatment infrastructure, and where rural communities with limited or no access to municipal water are dependent on nearby open water sources for their subsistence. The role of sewage-contaminated water and bivalve shellfish as vehicles for transmission of enteric viruses is well documented but, to our knowledge, has not been comprehensively reviewed in the African context. Here we provide an overview of enteric viruses and then review the growing body of research where these viruses have been detected in association with sewage-contaminated water or food in several African countries. These studies highlight the need for more research into the prevalence, molecular epidemiology and circulation of these viruses in Africa, as well as for development and application of innovative wastewater treatment approaches to reduce environmental pollution and its impact on human health on the continent.

Keywords Enteric viruses · Gastroenteritis · Wastewater · Shellfish · African continent

Introduction
Enteric viruses collectively represent a diverse group of pathogens that are responsible for a variety of notifiable diseases in humans through ingestion of contaminated food or water. Well-known human pathogens in this group are members of the Caliciviridae (e.g. norovirus), Picornaviridae (e.g. enterovirus, Aichi virus, hepatitis A virus), Hepeviridae (e.g. hepatitis E virus), Reoviridae (e.g. rotavirus), Astroviridae (astrovirus) and Adenoviridae (e.g. adenovirus 40, 41) (Fong and Lipp 2005; Bishop and Kirkwood 2008). These viruses are a major public health concern globally due to the fact that they tend to be stable in the environment, are shed at high concentrations in the faeces of infected people and are transmitted by the faecal–oral route (Fong and Lipp 2005; Sánchez and Bosch 2016; Bouseettine et al. 2019). Although these viruses are known to occur naturally in aquatic environments, one of the greatest risks they pose to public health arises from discharge of either raw (untreated) sewage or inadequately treated wastewater into ground or surface water sources utilised by humans for various activities including drinking, recreation, or consumption of foods such as molluscan bivalve shellfish harvested from polluted rivers or marine estuaries. Isolation of enteric viruses from such sewage-contaminated water sources and association of disease outbreaks as a result of human exposure to them are well documented in the literature (see for example: Lees 2000;
Transmission of enteric viruses to humans is particularly concerning in developing countries of the world where it is estimated that 80-90% of untreated sewage is disposed into water sources such as rivers, streams and other aquatic ecosystems causing environmental pollution (WWAP and UNESCO 2017; Fayomi et al. 2019). Countries in Africa and especially those in the Sub-Saharan region where it is estimated that 55% of urban dwellers now live in informal settlements are most at risk from infectious diseases because of overcrowding and poor sanitation infrastructure (Neiderud 2015; Boyce et al. 2019; Weimann and Oni 2019). Furthermore, people living in rural communities and informal settlements in Africa often rely on environmental surface and ground water sources polluted by wastewater and other contaminants originating from human activities for drinking, cooking and washing (Wang et al. 2014; Sibanda et al. 2015; Pichel et al. 2019; UNESCO 2019). Growing concern about the human health impact of environmental pollution, in particular untreated sewage discharge into fresh and marine aquatic systems, has led to numerous studies describing the isolation and molecular detection of enteric viruses in water and food sources and their association with disease outbreaks in several African countries. For example, these viruses have been reported in bivalve shellfish and environmental water sources in North Africa (Morocco, Tunisia and Egypt) (Benabbes et al. 2013b; Zormati et al. 2018; Shaheen and Elmahdy 2019) as well as in several Sub-Saharan countries including Chad (Guerrero-Latorre et al. 2011), Nigeria (Adeniji and Faleyre 2014), Ghana (Lutterodt et al. 2018), Côte d’Ivoire (Momou et al. 2017), Benin (Verheyen et al. 2009), Kenya (Kiulia et al. 2014), Uganda (Katukiza et al. 2013b), Tanzania (Mattioli et al. 2014), Mozambique (Nenonen et al. 2006) and South Africa (Chigor et al. 2014; Onosi et al. 2020). Although the role of enteric viruses in food- and waterborne disease transmission has been extensively reviewed for the developed world (Lipp and Rose 1997; Potasman et al. 2002; Sinclair et al. 2009; Westrell et al. 2010; Bellou et al. 2013; Romalde et al. 2017; Meghnath et al. 2019) and Egypt (Aboubakr and Goyal 2019), to our knowledge, a review of the African continent where people are most at risk from exposure to these viruses is not available. Here, we present an overview of enteric viruses and then review what is known about bivalve shellfish and sewage-contaminated water sources as possible vehicles for transmission in African countries, with a view to highlighting the importance of further studies investigating the true prevalence, molecular epidemiology and circulation of these important human pathogens on the continent.

**Classification, Structure and Biology of Enteric Viruses**

Enteric viruses encompass a broad range of viral pathogens which infect the human gastrointestinal tract, causing a wide variety of diseases and symptoms (Okoh et al. 2010). Commonly studied enteric viruses are non-enveloped and display high levels of stability in the environment (Fong and Lipp 2005; Alidjinou et al. 2019). Most enteric viruses have RNA genomes and belong to the Reoviridae (ds: double-stranded), Astroviridae, Caliciviridae, Hepeviridae or Picornaviridae (ss: single-stranded) families, while enteric viruses with DNA genomes are members of the Adenoviridae (ds) or Parvoviridae (ss).

**Rotaviruses**

Globally, rotaviruses (RVs; family Reoviridae) are a major cause of severe viral gastroenteritis in the paediatric population (Bányaí et al. 2018) and account for a significant proportion of diarrhoea-related deaths among children under the age of five (29.3% in 2015) (Wang et al. 2016). The introduction of RV vaccines has, however, reduced the burden of associated gastroenteritis in many countries (Troeger et al. 2018; Aliabadi et al. 2019), including those within sub-Saharan Africa (Tate et al. 2016; Godfrey et al. 2020). The RV genome consists of 11 dsRNA segments encoding six structural (VP1-4, 6 and 7) and six non-structural (NSP1-NSP6) proteins (Crawford et al. 2017; Gómez-Rial et al. 2020). Based on antigenic differences and the diversity of the VP6 capsid protein sequence, RVs are classified into ten species (A-J), of which species A, B, C and H cause human RV infections. Group A is the predominant cause of RV-gastroenteritis and is further classified into 32 G and 47 P genotypes based on differences in the sequences encoding the surface exposed capsid proteins VP7 (G antigen) and VP4 (P antigen) (Crawford et al. 2017), respectively. Various G and P combinations have been identified, however, genotypes G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] are more commonly detected worldwide (Bányaí et al. 2018). RV genotypes isolated from wastewater correspond to those reported in clinical samples from the same regions (Sdiri-Louliz et al. 2010a; Prez et al. 2020), thus environmental monitoring provides useful data on the epidemiology of RVs circulating within populations (Abdel-Daim et al. 2019).

**Caliciviruses: Noroviruses and Sapoviruses**

Noroviruses (NoVs; family Caliciviridae) are currently recognised as the leading cause of sporadic and epidemic
Sapoviruses (SaVs) also belong to the *Caliciviridae* and are aetiological agents of acute viral gastroenteritis, causing both sporadic and outbreak cases in a variety of settings (Oka et al. 2015). Symptoms are generally milder than those caused by NoV and although infections occur in all age groups, SaV-associated gastroenteritis is more prevalent in children and infants (Liu et al. 2016). The SaV genome usually contains two ORFs. The first (ORF1) encodes a single non-structural polyprotein and a major capsid protein (VP1), while a smaller capsid protein (VP2) is encoded by ORF2 (Makhaola et al. 2020). SaVs are routinely detected by RT-PCR or polymerase chain reaction (RT-qPCR) assays targeting the ORF1-ORF2 junction region have become standard in the detection and quantification of NoV from clinical and environmental samples. NoVs display a wide degree of genetic and antigenic variability and can be classified into different genogroups (GI–GX) as well as P groups based on amino acid diversity of the complete VP1 gene and nucleotide diversity of the viral polymerase sequence (Kroneman et al. 2013; Chhabra et al. 2019), respectively. Human NoV infections are predominantly caused by the genogroups GI and GII, with GII.4 variants causing most cases in the last decade (Siebenga et al. 2009; Vinjé 2015; Mans et al. 2016). In 2014, a novel variant of genotype GII.17 (GII.P17-GII.17) emerged as a major cause of NoV-associated gastroenteritis in Asia, and has since been reported globally (Chan et al. 2015; Lu et al. 2015; Chan et al. 2017; Hoa-Tran et al. 2017; Zhou et al. 2019). Genotype GII.4 is most associated with transmission through person–person contact, while strains of Genogroup GI are more closely associated with waterborne transmission (De Graaf et al. 2016). Strains belonging to GIV have been detected in both clinical and environmental samples; however, further studies are needed to fully understand the prevalence, epidemiology and medical significance of this genogroup (Musciolo et al. 2013; Kitajima et al. 2016; Teixeira et al. 2016).

Human Astroviruses (HAstVs) are common aetiological agents of acute paediatric gastroenteritis, after rotavirus and the caliciviruses (Platts-Mills et al. 2015; Vu et al. 2017; Wohlgemuth et al. 2019), and are detected at rates comparable to adenoviruses in cases of paediatric diarrhoea (Andreadi et al. 2008; Levidiotou et al. 2009; Hamkar et al. 2010; Akdag et al. 2020). HAstV-associated gastroenteritis is also reported in the adult population, particularly in the elderly and individuals with compromised immunity (Vu et al. 2017; Wohlgemuth et al. 2019). More recently, HAstVs have become associated with central nervous system (CNS) infections such as encephalitis and acute flaccid paralysis, in immunocompromised individuals (Cordey et al. 2016; Vu et al. 2016). The HastV genome contains three ORFs. ORF1a and 1b encode the non-structural proteins involved in viral replication, while ORF2 encodes the capsid proteins, which are expressed on a sub-genomic RNA (Bosch et al. 2014). Astroviruses exhibit a high degree of genetic diversity and infect many host species which has complicated the classification of these viruses. Based on the full-length amino acid sequence of ORF2, HAstVs belong to the species MAstV-1 (HAstV-1–8), MAstV-6 (MLB1–3), MAstV-8 (VA2/HMO-A, VA4, VA5, BF34) and MAstV-9 (VA1/HMO-C, VA3/HMO-B) of the *Mastastrovirus* genus (reviewed by Donato and Vijaykrishna, 2017). Additionally, classic HAstVs (MAstV-1 strains) have been further typed, according to ORF1a, into genogroups A (HAstv-1, -2, -3, -4, -5 and -8) and B (HAstv-6 and -7). Globally, HastV-1 strains account for more than half of all recently reported cases (Vu et al. 2017).

