Vaccine preventable viral diseases and risks associated with waterborne transmission

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Abstract. Rotavirus and poliovirus are paradigmatic viruses for causing major diseases affecting the human population. The impact of poliovirus is remarkably diminished because of vaccination during the last half century. Poliomyelitis due to wild polio currently affects a limited number of countries, and since 2000 sporadic outbreaks have been associated to neurovirulent vaccine-derived polioviruses. Conversely, rotavirus is presently very diffuse, accounting for the largest fraction of severe gastroenteritis among children <5 years-old. Vaccination towards rotavirus is still in its dawn, and zoonotic strains contribute to the emergence and evolution of novel strains pathogenic to man. The environment, particularly surface water, is a possible vehicle for large transmission of both viruses, but environmental surveillance of circulating strains can help promptly monitor entry of new virulent strains into a country, their shedding and spread.

Key words: rotavirus, poliovirus, vaccine, waterborne transmission, surveillance.

In this review, we report studies on the impact of vaccination in the distribution of poliovirus and rotavirus in the environment. The role of the environment as a possible vehicle for large transmission of both viruses is discussed, and the importance of environmental surveillance in monitoring their spread is highlighted.

INTRODUCTION

Water represents an important vehicle for transmission of viruses responsible for a wide range of diseases in humans and animals, particularly viruses colonizing the gastrointestinal tract. Intestinal agents such as poliovirus and other enteroviruses, norovirus or rotavirus can be shed with stools at high concentrations for days or weeks. These may heavily contaminate the environment, reaching watercourses and other surface water basins, and eventually enter the potable water distribution pipelines, or crops, soft fruit and vegetables, or water-filtering seafood, posing a risk for possible epidemic outbursts of disease.

Viruses infecting the intestine of man or animals are normally quite resistant in the harsh condition of the environment, also due to an envelope-free capsid made of only proteins, which provides efficient protection for the viral genome.

In the absence of effective therapeutic treatments for most viral diseases, and in consideration of the large epidemic potential of many of the viral agents shed with stools, vaccination is the most reliable approach that can be adopted to halt or limit viral diffusion through the susceptible populations. However, the great genetic variability of many viruses, often resulting in the emergence of distinct serotypes, makes the development of largely effective cross-reactive vaccines problematic. Finally, the globalization with the consequent massive travelling of people, animals and goods between different areas of the world, has further enhanced the emergence and spread of novel viruses in naïve populations, sometimes showing full or mixed characters of agents from the animal world.

In this review, we report studies on the impact of vaccination in the distribution of poliovirus and ro-
tavirus, two typical enteric viruses able to severely affect humans, and the role of environmental waters in strain circulation among susceptible individuals.

**Rotavirus**

Rotavirus was discovered in the early ‘70s, and soon identified as the major etiological agent of infectious acute gastroenteritis in childhood [1]. Today, rotavirus remains the single most important cause of morbidity among pediatric patients with diarrhea worldwide, more than any other viral, bacterial or parasitic organism, and is estimated to cause approximately 450,000 deaths per year, predominantly in developing countries [2]. It is believed that every single child in developed countries contracts rotavirus at least once during its first 2½ years of life, although only a lesser number of subjects develop diarrhea severe enough to require hospital admission and medical intervention, including rehydrating therapies.

**Structure, proteins and genome**

Rotavirus represents a genus of the Reoviridae Family [3], and derives its name from its wheel-like (rota, in Latin) shaped virion, approximately 75 nm in diameter. The virion is rather complex, and the variability in the functions and antigenicity of its proteins largely influences the host-pathogen relationships, including host range restriction.

The capsid is made of three concentric protein layers, engulfing 11 segments of double-stranded RNA (dsRNA) with molecular size ranging between approximately 670 by 3300 base pairs (www.iah. bbrc.ac.uk/dsRNA virus proteins/Rotavirus.htm) [3]. This complete form of infectious rotavirus is also known as TLP (triple-layered particle).

The outermost rotavirus shell comprises two proteins, VP7 and VP4, containing epitopes which elicit neutralizing protective antibodies. The glycosylated protein VP7 forms a continuous layer of trimers, and represents the major neutralization antigen, specifying the virus G-serotype [3]. The VP7 layer is crossed by 60 spike-like projections of the protein VP4, which determines the P-serotype, and is cleaved by pancreatic trypsin yielding two fragments, VP8* and VP5*, that results in profound conformational modifications and enhanced infectivity. VP4 is the viral protein responsible for attachment to the susceptible cells, and has been recently shown to bind histo-blood group antigens (HBGA) in a serotype-specific manner, that might explain differential host susceptibility to infection [4].

The inner rotavirus shell is only made of the VP6 protein, recognized as the “group antigen” of rotavirus which, and due to its ample antigenic conservation between distinct G- and P-serotypes it is conveniently used for antigen-based diagnostics [5]. Based on VP6, 7 groups of rotaviruses are distinguished designated A through G, among which group A contains by far the most important viral strains pathogenic for humans, and many other animal species. Man is also infected with group B and C rotaviruses, known for being implied in large water-borne outbreaks in Asia and for causing sporadic or epidemic gastroenteritis cases in children and adults, respectively [3].

In addition to 3 further structural proteins (VP1-3), the 11 segments rotavirus genome also codes for 6 non-structural proteins (NSP1-6), involved in either RNA transcription or progeny virus maturation [3]. Importantly, NSP1 and NSP3 appear to have specific roles in rotavirus virulence and possibly host range restriction. NSP4 is functional to virus final morphogenesis [3], and is also acting as an enterotoxin, being able to induce diarrhea in the infant mouse pathogenesis model [6], and likely exerting its effects in vivo by interacting with the luminal enterochromaffin (EC) cells and the enteric nervous system (ENS) [7].

**Serotypes, genotypes and immunity**

Group A rotaviruses can be differentiated into serotypes based on reactivity with hyperimmune sera or neutralizing monoclonal antibodies directed at either VP7 or VP4 [8]. Since the two genes encoding VP4 and VP7 segregate independently, a binomial serotype system was adopted to identify strains, defined as GxPx serotypes. However, viral characterization is normally conducted by more friendly molecular approaches using semi-nested RT-PCR and panels of genotype-specific oligonucleotide primers [9]. Genotyping is considered to be a valid proxy for serotyping, and is adopted universally. As a result, a large amount of data is being accumulated concerning rotavirus genotypes and serotypes circulating worldwide [10, 11], which is valuable to confirm the adequacy of current vaccines in relation to emergence and evolution of viral strains [12, 13]. Currently, at least 27 different G-types and 35 P-types are recognized among human or animal worldwide [14], and increasing evidence of zoonotic transmission of animal rotaviruses to humans, involving reassortment mechanisms during dual infection, has strengthened the threat of novel rotavirus strains emergence from domestic and wild animals with respect to vaccine efficacy [15]. It underlines the need for surveillance of circulating rotavirus strains in order to identify emerging or reassortants strains with unusual serotype.

Due to the high death toll and economic burden of rotavirus disease worldwide, vaccine strategies for disease control were implemented through joint efforts of scientists, industry and public health authorities as early as in the mid-90’s [12, 13].

Two live-attenuated vaccines (RotaTeq®, by Merck; Rotarix®, by GlaxoSmithKline) have been successfully introduced in a growing number of countries since 2006 [16], and although they are respectively pentavalent and monovalent they appear to be largely protective against co-circulating rotavirus genotypes in human populations [17]. However, some lower efficacy of vaccination is reported in less developed countries [17], possibly related to unsatisfactory herd immunity established against uncommon rotavirus genotypes...
circulating in these areas [11, 18]. Vaccination is also considered an important line of defense for several animal species with an economic value.

Protection to rotavirus disease can be afforded by both symptomatic and asymptomatic natural infection, as well as by vaccination [13, 19].

Also, animal strains are normally less pathogenic for humans than they are in their species of origin, a knowledge that drove towards a Jennerian approach to rotavirus vaccines in childhood [20].

However, early volunteer studies and field investigations demonstrate that at least some strains deriving from sick children can re-infect adults despite the presence of pre-existing immunity [21], although often in the absence of symptoms. This is confirmed by community studies showing that rotavirus infection was shed by mild sporadic cases of diarrhea not necessitating hospitalization or asymptomatic subjects, spanning all ages [22]. In the UK, rotavirus-specific IgM was detected in the normal adult population with no seasonal trend [23], suggesting constant circulation of the virus among adults independent on the winter peak of disease typical for children. Although immunity to rotavirus gastroenteritis has been considered to be life-long, adults might play a role of healthy carriers and reservoir for rotavirus [24].