**Picornaviruses: Enteroviruses, Aichivirus and Hepatitis A Virus**

Enteroviruses (EVs; genus *Enterovirus*) belong to the family *Picornaviridae* and include enterically transmitted viruses of clinical significance (Wells and Coyne 2019). EVs infect millions of people globally, causing sporadic and outbreak cases in both developed and low to middle-income countries (Lugo and Krogstad 2016; Cassidy et al. 2018; Smuts et al. 2018; Puenpa et al. 2019). Although infections are usually
asymptomatic, coxsackieviruses (e.g. CV-A6), echoviruses (e.g. E-30) and enteroviruses (e.g. EV-A71 and EV-D68) cause a broad range of illnesses such as hand-foot-and-mouth disease, myocarditis, encephalitis and acute flaccid paralysis, which tend to be more severe in neonates and immunocompromised individuals (Tang and Holmes 2017; Baggen et al. 2018). To date, more than 300 EV types have been identified and are classified within 15 species (Chen et al. 2020). Human EVs belong to seven species, but only four (Enterovirus A-D) include viruses infecting the gastrointestinal tract (Baggen et al. 2018). Originally, EVs were grouped into serotypes on the basis of the VP1 capsid protein, however, sequence-based methods targeting the VP1 capsid-encoding region are now typically used to classify EV by genetic similarity (Oberste et al. 1999a, 1999b; Nix et al. 2006). RT-PCR-based assays targeting additional sequences such as the 5’ untranslated region (UTR) of the genome (Zhou et al. 2011) and the VP2 and VP4 capsid proteins (Royston et al. 2017) have also been developed, but their roles in genotyping are variable (Lindberg et al. 2003; Kottaridi et al. 2004; Nasri et al. 2007; Perera et al. 2010). Globally, many studies have isolated EVs from sewage, fresh surface waters, ground water and seas, highlighting the widespread presence of these viruses in the environment (Rajtar et al. 2008).

Aichi virus (AiV), a subtype of the species Aichivirus A (genus Kobuvirus) (Rivadulla and Romalde 2020), was first isolated in 1989 during an outbreak of oyster-associated gastroenteritis in Japan (Yamashita et al. 1991) and has since been detected worldwide (Oh et al. 2006; Sdiri-Loulizi et al. 2009; Yang et al. 2009; Chuachaona et al. 2017; Bergallo et al. 2018; Kitajima et al. 2018; Japhet et al. 2019; Onosı et al. 2019; Northill et al. 2020). Globally, the incidence rates of AiV in gastroenteritis cases are low (0.4–6.5%) (Rivadulla and Romalde 2010; Taghinejad et al. 2020), yet AiV-specific antibodies are present in 80–99% of the adult population (Kitajima and Gerba 2015) suggesting that most infections are asymptomatic (Bergallo et al. 2018). In addition to the low prevalence of AiV-associated gastroenteritis, the virus is often detected in conjunction with other well-defined enteric pathogens (Oh et al. 2006; Ambert-Balay et al. 2008; Kaikkonen et al. 2010; Japhet et al. 2019). Consequently, the clinical importance of AiV is unclear (Rivadulla and Romalde 2020), although some studies do support the virus as an enteric pathogen causing gastroenteritis (Yang et al. 2009; Drexler et al. 2011; Rivadulla et al. 2019). Human AiV is currently divided into three genotypes (A–C), based on differences in the sequences encoding the C-terminus of the 3C protease and the N-terminus of the 3D RNA-dependent RNA-polymerase (RdRp) (3CD junction) (Yamashita et al. 2000; Kitajima and Gerba 2015). RT-PCR-based assays targeting this junction and the VP1 capsid protein are widely used for AiV detection and genotyping (Oh et al. 2006; Pham et al. 2007; Ambert-Balay et al. 2008; Lodder et al. 2013; Ibrahim et al. 2017a). Genotypes A and B are prevalent in clinical and environmental samples from Africa, America, Asia and Europe (Rivadulla and Romalde 2020), while genotype C has only ever been detected in one clinical sample from France (Ambert-Balay et al. 2008).

Hepatitis A (HAV) is one of the major aetiological agents of acute hepatitis worldwide, infecting an estimated 1.5 million people per annum (WHO 2017; Lemon et al. 2018). HAV is classified within the Hepatovirus genus of the Picornaviridae family and is subdivided into one serotype and six genotypes, of which three (I-III) infect humans (Smith and Simmonds 2018). Several subtypes have been characterised, but HAV IA, IB, IIIA and IIIB are more commonly detected (Smith and Simmonds 2018). HAV encodes a single polyprotein, which is cleaved into the capsid (VP1Px, VP2-4) and non-structural (2B, 2C and 3A-D) proteins (McKnight and Lemon 2018). RT-PCR-based assays targeting the partial or full-length VP1 capsid protein, VP1-2A(Px) junction and VP1-2A(Px)-2B proteins are widely implemented for the detection and genotyping of HAV (Nainan et al. 2006; Coudray-Meunier et al. 2014).

**Hepatitis E Virus**

Hepatitis E Virus (HEV; family Hepeviridae) is classified within the genus Orthohepevirus and species Orthohepevirus A (Denner 2019). Eight genotypes have been characterised (HEV 1-8), although only five (HEV 1-4 and 7) are associated with human infection. Genotypes 1 and 2 are responsible for most sporadic and epidemic cases of hepatitis E, and are highly endemic in certain regions of Africa, Asia and Mexico (Smith and Simmonds 2018). Genotypes 3, 4 and 7 are primarily zoonotic and are more typically associated with sporadic and clustered infections in developed regions (Nimgaonkar et al. 2018). The HEV genome contains three ORFs (ORF1-3), although a fourth, ORF4, is present in genotype 1 (Nimgaonkar et al. 2018). RT-PCR-based assays targeting the full or partial sequences of ORF1 (non-structural proteins) and ORF2 (capsid proteins) are typically used for the molecular characterisation and genotyping of HEV in clinical and environmental samples. However, other genomic regions are sometimes targeted as there is no consensus classification system for the differentiation of HEV genotypes (Al-Sadeq et al. 2018).

**Enteric Adenoviruses**

Human adenoviruses (HAdVs; family Adenoviridae) are endemic aetiological agents of enteric, respiratory and ocular diseases that are typically mild and self-limiting, but can develop into severe infections with unusual manifestations in immunocompromised individuals (reviewed by Khanal...
et al., 2018). The global prevalence of enteric HAdVs in childhood diarrhoea varies from 1–8% and 2–31% in developed and developing countries (Meqdam and Thwiny 2007; Hassou et al. 2019), respectively. In sub-Saharan Africa, HAdV was detected in approximately 10.8% of all gastroenteritis cases among children under five years of age between 2007 and 2019 (Oppong et al. 2020). Currently, 103 HAdV types are reported and classified into seven species (A–G) within the Mastadenovirus genus (Kosulin, 2019; Mennechet et al., 2019; Brister et al. 2019). Classification of the first 51 types was based upon serological profiles of the dominant capsid proteins, however, sequence analysis is now the standard method for characterising novel types (Mennechet et al. 2019). The large dsDNA genomes of HAdV encode many genes, including those for the hexon, penton and fibre proteins which constitute most of the viral capsid (Yellinger et al. 2005; Mennechet et al. 2019). PCR-based assays targeting the sequences encoding the hexon and fibre proteins have become the accepted method for HAdV detection, in both clinical and environmental samples (Jiang 2006). HAdV species F, types 40 and 41 account for almost all cases of HAdV-related gastroenteritis. Types within groups A, C, D and G have been detected in patients presenting with diarrhoea, but potential roles for these viruses in the aetiology of gastroenteritis remain unclear (Lion 2014; Afrad et al. 2018). Studies using next-generation sequencing (NGS) have documented the circulation of a wide range of HAdV species and types in sewage and wastewater in different countries (Ogorzaly et al. 2015; Iaconelli et al. 2017).

**Human Bocaviruses**

Human bocaviruses (HBoVs; genus Bocaparvovirus) are recent members of the Parvovirus family and have been detected globally in a variety of clinical samples (Guido et al. 2016). Four HBoVs are described and classified into two species (Primate bocaparvovirus 1: HBoV-1 and HBoV-3; Primate bocaparvovirus 2: HBoV-2 and HBoV-4) (Söderlund-Venermo 2019). HBoV-1 was first isolated from nasopharyngeal aspirates and is associated with respiratory tract infections in children (Christensen et al. 2019). HBoV-2 and HBoV-4 are frequently detected in stools from diarrheic patients (Guido et al. 2016) but also asymptomatic individuals (Paloniemi et al. 2014) and are often found in conjunction with other enteric viruses, such as RV and NoV (Huang et al. 2010; Campos et al. 2016). Consequently, the role of HBoVs in gastroenteritis remains to be clarified (Ong et al. 2016). Several PCR-based assays have been developed for the molecular detection of HBoV DNA in clinical and environmental samples (Lu et al. 2006; Iaconelli et al. 2016; La Rosa et al. 2016, 2018). These assays are specific for the sequences encoding the conserved non-structural protein (NS1) and moderately conserved nuclear phosphoprotein (NP1), while the hypervariable region encoding the VP1/VP2 capsid proteins is commonly targeted for the differentiation of genotypes (Guido et al. 2016; Lee et al. 2016; Wagner et al. 2016). Globally, HBoVs have been detected in environmental samples such as sewage wastewater (Blinkova et al. 2009; Iaconelli et al. 2016), river water (La Rosa et al. 2017) and shellfish (La Rosa et al. 2018; Onosi et al. 2020), highlighting potential routes of transmission.

**Sewage-Polluted Water and Bivalve Shellfish as Vehicles for Enteric Virus Transmission**

Human enteric viruses are shed at high concentrations ($10^5$–$10^{13}$ viral particles per gram of stool) in the faeces of infected individuals (Bosch 1998; Atmar et al. 2008; Drexler et al. 2011; Bosch et al. 2014), with or without illness and often long after the cessation of symptoms (Yotsuyanagi et al. 1996; Shastri et al. 1998; Murata et al. 2007; Takahashi et al. 2007; Kapusinszky et al. 2012; Crawford et al. 2017). Untreated sewage wastewater is therefore a significant source of enteric pathogens and requires decontamination prior to discharge in the environment. However, traditional treatment processes do not completely reduce viral loads as many enteric viruses can withstand physical treatment procedures and disinfectants such as chlorination and UV irradiation (La Rosa et al. 2010; Okoh et al. 2010; Qiu et al. 2015; Sidhu et al. 2018; Prado et al. 2019). Consequently, a high concentration of surviving virions may be discharged into receiving water bodies such as springs, rivers, dams or marine estuaries upon the release of inadequately treated effluent. Alternatively, raw sewage may enter the environment through damaged sewage infrastructure and wet weather overflows such as heavy rainfall events (Ahmed et al. 2020b). Moreover, many developing countries lack adequate sanitation infrastructure altogether, and surrounding water sources are easily contaminated with excreta containing enteric pathogens (Wang et al. 2014; Kayembe et al. 2018; Farkas et al. 2020). Indeed, it is estimated that only 28% of sewage wastewater is treated prior to discharge into surface waters in low- and middle-income countries (WWAP and UNESCO 2017).

Enteric viruses are highly stable in water environments, particularly when bound to particulate matter (Bosch 1998; Okoh et al. 2010; Seitz et al. 2011). Adsorption to solid particles facilitates the dispersion of viruses along water systems and the settling and accumulation of virions within sediments, where they may persist for prolonged periods (Goyal et al. 1984; Hassard et al. 2016). Disturbance of these sediments releases viruses back into the water column, enabling further dissemination through the water system (Bosch 1998). Enteric viruses have very low infectious doses (Okoh et al. 2010); for example, the 50% human infectious dose...
(HID)\textsubscript{50} of NoV is 18 virus particles, while for RV, only 1 focus forming unit (ffu) is required to infect 25\% of susceptible adults (Yezli and Otter 2011; Hall 2012). Therefore, the use of untreated water from environmental sources often leads to outbreaks of viral diseases, even in cases where contamination is marginal (Sinclair et al. 2009).

The risk of enteric virus transmission arises not only from contact with polluted water but also from the consumption of bivalve shellfish grown and harvested in virus-contaminated waters (Lees 2000; Bellou et al. 2013). Bivalve shellfish (molluscs) including clams, mussels, oysters and scallops are active filter-feeding species that remove and sequester food particles together with a myriad of waterborne microbes. These bivalves sieve many gallons of water a day through their gills, (Burkhardt and Calci 2000; Rice 2001; Greening and Cannon 2016), which can lead to the bioaccumulation of environmentally stable, viral pathogens within shellfish tissues, at concentrations far exceeding those in the overlying water column (Burkhardt and Calci 2000; Rehnstam-Holm and Hernroth 2005; Araud et al. 2016; Amoroso et al. 2020). Because of their ability to accumulate organic and inorganic contaminants, wild bivalve shellfish may be exploited as biological monitors for the surveillance of pollution in coastal environments (Viarengo and Canesi 1991; Donia et al. 2012; Gao et al. 2015; Kim et al. 2016).

The consumption of shellfish harvested from faecally contaminated waters is directly associated with outbreaks of viral gastroenteritis (reviewed in Bellou et al., 2013). Such outbreaks occur on a regular basis worldwide (Le Guyader et al. 2008; Westrell et al. 2010; Iritani et al. 2014; Lunestad et al. 2016; Woods et al. 2016; Meghnath et al. 2019) with potentially significant associated economic costs (Rheingans et al. 2009; Navas et al. 2015; Bartsch et al. 2016; Papadopoulos et al. 2019). Among the enteric viruses, NoV and HAV represent the majority of viral illnesses (Iizuka et al. 2010; Pepe et al. 2012; Fusco et al. 2013, 2019; La Bella et al. 2017), although other viruses like HEV, HAstV, RV, SaV and AiV impact shellfisheries with symptoms that are more or less similar to those caused by NoVs (Pina et al. 1998; Le Guyader et al. 2008; Nakagawa-Okamoto et al. 2009; Iritani et al. 2014; La Bella et al. 2017; La Rosa et al. 2018).

To reduce the risk of shellfish-associated infections, sanitary regulations based on bacterial indicators (e.g. Escherichia coli and Salmonella) in shellfish or their growing waters have been instituted in many countries. However, the inadequacy of the bacterial indicators for virus detection makes it necessary to develop new prevention strategies based on viral analysis, for improved regulatory standards (Flannery et al. 2009; Baert et al. 2011). Furthermore, methods to process shellfish post-harvest (e.g. depuration, relaying and heat treatment) are effective at reducing bacterial loading, but their efficacy at reducing viral loading to non-infectious levels may be limited (Baker 2016; Richards 2016). Consequently, improved processing procedures need to be developed to enhance shellfish safety.

### Human Enteric Viruses in African Aquatic Environments

Over one-hundred studies from twelve African countries have reported on the presence of enteric viruses in environmental water samples (Figure 1). These studies were identified using the electronic databases PubMed and Google Scholar and the search words “enteric virus”, “rotavirus”, “norovirus”, “sapovirus”, “calicivirus”, “astrovirus”, “enterovirus”, “hepatitis A virus”, “aichivirus”, “hepatitis E virus”, “adenovirus”, “bocavirus”, “wastewater”, “sewage”, “effluent”, “influent”, “drainage water”, “drinking water”, “ground water”, “surface water”, “irrigation water”, “Africa” and “name of country” in various combinations. Most of the studies were conducted in South Africa (39), Egypt (23) and Tunisia (18), followed by Kenya, Ghana, Morocco and then Uganda with seven, five, five and three studies, respectively. Two studies were conducted each in Tanzania and Nigeria, while only one study was conducted each in Côte d’Ivoire, Benin and Chad. These studies were conducted between 1995 and 2020, and the duration of sampling ranged from

![Fig. 1](https://example.com/image.png)
one month to four years. Collectively, the reports describe the presence, prevalence and diversity of enteric viruses in sewage, wastewater effluent, ground and surface water, and even piped municipal water (reviewed below), indicating the widespread contamination of African water sources with these viral pathogens (Table 1).

**Wastewater and Sewage**

A wide range of viruses have been detected in sewage from wastewater treatment plants across Africa. Kiulia et al. (2010) detected HAdV in 87.5-100% and RVA in 20-100% of raw sewage samples from two sewage treatment plants in Nairobi, Kenya, while another study detected calicivirus (NoV and SaV) RNA in 82% of raw sewage samples collected from 21 sewage treatment facilities across South Africa (Murray et al. 2013a). More recently, Shaheen et al. (2019a, b) detected AiV RNA and HBoV DNA in 16.6% and 41.6% of untreated raw sewage samples collected at the Abu-Rawash wastewater treatment plant in Giza, while HAdV and RV A were detected in 50% of urban sewage samples collected from the same site, with concentrations ranging from $10^3$ to $10^8$ genome copies/litre (GC/L) (Elmahdy et al. 2020). Enteric viruses were also recovered from final wastewater effluents in Egypt (Kamel et al. 2010; Shaheen and Elmahdy 2019; Shaheen et al. 2019a), Ghana (Silverman et al. 2013), Morocco (Amdiouni et al. 2017), South Africa (Olaniran et al. 2012; Osuolale and Okoh 2015; Adelisoye et al. 2016) and Tunisia (Béji-Hamza et al. 2014, 2015; Hassine-Zafrane et al. 2014; Ouardani et al. 2015; Varela et al. 2018), even after treatment and chlorination. Hamza et al., (2017) detected HBoV-1, -2 and -3 at concentrations of $2.9 \times 10^3$ GC/L, $4.1 \times 10^3$ GC/L and $2.1 \times 10^3$ GC/L in 34.3%, 84.4% and 87.5% of treated effluent samples, respectively. These samples were collected from three wastewater treatment plants in Greater Cairo which ultimately discharge treated effluents into the River Nile and Al Manzala Lake (Hamza et al. 2017). A separate study examined the prevalence of SaV at four Tunisian wastewater treatment plants between 2009 and 2010 and found that treatment did not sufficiently reduce SaV loads as concentrations of SAV RNA were relatively high in both influent and effluent samples (Varela et al. 2018). These studies highlight the need for improved wastewater treatment methodologies that eliminate enteric viruses from wastewaters prior to discharge into African water environments.

**Ground and Surface Water**

There is a significant lack of basic sanitation services and proper wastewater treatment in many African countries (Wang et al. 2014). For example, only 18%, 26%, 39% and 61% of people living in Algeria, Libya, Morocco and Egypt, respectively, have access to safely managed sanitation facilities (WHO et al. 2019), while in sub-Saharan Africa, approximately 709 million people are without basic sanitation services, sewer systems or wastewater treatment facilities (UN Habitat 2013; Ezeh et al. 2017; WHO et al. 2019). Consequently, millions of people resort to open defecation, or the use of pit latrines and septic tanks (WHO et al. 2019), which are recognised as major sources of microbial contamination in surrounding water resources within the region (Graham and Polizzotto 2013; Njuguna 2016; Lapworth et al. 2017). Indeed, many studies have described the detection of enteric viruses in contaminated water sources in Africa. For example, studies in Chad (Guerrero-Latorre et al. 2011), Ghana (Gibson et al. 2011; Lutterodt et al. 2018) Kenya (Kiulia et al. 2014), Nigeria (Muhammad et al. 2020), South Africa (Ehlers et al. 2005; Murray and Taylor 2015) and Uganda (Katukiza et al. 2013a, 2013b; Sadik 2016) have reported the contamination of groundwater sources such as boreholes, springs and wells with enteric viruses including EV, HAdV, NoV, SaV and RV.