Epidemiology of rotavirus infection and disease

Rotavirus transmission follows the fecal-oral route, and is mainly associated to direct inter-human passage. However, infectious viruses are shed in large amounts into the environment by both humans and animals, contaminating water, food and feed [3, 25]. Rotavirus pediatric cases show a seasonal trend, with an epidemic peak in cooler, drier months, particularly in countries with a temperate climate [26], that might involve a longer persistence of live virions in the environment in particular conditions. However, in addition to environmental conditions favoring virus survival, differences in rotavirus seasonality between areas may be also ascribed to individual country birth rate and virus transmission dynamics [27].

The age range associated to higher risk of severe rotavirus gastroenteritis is 6 to 24 months [28], but older children can also need medical care or hospitalization [29]. The estimated number of global rotavirus diarrhea cases approaches 110 million yearly, 2 million of which necessitate admission to hospitals [2, 30]. Although most of fatal cases are restricted to developing areas (Table 1), a high rotavirus burden is recorded in industrialized countries; as an example of the magnitude, before mass vaccination was introduced in 2006 in the US, rotavirus caused in this country 410 000 physician visits, 70 000 hospitalizations, and 272 000 emergency department visits per year, costing the society more than $1 billion [30].

The main symptoms during rotavirus infection are diarrhea and vomiting, and disease can vary from mild disease up to severe dehydration, osmotic shock and death. On a clinical basis, the severity of cases is conventionally determined by the Vesikari scoring system, that assigns between 0-20 score considering several factors, such as duration of diarrhea, number of stools in 24 hours, vomiting duration, number of vomiting episodes in 24 hours, maximum fever, medical visits, and treatment (none, outpatient and hospitalization) [28]. Asymptomatic rotavirus infection also occurs frequently in both children and adults, possibly related also to partial immune protection following earlier infection. These data highlight the risk that rotavirus shed by healthy people may be transmitted to susceptible non-immune subjects, as the newborn, directly or through environmental and/or foodstuff contamination circuits [31, 32].

The mechanisms and duration of protection in rotavirus infection are not completely understood, particularly regarding the extent to which different serotypes or “genotypes” of rotavirus underpin antigenic diversity that may affect the immune system reaction against infection with different strains [33, 34]. It also raises problems in evaluating the risks of zoonotic transmission of animal strains, and as a consequence the risks for human health associated with environmental contamination with animal feces and manure, animal farming effluents, in addition to human sewage. Since group A rotaviruses infect many animal species, including domestic animals and pets, susceptible subjects are also exposed to a large variety of strains of animal origin [15, 35]. Reassortment of genome segments during co-infection with several RV strains is crucial in favoring adaptation of zoonotic animal rotavirus strains in man, via environmental or food-borne transmission [33, 36].

Rotavirus zoonotic transmission

Despite some constraints in rotavirus replication and spread between different animal species or hu-

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of deaths</th>
<th>Percent on all deaths</th>
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<tr>
<td>India</td>
<td>98 621</td>
<td>26.1%</td>
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<tr>
<td>Nigeria</td>
<td>41 057</td>
<td>30.8%</td>
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<td>Pakistan</td>
<td>39 144</td>
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<td>Democratic Republic of Congo</td>
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<tr>
<td>Bangladesh</td>
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<td>65.3%</td>
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<tr>
<td>Angola</td>
<td>8 788</td>
<td>67.2%</td>
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<tr>
<td>Total</td>
<td>304 368</td>
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*Percentage of rotavirus specific deaths over total deaths for all causes. The total of deaths in the 10 countries reported in the Table represents 67.2% of the 453 000 children dead with rotavirus infection in that year. Source: WHO IVB (http://www.who.int/immunization_monitoring/burden/rotavirus_estimates/en/index.html).
mans, interspecies and more specifically zoonotic transmission of rotaviruses is now recognized to occur frequently, sometimes resulting in overt disease in the heterologous species [15, 35]. As an example, G9 rotavirus is thought to have possibly originated from swine, being a rare cause of infantile gastroenteritis in US in 1983-1984, expanding significantly among symptomatic children in this and other countries after a decade, and becoming one of the 5 commonest human rotaviruses today. The P[6] gene normally found in swine was also circulating in the same period, and when G9 emerged throughout the world G9P[6] strains were frequently observed [37]. The occurrence of G9P[6] and G9P[8] in man, and other findings of typically human G types in animals are altogether suggestive of natural genetic reassortment between human- and porcine strains, eventually leading to a novel globally widespread virus type [11, 37].