Untreated or partially treated sewage effluent is a major contaminant of surface water sources in many African cities (Omosa et al. 2012; Adewumi and Oguntunase 2016; Herbig 2019; Okubo et al. 2019), and enteric viruses including EV, HAdV, HAV, HBoV and RV are frequently detected in African rivers, dams and lakes at high concentrations ranging from $1.3 \times 10^3$ to $1.5 \times 10^7$ GC/L (Hamza et al. 2009; Chigor and Okoh 2012a; Katukiza et al. 2013b; Sibanda and Okoh 2013; Shaheen et al. 2019b). The Nile is the main source of water for irrigation and domestic use in Egypt, however, many studies have found this river to be polluted with enteric viruses (El-Senousy et al. 2013a, 2014, 2015; Shaheen et al. 2019b). For example, 4%, 25%, 16.6%, 8.3% and 12.5% of water samples collected from the Rosetta branch of the Nile in 2017 and 2018 were contaminated with AiV, AstV, NoV, RV and HBoV, respectively (Shaheen et al. 2018, 2019a; Shaheen and Elmahdy 2019), while more recently, Rizk and Allayeh, (2020) detected RVA RNA in 18.75% of Nile water samples collected in Giza. Similarly, enteric viruses have been found in major surface waters in Kenya, Chad, Nigeria and Uganda (Kiulia et al. 2010, 2014; Guerrero-Latorre et al. 2011; Katukiza et al. 2013a, 2013b; Baker et al. et al. 2018; Bauza et al. 2019; van Zyl et al. 2019; Muhammad et al. 2020). For example, two separate studies detected EV and HAdV in water samples from Homa Bay, Lake Victoria (Sadik 2016; Opere 2019). Over the last 25 years, many studies have revealed the presence of enteric viruses in South African rivers and dams, which are essential water sources for the surrounding communities (Taylor et al. 2001; Sibanda and Okoh 2012; Sibanda et al. 2013; Saïd et al. 2014; Lin et al. 2015; Potgieter et al. 2020). For example, several studies have identified HAdV, HAV and
Table 1 Detection of enteric viruses in environmental water and sewage collected in Africa

| Virus | Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|-------|-----------------------|-------------------------|-----------------|-------------|---------|----------------|------|
| N.D.  | RT-qPCR               | N.R.                    | DW (untreated)  | South Africa | N.R.   | (Grabow et al. 2001) |
| N.D.  | ELISA                 | N.A.                    | GW              | Ghana       | N.R.   | (Lutterodt et al., 2018) |
| N.D.  | RT-qPCR, multiplex & nested RT-PCR† | Partial VP6, VP7† & VP4† | SWG & WW | Egypt       | N.R.   | (Okabo et al. 2019) |
| G1, G2, G3, G4, G5 & G9; P[4], P[6], P[8] & P[9] | Nested RT-PCR | Partial VP7 | SWG & DW | South Africa | Jul 2000–Jun 2002 | (van Zyl et al. 2004) |
| G1, G2, G3, G8, & G9; (P[4], P[6], P[8] & P[9] | Nested RT-PCR | Partial VP7† & VP4† | SW (river & dam), IW & DW (treated) | South Africa | Apr 2002–Feb 2005 | (van Zyl et al. 2006) |
| N.D.  | RT-PCR                | N.R.                    | DW              | Benin        | May 2003–Apr 2007 | (Verheyen et al. 2009) |
| G & P genotypes | Nested RT-PCR† | VP7† & VP4† | SWG & WW (effluent) | Tunisia | Jan 2003–Apr 2007 | (Sdiri-Loulizi et al. 2010a) |
| G1P[8], G2P[4], G2P[8], G9P[8] & G1P[4] | RT-PCR† | VP6†, VP7† & VP4† | SWG & WW (effluent) | Egypt | Apr 2006–Feb 2007 | (Kamel et al. 2010) |
| G1P[4], G1P[6], G1P[8], G3P[4] & G3P[8] | RT-PCR† | VP6†, VP7† & VP4† | SW & DW | Egypt | Oct 2006–Sep 2008 | (El-Senousy et al. 2014) |
| G3P[8], G2P[8], G1, G2, G3, G5, G8, G9, G10, G11 & G12 | Nested RT-PCR† | VP7† | SW (river) & SWG | Kenya | May 2007–Feb 2008 | (Kiulia et al. 2010) |
| G1, G2, G3, G4, G5, G6, G8, G9, G10 & G11; P[1], P[4], P[5], P[6], P[7], P[8], P[9] & P[11] | Nested RT-PCR† | VP7† & VP4† | SWG and WW (effluent) | Tunisia | April 2007–April 2010 | (Hassine-Zaafane et al. 2015) |
| N.D.  | RT-qPCR & cell-culture-RT-PCR | VP6 | SWG, WW (effluent) & SW (river) | Egypt | Jul 2009–Jun 2011 | (El-Senousy et al. 2013a) |
| Groups A & C | RT-PCR & RT-qPCR | Partial VP6 & VP7 | SWG & WW (effluent) | Egypt | Nov 2009–Oct 2011 | (El-Senousy et al. 2015) |
| N.D.  | RT-qPCR               | NSP3                    | DW              | Tanzania     | Mar 2010 | (Mattioi et al. 2013) |
| N.D.  | RT-PCR                | NSP3                    | DW              | Tanzania     | March–May 2010 | (Mattioi et al. 2014) |
| N.D.  | RT-qPCR               | NSP3                    | GW (spring) & DNW | Uganda | Jan 2010–Feb 2014 | (Katukiza et al. 2013a) |
| N.D.  | RT-qPCR               | NSP3                    | SW (river & dam) | South Africa | Aug 2010–Jul 2011 | (Chigor and Okoh 2012a) |
| N.D.  | RT-qPCR               | NSP3                    | SW (river)     | South Africa | Aug 2010–Jul 2011 | (Sibanda and Okoh 2013) |
| G1, G3, G8, G9 & G10 | RT-qPCR & semi-nested multiplex RT-PCR† | Partial VP2, VP4† & VP7† | WW | Tunisia | 2011 (Months N.R.) | (Ibrahim et al. 2016) |

RT-qPCR: Real-Time Quantitative Polymerase Chain Reaction
ELISA: Enzyme-Linked Immunosorbent Assay
RT-PCR: Reverse Transcription-Polymerase Chain Reaction
NSP3: Nucleocapsid Protein 3
VP6, VP7, VP4: Non-structural Proteins 6, 7, 4
VP2: Second Inner Capsid Protein
VP6, VP7: Inner Capsid Proteins
VP4: Outer Capsid Protein
VP1: Outer Capsid Protein
| Virus          | Group/Genotype/Strain                                | Detection/Typing method                      | Targeted region | Sample type      | Country       | Period sampled    | Ref.                          |
|---------------|-----------------------------------------------------|----------------------------------------------|-----------------|------------------|---------------|-------------------|-------------------------------|
| N.D.          | Nested RT-PCR, RT-qPCR & ICC PCR                    | VP7                                          | SW (river)      | South Africa     | Mar 2011–Jan 2012 | (Singh 2012)                |
| N.D.          | RT-qPCR                                             | VP7                                          | WW (effluent)   | South Africa     | Sep 2012–Aug 2013 | (Osuolale and Okoh 2017)    |
| N.D.          | RT-qPCR                                             | N.R.                                         | SW (river) & WW | Kenya             | Apr 2015–Apr 2016 | (van Zyl et al. 2019)       |
| G1, G3 & G9; P[4], P[6] & P[8] | Nested RT-PCR†                                     | VP4⁺, VP6⁺ & VP7⁺                             | Egypt           | Oct 2015–Mar 2017 | (El-Senousy et al. 2020)   |
| Group A       | Illumina Miseq                                       | N.A.                                         | DNW             | Kenya             | Jun–Aug 2016      | (Hendriksen et al. 2019)    |
| N.D.          | RT-qPCR                                             | N.R.                                         | SW (river)      | South Africa     | Jun–Nov 2016      | (Potgieter et al. 2020)     |
| G1, G2, G3 & G9; P[4], P[6], & P[8] | RT-PCR & multiplex semi-nested RT-PCR†             | VP6, VP7 & VP4†                               | Egypt           | June 2016–May 2017 | (Rizk and Allayeh 2020)    |
| N.D.          | RT-PCR                                              | NSP3                                         | SW (lagoon) & WW| South Africa     | Dec 2016–Jan 2017 | (Sekwadi et al. 2018)       |
| G1P[8], G2P[8], G2P[4], G4P[8] & G9P[8] | RT-PCR & semi-nested RT-PCR†                       | DW (tap water)                               | Egypt           | Dec 2016–Nov 2017 | (Gad et al., 2019)         |
| Group A       | RT-qPCR                                             | NSP3                                         | SWG (raw & activated sludge) & WW (effluent) | Egypt           | Sep–Dec 2017      | (Shaheen et al. 2019b)      |
| Group A       | RT-qPCR                                             | NSP3                                         | DW (tap water)  | Nigeria          | N.R.             | (Elmahdy et al. 2020)       |
| Group C       | Semi-nested RT-PCR                                  | VP7                                          | SWG (raw & activated sludge), WW (effluent) & SW (river) | Egypt           | Apr 2017–Sep 2018 | (Shaheen et al. 2018)       |
| Norovirus     | GI                                                   | Nested RT-PCR                                | ORF1-ORF2 junction | DW (tap water)  | Ghana          | N.R.                  | (Dongdem et al. 2011)       |
| N.D.          | RT-qPCR                                             | N.R.                                         | DW              | Nigeria          | N.R.             | (Ali et al. 2004)          |
| GI, GII & GIV | RT-qPCR                                             | N.R.                                         | DNW             | Nigeria          | N.R.             | (Okubo et al. 2019)        |
| GI, GL2, GL5, GL9 & GI14 | RT-PCR†                                           | RdRp & partial capsid gene                   | SWG & WW (effluent) | Tunisia          | Jan 2003–Apr 2007 | (Sdiri-Loulizi et al. 2010a) |
| GI, GL2, GL3, GL5, GL9, GI14 & GI1b | RT-qPCR & Semi-nested RT-PCR†                     | Partial capsid gene†                         | SWG & WW (effluent) | Egypt           | Apr 2006–Feb 2007    | (Kamel et al. 2010)         |
| GI & GII      | RT-qPCR                                             | ORF1-ORF2 junction                           | SW (river) & SWG | Kenya            | May 2007–Feb 2008 | (Kiulia et al. 2010)       |
| GI, GII       | RT-PCR†                                             | Partial RdRp & a conserved region of ORF2⁺   | SWG & WW (effluent) | Tunisia          | Apr 2007–Apr 2010 | (Hassine-Zaafrene et al. 2014) |
| GI & GII      | RT-qPCR                                             | ORF1-ORF2 junction                           | IW              | Egypt            | Dec 2008 – Nov 2009 | (El-Senousy et al. 2013b)   |
| GI & GII      | RT-qPCR                                             | ORF1-ORF2 junction                           | GW, SW & DW (treated) | Ghana          | Jul–Aug 2009    | (Gibson et al. 2011)       |
| Virus | Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|-------|----------------------|-------------------------|-----------------|-------------|---------|----------------|-----|
| GI & GII | RT-qPCR & RT-PCR† | capsid region† | SW (river) | South Africa | Jan 2008–Dec 2010 | (Mans et al. 2013) |
| GI & GII | RT-qPCR & Semi-nested RT-PCR† | ORF1-ORF2 junction | SW (river), DNW, SWG & WW (effluent) | Ghana | Jul 2010 | (Silverman et al. 2013) |
| GI & GII | RT-qPCR & nested RT-PCR† | Partial capsid region† | SWG (raw & activated sludge) & WW (effluent) | South Africa | Aug 2010–Dec 2011 | (Murray et al. 2013a) |
| GI & GII | RT-qPCR & RT-PCR† | Partial capsid & RdRp‡ | WW | Tunisia | Jan–Dec 2011 | (Ibrahim et al. 2015) |
| GI & GII | RT-qPCR | ORF1-ORF2 junction | SWG (sludge) & DNW | Ghana | Apr–Oct 2012 | (Kiulia et al. 2010) |
| GI & GII | RT-qPCR | N.R. | SW (river) & WW (effluent) | Kenya | Apr 2015–Mar 2016 | (van Zyl et al. 2019) |
| GI & GII | RT-qPCR | N.A. | DNW | South Africa | Jun–Aug 2016 | (Hendriksen et al. 2019) |
| GI & GII | RT-qPCR | Partial capsid region† | SW (lagoon) & WW (effluent) | Kenya | Dec 2016–Jan 2017 | (Sekwadi et al. 2018) |
| GI & GII | RT-qPCR | N.R. | SW (river) | South Africa | Jun–Nov 2016 | (Potgieter et al. 2020) |
| GI & GII | RT-qPCR | ORF1-ORF2 junction | SW (river), DNW & WW | Egypt | Sep–Dec 2017 | (Shaheen et al. 2019b) |
| GI & GII | RT-qPCR | Partial RdRp & capsid genes | SW (river), DNW & WW | Egypt | Apr 2017–Mar 2018 | (Shaheen and Elmahdy 2019) |
| GI & GII | RT-qPCR | ORF1-ORF2 junction | SWG (raw & activated sludge), WW (effluent) & SW (river) | Egypt | Apr 2017–Sep 2018 | (Shaheen et al. 2018) |
| Sapovirus | RT-qPCR† | RdRp/capsid junction† | SW (river) & SWG | Kenya | May 2007–Feb 2008 | (Kiulia et al. 2010) |
Table 1 (continued)

| Virus | Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|-------|-----------------------|-------------------------|-----------------|-------------|---------|----------------|------|
| GI.1, GI.2, GI.3, GI.5, GI.6, GI.7, GII.3, GII.5 & GII.7 | RT-qPCR & nested RT-PCR† | RdRp/capsid junction† | SW (river) & WW | South Africa | Jan 2009–Dec 2010 | (Murray et al. 2013b) |
| GI.1, GI.2, GI.1 & GII.1 | RT-qPCR & nested RT-PCR† | RdRp/capsid junction & partial capsid† | SWG (influent) & WW (effluent) | Tunisia | Dec 2009–Dec 2010 | (Varela et al. 2018) |
| N.D. | RT-qPCR | RdRp/capsid junction | SWG (sludge) & WW (effluent) | South Africa | Aug 2010–Dec 2011 | (Murray et al. 2013c) |
| GI.2, GI.3, GI.6, GII.1 & GII.2 | RT-qPCR & nested RT-PCR† | RdRp & capsid junction† | SWG (raw & activated sludge) & WW (effluent) | South Africa | Aug 2010–Dec 2011 | (Murray et al. 2013a) |
| GIV.1 & GGI.3 | RT-qPCR & RT-PCR† | RdRp† | WW (influent & effluent) | Tunisia | Jan–Dec 2011 | (Ibrahim et al. 2019) |
| GL2 & GIV | RT-qPCR & nested RT-PCR† | RdRp & partial capsid† | SW (river & dam), GW (borehole) & WW (effluent) | South Africa | Jan–Mar 2012 | (Murray and Taylor 2015) |
| N.D. | RT-qPCR | RdRp/capsid junction | SW (river) & WW (effluent) | Kenya | Apr 2015–Apr 2016 | (van Zyl et al. 2019) |
| N.D. | Illumina MiSeq | N.A. | DNW | Kenya | Jun–Aug 2016 | (Hendriksen et al. 2019) |
| N.D. | Nested-RT-PCR | RdRp/capsid junction | SW (river) | South Africa | Jun–Nov 2016 | (Potgieter et al. 2020) |
| N.D. | RT-PCR | Partial 3′UTR | SW | South Africa | N.R. | (Marx et al. 1995) |
| N.D. | RT-PCR | 3′UTR | DW | South Africa | N.R. | (Grabow et al. 2001) |
| N.D. | RT-PCR | N.R. | DW (tap water) | Ghana | N.R. | (Dongdem et al. 2011) |
| N.D. | RT-PCR (following amplification by cell culture) | Partial 3′UTR | SW (river & dam) | South Africa | Jun 1997–May 1998 | (Taylor et al. 2001) |
| Genogroups A & B | Competitive multiplex RT-PCR† | Partial ORF1α† | SWG, WW (effluent) & DW | Egypt | Nov 1998–Oct 1999 | (El-Senousy et al. 2007) |
| Genotypes 1, 2, 3, 4, 5, 7, & 8 | RT-PCR† (following cell culture amplification)† | 3′ end of ORF2†, partial capsid & ORF1α† | SWG & WW (effluent) | South Africa | Apr 1999–Oct 2000 | (Nadan et al. 2003) |
| N.D. | RT-qPCR | Partial 3′UTR | SW (river) & SWG | Kenya | May 2007–Feb 2008 | (Kiulia et al. 2010) |
| Genotypes 1 & 6 | RT-qPCR Partial capsid† | WW | Tunisia | Jan–Dec 2011 | (Ibrahim et al. 2017b) |
| N.D. | RT-qPCR | N.R. | SW (river) & WW (effluent) | Kenya | Apr 2015–Apr 2016 | (van Zyl et al. 2019) |
| 1, MLB3 | Illumina MiSeq | N.A. | DNW | Kenya | Jun–Aug 2016 | (Hendriksen et al. 2019) |
| N.D. | RT-qPCR | N.R. | SW (river) | South Africa | Jun–Nov 2016 | (Potgieter et al. 2020) |
| N.D. | RT-PCR | RdRp & capsid | SW (lagoon) & WW (effluent) | South Africa | Dec 2016–Jan 2017 | (Sekwadi et al. 2018) |
| Virus                      | Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country   | Period sampled | Ref.                          |
|---------------------------|-----------------------|-------------------------|-----------------|-------------|-----------|----------------|------------------------------|
| Genogroups A & B          | Semi-nested RT-PCR†   | Partial ORF1α†          | SWG (raw & activated sludge), WW (effluent) & SW (river) | Egypt       | Apr 2017–Sep 2018 | (Shaheen et al. 2018)       |
| Genogroups A & B          | Semi-nested RT-PCR†   | Partial ORF1α†          | SW (river), WW & DNW | Egypt       | Apr 2017–Mar 2018 | (Shaheen and Elmahdy 2019)   |
| Enterovirus               | N.D.                  | Nested RT-PCR           | 5’UTR           | DW (untreated & treated) | South Africa | N.R.           | (Grabow et al. 2001)         |
| N.D.                     | RT-qPCR               | N.R.                    | DNW             | Egypt       | N.R.               | (Ali et al. 2004)           |
| N.D.                     | RT-qPCR               | N.R.                    | DNW             | Egypt       | N.R.               | (Azzam et al. 2014)         |
| CBV & PV                  | Nested RT-PCR         | Partial 5’UTR†          | SW (river & dam) & WW | South Africa | Jan 1996–Oct 1997 | (Grabow et al. 1999)        |
| CBV & PV                  | Nested RT-PCR         | 5’UTR & VP1†            | DW              | South Africa | Apr 1999–Mar 2000 | (Vivier et al. 2004)        |
| Echovirus (4, 6, 11,13, 25 & 33), PV, CAV 5 & 6; CBV1, 2, 3, 4 & 5; ECV 11 | Nested RT-PCR & restriction enzyme analysis† | Partial 5’UTR† | SWG, SW (river & dam), GW (spring & borehole) & DW | South Africa | Jul 2000–Jun 2002 | (Ehlers et al. 2005)         |
| PV                        | Triplex RT-PCR†       | 5’UTR & VP1†            | SWG & SW (river) | South Africa | 2001–2003 (Months N.R) | (Pavlov et al. 2005)        |
| N.D.                     | RT-PCR                | 5’UTR                   | GW (public baths) | Morocco     | Jan–Jun 2004 | (Karamoko et al. 2006b)    |
| N.D.                     | RT-PCR                | N.R.                    | WW              | Tunisia     | Oct 2005–Jul 2006 | (Hassine et al. 2010)       |
| N.D.                     | RT-qPCR               | 5’UTR                   | SWG & WW (effluent) | Egypt       | Jul 2006–Jan 2007 | (Kamel et al. 2010)         |
| N.D.                     | RT-qPCR               | 5’UTR                   | SW (river) & SWG | Kenya       | May 2007–Feb 2008 | (Kiuila et al. 2010)        |
| N.D.                     | RT-qPCR               | 5’UTR                   | WW              | Morocco     | Aug 2007–Oct 2008 | (Hassaine et al. 2011)      |
| CBV5 and PV               | RT-PCR†               | VP1†                    | SWG & WW (effluent) | Morocco     | Apr–July 2008 | (Amdiouni et al. 2012)     |
| N.D.                     | RT-PCR                | 5’UTR                   | SWG & lagoon sediments | Côte d’Ivoire | Sep 2008–Jan 2009 | (Momou et al. 2017)        |
| N.D.                     | RT-PCR                | 5’UTR                   | SWG (raw & sludge) & WW (effluent) | Tunisia       | Jan–Aug 2009 | (Jebri et al. 2012)        |
| N.D.                     | RT-qPCR               | 5’UTR                   | WW              | Morocco     | Jan–Dec 2009 | (Amdiouni et al. 2017)     |
| N.D.                     | RT-qPCR               | 5’UTR                   | DW              | Tanzania    | Mar 2010    | (Mattoli et al. 2013)       |
| Echovirus 7, 11, 13, 19, 20 | RT-qPCR & serological detection† | Partial VP1†           | DNW & SWG      | Nigeria     | Jun–Sept 2010 | (Adeniji and Faleye 2014)   |
| N.D.                     | RT-qPCR               | 5’UTR                   | SW (river)      | South Africa | Aug 2010–Jul 2011 | (Chigor and Okoh 2012a)     |
| N.D.                     | Nested RT-PCR         | 5’UTR                   | SW (lake)       | Kenya       | Oct 2011–Apr 2012 | (Opere 2019)                |