Also because of improved sequencing and whole genome genotyping analysis of rotavirus to explore rotavirus evolution, it is now possible to identify emerging zoonotic strains with a possible potential for rapid global spread, also in consideration of an increasing herd immunity by extending vaccination [13]. Using these approach, G8P[8] and G8P[6] strains identified in children with diarrhea in the Democratic Republic of Congo in 2003 [36] could be studied in detail, demonstrating for 9 of their genes a close evolutionary relationship with rotavirus strains belonging to the DS1-like (G2P[4]) sub-group, and suggesting at least three, and possibly four, consecutive reassortment events involving both DS1-like and Wa-like human rotaviruses and more animal strains of bovine (G8) and swine origin.

This process of “humanization” following zoonotic transmission may further proceed generating new virus reassortants, as was shown in two distinct G8P[8] and G8P[4] rotaviruses reported in 2006 and 2009 in Europe, showing partial or little similarity with the DRC strains and close phylogenetic links with other common human rotavirus circulating in Europe belonging to G types other than G8 [38, 39]. One of these latter strains, G8P[8] with a full Wa-like genome, unexpectedly became predominant among children with severe gastroenteritis in Croatia in 2006, suggesting that its emergence was [36, 38] favored by an unusual gene repertoire [13, 17, 36].

There is increasing evidence that some animal species may play a relevant role of reservoir of rotavirus strains transmitted zoonotically. Close genetic relatedness between strains of different origin suggests that ruminants and ungulates may be the reservoir of G6 rotaviruses for humans [15]. Besides the G9 strains reported above, the swine is also regarded as a possible reservoir of G3, G5, G12 and P[6], P[16], P[19] rotaviruses.

The emerging G12 genotype increasingly reported in humans worldwide are also thought to have originated from swine establishing in man after animal-human reassortments [15, 18].

Also the rabbit has been proposed to harbor rotavirus with similar characteristics to strains found in gastroenteritis cases in children, as well as pet animals like dogs and cats [15, 33]. All of these animal species and others may take part in generating novel zoonotic strains evolving across multiple animal reservoirs, as far as different animal species get into direct contact or share a same environment or vehicles of fecal contamination.

**Rotavirus and the environment**

The environment, and more specifically surface or recreational waters, can be contaminated by introduction of fecal pathogens, including rotavirus, via sewage spill-out, which may sporadically become massive as a consequence of flood, sewage-treatment plants failure, pipeline leakage, and others. These aspects are reviewed in more detail in a separate article of this same issue, and will not be presently examined in further detail.

Occurrence of rotavirus in environmental water and its association to a community waterborne gastroenteritis outbreak [40] were established even before sensitive molecular detection methods became available. It has long been known that rotavirus passing through urban waste-water treatment plants is only subject to partial viral load reduction before proceeding into receiving environmental water [41, 42]. Also, it is noteworthy that rotavirus is stable in contaminated food, fomites and environmental matrices, and is resistant to disinfection [43]. Persistence of rotavirus in surface or drinking waters as well as in food or on surfaces is remarkable [44-46], and it is widely known that irrigation water contaminated with feces or organic fertilizers can cause pre-harvest contamination of fruits and vegetables with enteric viruses in general [44, 45]. For these reasons, quantitative risk assessment models have been proposed for water, wastewater and manure to the food safety operators with the final aim of preventing contamination of the food chains with rotavirus and other viral pathogens [47].

Besides crops, fecal shedding of rotavirus into surface waters would also have an impact on natural banks or farming sites of mollusk seafood. In Italy, as many as 18% of natural bank mussels were found to be positive for rotavirus [46], and a relevant rate of natural contamination of seafood with rotavirus was confirmed in several other field studies conducted in other countries [48].