†semi-nested RT-PCR; †nested RT-PCR; †RT-qPCR; †cell culture & serotype identification; †restriction enzyme analysis; †RT-PCR & serological detection; †Nested RT-PCR & restriction enzyme analysis; †Triplex RT-PCR; †RT-PCR & serological detection; †RT-qPCR; †RT-qPCR & serological detection.
Table 1 (continued)

| Virus | Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|-------|-----------------------|-------------------------|-----------------|-------------|---------|---------------|------|
| CBV 5 | Nested RT-PCR & ICC RT-PCR & ICC | 5'UTR† | SW (river) | South Africa | April 2011–Jan 2012 | (Lin and Singh 2015) |
| N.D. | RT-qPCR | 5'UTR | GW, SW & DW (tap water) | Uganda | Nov 2014–May 2015 | (Sadik 2016) |
| N.D. | RT-qPCR | N.R. | SW (river) & SW WW | Kenya | Apr 2015–Apr 2016 | (van Zyl et al. 2019) |
| EV A, B, C | Illumina Miseq | N.A. | DNW | Kenya | Jun–Aug 2016 | (Hendriksen et al. 2019) |
| N.D. | RT-qPCR | 5'UTR | SW (river) | South Africa | Jun–Nov 2016 | (Potgieter et al. 2020) |
| N.D. | RT-qPCR | VP3/VP1 & VP1/P2A regions | DW (treated & untreated) | South Africa | Jul 2010–Aug 2012 | (Shaheen et al. 2019b) |
| N.D. | RT-PCR | N.R. | DW (treated & untreated) | Egypt | N.R. | (Ali et al. 2004) |
| N.D. | RT-PCR | 5'UTR & VP3/VP1 region | SW (river & dam) | South Africa | Jun 1997–May 1998 | (Taylor et al. 2001) |
| IB | RT-PCR† | VP1X2A region† | SWG | Egypt | Nov 1998–Oct 1999 | (Pintó et al. 2007) |
| IA | RT-PCR† | VP3/VP1 region† | SWG & WW (effluent) | Tunisia | Sep 2000–Aug 2001 | (Gharbi-khelifi et al. 2007) |
| N.D. | RT-PCR | N.R. | WW | Tunisia | Oct 2005–Jul 2006 | (Hassine et al. 2010) |
| IB | Semi-nested RT-PCR† | N.R. | SWG & WW (effluent) | Egypt | Jul 2006–Feb 2007 | (Kamel et al. 2011) |
| IA & IB | Nested RT-PCR† & RT-qPCR | VP1/2A | SWG & WW (effluent) | Tunisia | 2007–2008 (Months N.R.) | (Béji-Hamza et al. 2014) |
| IA & IB | RT-qPCR & semi-nested RT-PCR† | 5'UTR & VP3/VP1 region† | SWG & WW (effluent) | Tunisia | Dec 2009–Dec 2010 | (Ouwardani et al. 2015) |
| N.D. | RT-qPCR | 5'UTR | SW (river & dam) | South Africa | Aug 2010–Jul 2011 | (Chigor and Okoh 2012a) |
| N.D. | RT-qPCR | 5'UTR | SW (river) | South Africa | Aug 2010–Jul 2011 | (Sibanda and Okoh 2013) |
| N.D. | RT-qPCR | 5'UTR | SW & WW | Uganda | Jan–Feb 2011 | (Katukiza et al. 2013b) |
| IB | RT-qPCR & nested RT-PCR† | VP1/P2B† | SW (river & dam) & WW (effluent) | South Africa | Jan 2010–Aug 2012 | (Sáló et al. 2014) |
| V | RT-qPCR & RT-PCR† | VP1 & VP1/2B† | SW (dam) | South Africa | Jan–Mar 2013 | (Rachida et al. 2016) |
| N.D. | Nested RT-PCR | VP1/2A | SW (river) | South Africa | Apr–Sep 2014 | (Marie and Lin 2017) |
| IB | RT-PCR† | VP1/2A† | WW | Egypt | 2014 (Months N.R.) | (Hamza Ewess 2016) |
| N.D. | RT-qPCR | N.R. | SW (river) & WW (effluent) | Kenya | Apr 2015–Apr 2016 | (van Zyl et al. 2019) |
| N.D. | RT-qPCR | N.R. | SW (river) | South Africa | Jun–Nov 2016 | (Potgieter et al. 2020) |
| N.D. | RT-qPCR | 5'UTR | SW (river) | Egypt | Sep–Dec 2017 | (Shaheen et al. 2019b) |
Table 1 (continued)

| Virus Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|-----------------------------|-------------------------|-----------------|-------------|---------|----------------|-----|
| Aichivirus A                | RT-PCR†                 | 3CD junction†    | SWG & WW    | Tunisia | Jan 2003–Apr 2007 | (Sdiri-Loulizi et al. 2010b) |
| B                           | RT-PCR†                 | 3CD junction†    | WW          | Tunisia | 2011 (Months N.R.) | (Ibrahim et al. 2017a) |
| B                           | RT-PCR†                 | 3CD junction† & VP1† | SWG    | South Africa | May 2016 | (Onosi et al. 2019) |
| A                           | Illumina Miseq          | N.A.            | DNW         | Kenya   | Jun–Aug 2016 | (Hendriksen et al. 2019) |
| N.D.                        | Semi-nested RT-PCR      | 3CD junction    | SWG (raw, sludge & treated), DNW, SW (river) & river sediment | Egypt | Oct 2017–Sep 2018 | (Shaheen et al. 2019a) |
| Hepatitis E virus           | Genotype 3              | Nested RT-PCR†  | ORF1, ORF2 & ORF2/3 regions† | SWG & WW (effluent) | Egypt | Jul 2006–Feb 2007 | (Kamel et al. 2011) |
| N.D.                        | RT-PCR                 | ORF1, ORF2 & ORF2/3 regions | SWG, SW & DW | Egypt | Oct 2006–Sep 2008 | (El-Senousy et al. 2014) |
| Genotypes 1 & 3             | RT-PCR†                | ORF1, ORF2 & ORF2/3 regions† | SWG    | Tunisia | 2007–2008 (Months N.R.) | (Béji-Hamza et al. 2015) |
| Human adenovirus            | N.D.                   | N.R.            | N.R.        | DW (treated & untreated) | South Africa | N.R. | (Grabow et al. 2001) |
| N.D.                        | qPCR & nested PCR       | Hexon           | GW          | Ghana   | N.R.           | (Lutterodt et al. 2018) |
| N.D.                        | qPCR                   | N.R.            | DNW         | Egypt   | N.R.           | (Okubo et al. 2019) |
| N.D.                        | Cell culture neutralisation assay | N.A. | SW (river) | South Africa | Jan 1996–Oct 1997 | (Grabow et al. 1999) |
| N.D.                        | Nested PCR              | Hexon           | SW (river & dam) & DW (treated) | South Africa | Jul 2001–Jun 2002 | (van Heerden et al. 2004) |
| N.D.                        | Nested PCR              | Hexon           | SW (river & dam) & DW (treated) | South Africa | Jul 2000–Jun 2001 | (van Heerden et al. 2003) |
| N.D.                        | PCR                    | N.R.            | WW (effluent) | Morocco | 2001 (Months N.R.) | (Karamoko et al. 2005) |
| N.D.                        | Nested PCR              | Hexon           | Recreational water (swimming pool) | South Africa | Jan 2002–Mar 2003 | (van Heerden et al. 2005a) |
| HAdV C (2), D & F (40 & 41) | Nested PCR†             | Hexon†          | SW (river) & DW | South Africa | Jun 2002–Jul 2003 | (van Heerden et al. 2005b) |
| N.D.                        | qPCR                   | N.R.            | DW          | Benin   | May 2003–Apr 2007 | (Verheyen et al. 2009) |
| HAdV F (41)                | PCR†                   | Hexon†          | SWG         | Tunisia | Jan 2003–Apr 2007 | (Sdiri-Loulizi et al. 2010a) |
| HAdV A, B, C, D & F        | Nested PCR†             | Hexon†          | WW (effluent) & DW (treated) | South Africa | Jan 2006–Dec 2007 | (Mgwaliiba 2009) |
| N.D.                        | Nested PCR              | Hexon           | SW (river) & SWG | Kenya | May 2007–Feb 2008 | (Kialia et al. 2010) |
| HAdV B & D                 | PCR†                   | Hexon†          | SWG and WW (effluent) | Morrocco | Apr–July 2008 | (Amdouni et al. 2012) |
| Virus Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|---------------------------|-------------------------|-----------------|-------------|---------|----------------|-----|
| N.D.                      | Presumptive testing using TEM | N.A.            | WW (effluent) | South Africa | Jul–Sep 2009 | (Olaniran et al. 2012) |
| HAdV D                    | qPCR & nested PCR†       | Hexon†          | SW (river) & GW (borehole) | Chad | Sep 2009 | (Guerrero-Latorre et al. 2011) |
| N.D.                      | PCR                     | Hexon           | SW          | Ghana | Jul–Aug 2009 | (Gibson et al. 2011) |
| N.D.                      | PCR                     | Hexon           | WW (effluent) | Morocco | Jan–Dec 2009 | (Amdiouni et al. 2017) |
| HAdV C (2) & F (41)       | Nested PCR (following cell culture amplification) | Hexon           | SWG, WW (effluent) & SW (river) | Egypt | Jul 2009–2011 Jun | (El-Senousy et al. 2013a) |
| N.D.                      | PCR                     | Hexon           | DW          | Tanzania | March–May 2010 | (Mattioli et al. 2014) |
| N.D.                      | qPCR                    | Hexon           | SWG, DNW, WW (effluent) & SW (river) | Ghana | Jul 2010 | (Silverman et al. 2013) |
| HAdV C (1, 2 & 6), B (7) & F (41) | qPCR & multiplex PCR†       | Hexon & fibre†  | SW (river) | South Africa | Aug 2010–Jul 2011 | (Sibanda and Okoh 2012) |
| HAdV F (40 & 41) & B (52) | qPCR & multiplex PCR†       | Hexon & fibre†  | SW (river) | South Africa | Aug 2010–Jul 2011 | (Chigor and Okoh 2012a) |
| N.D.                      | qPCR                    | Hexon           | GW (spring) & DNW WW | Uganda | Jan 2010–Feb 2011 | (Katukiza et al. 2013a) |
| HAdV F (41)               | Nested PCR              | Hexon           | WW          | Tunisia | 2011 (Months N.R.) | (Ibrahim et al. 2018) |
| HAdV F (40 & 41) & G (52) | qPCR                    | Hexon           | SW, GW (spring) & WW | Uganda | Jan–Feb 2011 | (Katukiza et al. 2013b) |
| HAdV C (2)                | Nested PCR & ICC qPCR†   | Hexon†          | SW (river) | South Africa | April 2011–Jan 2012 | (Lin and Singh 2015) |
| N.D.                      | PCR                     | Hexon           | SWG (sludge) & DNW SW (lake) | Ghana | Apr–Oct 2012 | (Berendes et al. 2018) |
| HAdV F                    | Nested PCR              | Hexon           | WW (effluent) | South Africa | Oct 2012–Jul 2013 | (Opere 2019) |
| HAdV B (3) & F (41)       | qPCR & genotype-specific PCR†       | Hexon & fibre†  | WW (effluent) | South Africa | Sep 2012–Aug 2013 | (Oswolade and Okoh 2015) |
| HAdV C                    | Nested PCR              | Hexon           | SW (river) | South Africa | Apr–Sep 2014 | (Adefisyoye et al. 2016) |
| N.D.                      | qPCR                    | Hexon           | SW (stream), DNW & stored water | Kenya | Jun 2015 | (Marie and Lin 2017) |
| N.D.                      | qPCR                    | Hexon           | SW (river) & WW (effluent) | Kenya | Apr 2015–Apr 2016 | (Bauza et al. 2019) |
| HAdV D (17)               | Nested PCR†             | Hexon†          | SWG         | South Africa | May 2016 | (Vos and Knox 2018) |
| N.D.                      | qPCR                    | Hexon           | SW (river) | South Africa | Jun–Nov 2016 | (Potgieter et al. 2020) |
| HAdV F (40 & 41)          | PCR†                    | Hexon†          | DW (tap water) | Egypt | Dec 2016–Nov 2017 | (Gad et al. 2019) |
| N.D.                      | PCR                     | Hexon           | SWG (sludge) | Egypt | Jan–Dec 2017 | (Elmahdy et al. 2019) |
| N.D.                      | qPCR                    | Hexon           | SW (river) | Egypt | Sep–Dec 2017 | (Shaheen et al. 2019b) |
in some cases EV, NoV and RV in water samples from the Tyume and Buffalo rivers of the Eastern Cape (Chigor and Okoh 2012a, 2012b; Sibanda and Okoh 2012, 2013), the Klip River and Vaal dam in the Gauteng province (Taylor et al. 2001), and the Umhlangane river in KwaZulu-Natal (Marie and Lin 2017). From 2008 to 2010, Mans et al. (2013) reported the presence of NoV RNA in 66%, 95% and 21% of water samples from the Klip, Rietspruit and Suikerbosrant rivers, respectively. During this period, SaV RNA was detected in 39-54%, 83-92% and 14-20% of water samples from the same rivers (Murray et al. 2013b).

More recently, HAdV, NoV GI and GII together with EV, HAV, HaStV, HAdV and SaV were detected in water samples from the Madadzhe river in the Limpopo province, which is exploited for domestic and agricultural activities (Potgieter et al. 2020).

The widespread contamination of African rivers, canals, dams and lakes with viral pathogens constitutes a significant health risk to the millions of people who depend on these surface waters for drinking, irrigational, domestic and recreational activities (Chigor et al. 2014; Sibanda et al. 2015; Pichel et al. 2019; UNESCO 2019). From 2002 to 2004 van Zyl et al. (2006) detected RV A in 14% of irrigation water samples and 1.7% of corresponding raw vegetable samples collected in the Western Cape, Gauteng and Limpopo provinces of South Africa. In Egypt, marginal water from drainage canals, often contaminated with enteric pathogens, is unofficially used for the irrigation of crops, despite the increased risk of viral contamination of agricultural products. El-Senousy et al. (2013b) detected NoV RNA in 31.9% of irrigation water samples from the Daqahlia governorate in 2008 and 2009, while more recently, Okubo et al. (2019) identified AiV, EV, HAdV, HAV, HEV, NoVs (GI, GII and GIV) and RV in 100% of irrigation water samples from the Umoum agricultural drainage canal, and its tributaries. Enteric viruses have even been identified in treated drinking water produced at several treatment facilities across Africa (Ali et al. 2004; Ehlers et al. 2005; van Zyl et al. 2006; El-Senousy et al. 2014). In South Africa, Grabow et al. (2001) recovered viable EV, HAdV and HAV in 23% of chlorinated drinking water samples, while van Heerden and colleagues detected HAdV in 29.8% and 5.32% of drinking water samples between 2001-2002 and 2002-2003, respectively (van Heerden et al. 2004, 2005b). Similar studies have reported the presence of EV, HAV, HEV, HaStV, NoV and RV in chlorinated drinking water in Egypt (Ali et al. 2004; El-Senousy et al. 2007, 2014; Rizk and Alidayeh 2018). Recently, Gad et al. (2019) detected HAdV DNA in 8.9%, and RV RNA in 15.6% of tap water samples from five Egyptian cities including Cairo and Giza. Furthermore, treated tap and kiosk water in Uganda and Ghana have been found to be contaminated with enteric viruses including EV and NoV (GII), respectively (Gibson et al. 2011; Sadik 2016).

### Table 1 (continued)

| Virus Group/Genotype/Strain | Detection/Typing method | Targeted region | Sample type | Country | Period sampled | Ref. |
|----------------------------|-------------------------|-----------------|-------------|---------|----------------|------|
| N.D.                       | qPCR & PCR              | VP1/VP2         | SWG (raw & sludge), WW (effluent) | Egypt | Oct 2017–Sep 2018 (Onosi et al. 2020) |      |
| N.D.                       | Nested PCR              | VP1/VP2         | SWG (raw & sludge), WW (effluent), SW (river) & river sediment | South Africa | May 2016–Sep 2018 (Shaheen et al. 2019a) |      |
| CAV/CBV                    | qPCR & PCR              | VP1/VP2†        | SWG (raw & sludge), WW (effluent) | Egypt | Oct 2017–Sep 2018 (Mans et al. 2013) |      |
| Human Bocavirus            | Nested PCR              | VP1/VP2†        | SWG (raw & sludge), WW (effluent) | Egypt | Oct 2017–Sep 2018 (Mans et al. 2013) |      |

† Method of genotyping/typing

N.D. not determined, N.R. not reported, ORF open reading frame, RdRp RNA dependent RNA polymerase, UTR untranslated region, DNW drainage water, DW drinking water, GW ground water, IW irrigation water, SW surface water, SWG sewage, WW wastewater.

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**Enteric viruses in African shellfish**

Over the past two decades, only seventeen studies detecting human enteric viruses in African shellfish have been reported (Figure 2 and Table 2). These studies were identified by searching the PubMed and Google Scholar databases for studies which included terms such as “enteric virus”, “rotavirus”, “norovirus”, “sapovirus”, “calicivirus”, “astrovirus”, “enterovirus”, “hepatitis A virus”, “aichivirus”, “hepatitis E virus”, “adenovirus”, “bocavirus”, “shellfish”, “mussel”, “cockle”, “periwinkle”, “oyster” “Africa” and the names of African countries. The first report was published by Karamoko et al. (2005), who detected HAdV in 20% of mussel samples grown in Casablanca, Morocco (Karamoko et al. 2005). Subsequently, two additional studies reported the presence of HAdV in African shellfish; Vos and Knox (2018) detected HAdV D17 in mussels collected from the Swartkops river estuary in Port Elizabeth, South Africa, while Benabbes et al. (2013a) identified HAdV and EV in 52.3% and 36.3% of Moroccan shellfish samples (clams and cockles), respectively. Collectively, several other studies have detected NoV (GI and GII), SaV, EV and HAV in cockles, clams and oysters harvested from different growing waters in Morocco (Karamoko et al. 2006a; Benabbes et al. 2013b; El Moqri et al. 2019). Polo et al. (2010) detected NoV (GI and GII) in 15% and HAV in 6% of Moroccan shellfish at high concentrations, ranging from $4.7 \times 10^3$ to $7.7 \times 10^7$ GC/g digestive tissue. Similarly, enteric viruses have been detected in bivalve molluscs grown along the Tunisian coastline (Gharbi-khelifi et al. 2007; Zormati et al. 2018). Elamri (2006) detected HAstV in 61%, NoV in 35%, HAV in 26% and EV in 4.3% (one mussel) of shellfish (mussels and clams) collected from Tunisian waters from July 2000 to September 2001. In two separate studies, 1.6% and 6.6% of shellfish collected from a fishing zone located near a wastewater outlet site in Monastir, Tunisia, tested positive for NoV (GI.2) and AiV A, respectively (Sdiri-Loulizi et al. 2010a, 2010b). Furthermore, Onosi et al (2019) reported the presence of AiV B in mussels collected from the Swartkops river estuary in Port Elizabeth, South Africa. In another study, the authors detected HBoV-2 in the same mussel samples, making it the first report of HBoV in shellfish from Africa (Onosi et al. 2020). Finally, there are only single reports of enteric viruses in shellfish from Mozambique (Nenonen et al. 2006) and Nigeria (Babalola et al. 2019), where HAV IB and NoV were detected in clams and periwinkles, respectively.

To date, there are only a few reports of shellfish-associated gastroenteritis from Africa (Potasman et al. 2002; Bel-lou et al. 2013); however, the aforementioned studies highlight the risk of transmission and disease outbreak through the consumption of virus-contaminated shellfish grown in African waters. The presence of viral pathogens in edible molluscs is a health risk not only for local populations but also for people in countries where such foods are imported. For example, Moroccan molluscs exported to Spain were previously found to be contaminated with NoV and HAstV (Polo et al. 2010), while recent outbreaks of gastroenteritis in North America, Australia and Europe were attributed to the consumption of food products imported from Egypt (Aboubakr and Goyal 2019). Collectively, these reports highlight the need for efficient surveillance programmes and epidemiological studies to fully understand the burden of virus-contaminated shellfish on public health and economies in Africa.

![Fig. 2](image_url)  
Fig. 2 African countries where enteric viruses have been detected in shellfish. Countries are coloured according to the number of studies published. MA Morocco, MZ Mozambique, NG Nigeria, TN Tunisia, ZA South Africa

**Conclusions and future perspectives**

Enteric viruses remain a serious public health threat globally, posing an enormous burden on the economies of many countries. This is particularly true of the developing world where most of the population tend to inhabit urban informal settlements or rural areas, and where the predominant mode of transmission is linked to sewage-contaminated water. This review testifies to the extent of the problem on the African continent by reviewing over 100 investigations conducted in 13 African countries in which the occurrence...
### Table 2 Detection of enteric viruses in shellfish collected in Africa

| Virus Group/Genotype/ Strain | Detection/Typing method | Targeted region | Sample Type | Country | Period sampled | Ref. |
|-----------------------------|-------------------------|-----------------|-------------|---------|----------------|-----|
| Norovirus N.D. RT-PCR       | Partial RdRp            | Clams & mussels | Tunisia     | Jul 2000–Sep 2001 | (Elamri et al. 2006) |
| GI.2 GI & GII RT-qPCR       | Partial RdRp, capsid genes | Clam            | Tunisia     | Jan 2003–Apr 2007 | (Sdiri-Loulizi et al. 2010a) |
| GI & GII RT-qPCR            | ORF1-ORF2 junction      | Clams           | Morocco     | Sep 2006–Mar 2009 | (Polo et al. 2010) |
| GI & GII RT-qPCR            | ORF1-ORF2 junction      | Clams, cockles & oysters | Morocco | Oct 2006–Aug 2010 | (Benabbes et al. 2013b) |
| GI & GII RT-qPCR            | N.R.                    | Clams           | Tunisia     | Mar 2013–Dec 2016 | (Zormati et al. 2018) |
| GI & GII RT-qPCR            | ORF1-ORF2 junction      | Clams & oysters | Morocco     | Nov 2015–Feb 2017 | (El Moqri et al. 2019) |
| N.D. NPEV Combo Card Test   | N.A.                    | Periwinkles     | Nigeria     | Oct 2018–Mar 2019 | (Babalola et al. 2019) |
| Sapovirus N.D. RT-qPCR      | Partial RdRp            | Clams, cockles & oysters | Morocco | Oct 2006–Aug 2010 | (Benabbes et al. 2013b) |
| Astrovirus N.D. RT-PCR      | 3'UTR                   | Clams & mussels | Tunisia     | Jul 2000–Sep 2001 | (Elamri et al. 2006) |
| Enterovirus N.D. RT-PCR     | 5'UTR                   | Mussels         | Morocco     | N.R. | (Elamri et al. 2006a) |
| Enterovirus N.D. RT-PCR     | 5'UTR                   | Mussels         | Tunisia     | Jul 2000–Sep 2001 | (Elamri et al. 2006) |
| Enterovirus N.D. RT-PCR     | 5'UTR                   | Cockles & clams | Morocco     | Oct 2006–Apr 2008 | (Benabbes et al. 2013a) |
| Enterovirus N.D. RT-qPCR    | 5'UTR                   | Cockles & oysters | Morocco | Oct 2006–Aug 2010 | (Benabbes et al. 2013b) |
| Sabin 1 & NPEVs RT-qPCR (following cell culture amplification)† | N.R.                    | Mussels         | Morocco     | Feb 2014–Feb 2015 | (Azzouzi et al. 2017) |
| Hepatovirus IA RT-PCR       | VP3/VP1 region†         | Carpet shells   | Tunisia     | Sep 2000–Aug 2001 | (Gharbi-khelifi et al. 2007) |
| Hepatovirus IB Nested RT-PCR†| 5'UTR, VP3/VP1 & VP1/P2A regions† | Clams | Mozambique | Mar–Nov 2004 | (Nenonen et al. 2006) |
| N.D. Aichivirus A RT–PCR     | 3CD junction†           | Shellfish (type N.R.) | Tunisia     | Jan 2003–Apr 2007 | (Sdiri-Loulizi et al. 2010b) |
| Aichivirus A RT–PCR          | 3CD junction†           | Mussels         | South Africa | May 2016 | (Onosi et al. 2019) |
| Human Adenovirus B PCR      | 3CD junction†           | Mussels         | Morocco     | 2001 (Months N.R.) | (Karamoko et al. 2005) |
| Human Adenovirus HAdV D (17) Nested PCR† | Hexon† | Mussels         | South Africa | May 2016 | (Vos and Knox 2018) |
| Human Bocavirus HBoV-2 Nested PCR† | VP1/VP2 region† | Mussels         | South Africa | May 2016 | (Onosi et al. 2020) |

*Note: NPEV non-polio enterovirus, N.D. not determined, N.R. not reported, ORF open reading frame, RdRp RNA dependent RNA polymerase, UTR untranslated region
†Method of genotyping/typing
of enteric viruses was described in a wide range of samples including not only raw sewage but also groundwater, open water sources, bivalve shellfish and, alarmingly, drinking water. While many of these studies are limited to simple molecular detection of specific viruses in selected samples a few go further to describe the quantification, prevalence and links to disease outbreaks (see for example: El-Senousy et al. 2015, 2020; Elmahdy et al. 2019; Okubo et al. 2019). When assessing the public health risks posed by enteric viruses in the environment, it must be acknowledged that standard molecular-based detection methods cannot discriminate between infectious viruses and non-viable virus particles and that additional data is needed for accurate risk estimates (Rodriguez et al. 2009; Leifels et al. 2016). The first step in attempting to alleviate the burden of disease will be to further understand the true prevalence, molecular epidemiology, viability and circulation of enteric viruses by conducting systematic surveillance studies and evaluating the virological safety of water and food prior to consumption or utilisation for daily living purposes. Recently, sewage epidemiology or wastewater-based epidemiology (WBE) has been successfully used as a surveillance and non-invasive early warning approach for outbreaks of pathogenic enteric viruses such as poliovirus, HAV and NoV (Asghar et al. 2014; Hellmér et al. 2014). In light of the current COVID-19 pandemic, research groups worldwide are evaluating this tool as a way to predict future coronavirus outbreaks (Ahmed et al. 2020a; Bivins et al. 2020; Haramoto et al. 2020; Kumar et al. 2020; La Rosa et al. 2020; Medema et al. 2020; Nemudryi et al. 2020; Wu et al. 2020). Along with clinical testing, WBE may represent a viable means of large-scale population-wide surveillance of imminent infectious disease outbreaks, particularly in resource poor regions in African nations. Additionally, Quantitative Microbial Risk Assessment (QMRA) could be used to estimate population health risks posed by these waterborne pathogens and inform water management strategies (Howard et al. 2006; Owens et al. 2020). Although QMRA has been widely used in developed countries (Bichai and Smeets 2013; Owens et al. 2020), less-developed countries such as those in Africa, with limited data and resources, have greater challenges when applying this technique (Howard et al. 2006; Chigor et al. 2014; Van Abel and Taylor 2018). A recent review of QMRA in sub-Saharan Africa (Van Abel and Taylor 2018) outlined these challenges such as the lack of quantification data and defined several steps including the collection of further data on virus detection, concentration and infectivity, to improve this methodology within the region.

The fact that reports of enteric viruses in water environments to date are limited to only one quarter of Africa’s 54 countries highlights the need for further investigations to better understand the occurrence of these pathogens and their associated public health risk on the continent. Moreover, because wastewater treatment systems in African countries tend to be either non-existent or poorly managed, the threat of human exposure to enteric viruses will remain. There is thus an urgent need for all stakeholders including governments, the private sectors and the public to invest heavily in basic sanitation services as well as the development of innovative wastewater treatment strategies to reduce faecal contamination of vital water sources utilised for irrigation, drinking, food-processing and domestic or recreational activities.

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**Compliance with ethical standards**

Conflict of interest The authors declare no conflict of interest.

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