One aspect that has not yet been analyzed in sufficient details is the asymptomatic infection of adults and older children, since they can release into the sewage rotavirus strains that are not necessarily the same shed during infantile diarrhea. In fact, stools and virus shed from patients of younger age are normally collected on diapers, which are not disposed into the sewage pipeline, and thus may not re-enter the environmental contamination route as do adult feces. Information from rotavirus genotyping studies applied to sewage samples is very limited, yet it suggests that significant differences may exist between viral types released into sewage and those...
detected in symptomatic pediatric cases. Whether this may imply diminished or increased risk of infection of susceptible children is unclear, also because surface waters and water-contaminated vegetables or seafood are hardly a good vehicle for direct viral transmission to infants. However, environmental contamination would surely help rotavirus circulate through the adult population as in the case of noroviruses and hepatitis A viruses, whose clinical effects are remarkable permitting identification of sporadic cases, outbreaks, and their attribution to environmental pollution. Eventually, asymptomatic infected adults may unconsciously transmit rotaviruses to the susceptible children within households or institutions.

In some studies, rotavirus strain characteristics revealed in sewage and contaminated surface basins were closely mirroring the strains normally responsible for the majority of infant disease cases [41, 49], but this may actually be true particularly for countries with lower sanitation where disposal of adult stools and children diapers is not kept separated.

A preliminary comparison between G- and P-types of rotavirus detected in either sewage samples or feces of children with diarrhea in Italy in 2010-2011 is reported in Figures 1 and 2, showing similar yet not identical distribution of viral strains.

Release and spread of animal rotaviruses may also result in contamination of surface waters, and food, favoring introduction of uncommon rotaviruses into human populations, that might ultimately endanger efficacy of current vaccines adopted for human use [12].

Due to the multiplicity of rotavirus strains possibly present in sewage or other environmental matrices at any time, genomic characterization of fundamental genes for origin attribution (e.g. VP7, VP4, and possibly NSP4, and VP6 encoding segments) may be of great value in monitoring the presence of emerging or uncommon rotavirus types circulating in a population, which are not yet but might subsequently get involved in symptomatic cases and epidemics. Similarly, environmental rotavirus genotyping may help identify the source of rotaviruses linked to epidemic outbreaks of disease [50]. However, due to the segmented nature of the genome it may not be possible to identify the whole genomic/antigenic formula of any rotavirus strains in sewage or water samples, possibly contaminated with multiple virus types deriving from an entire human or animal community [50, 51].

**Rotavirus diagnostics in environmental samples**

Whereas in sewage the concentration of rotavirus may be expected to be reasonably high, surface waters normally present low viral load [52], thus requiring extensive virus and RNA concentration protocols to be applied prior to detection and genotyping procedures by sensitive methods. These are treated in more detail elsewhere, and will only be discussed quickly in this section.

Protocols for concentration of rotavirus and other enteric viruses from sewage or clear water include adsorption-elution using negatively charged membranes, precipitation-flocculation, two-phase separation, centrifugation, tangential flow ultrafiltration, and gel chromatography.

Methods essentially similar to these can be successfully used also on vegetable rinsing extracts, optimizing virus detachment into the medium, or on sea mollusks.

Viability assay on rotaviruses present in naturally contaminated waters is impracticable due to low permissiveness of cell cultures to wild human strains, but viral RNA detection is more reliable.
Concerning molecular diagnostics and typing, a widely used protocol for feces analysis reported in the web site of the Rotavirus Surveillance Network “EuroRotaNet” (http://www.eurorota.net/), based on multiplex nested-PCR system following an RT phase with random primers, can be extended to environmental sample testing. For VP7 (G-genotyping), the protocol encompasses primers for common human rotavirus G-types G1-4, and G9, and emerging strains G8, G10, and G12. For VP4-typing, the multiplex assay covers the two common human P-types P[4] and P[8], and types P[6], and P[9-11] of possible animal origin. For special necessities, a full-genome typing approach may better help understand the evolution and occurrence of reassortment events of rotaviruses [14], although this is of hard applicability in the presence of multiple viral strains.

**Rotavirus epidemic outbreaks**

Although the children community faces winter-related major epidemics under temperate climate, rotavirus can also cause smaller epidemic outbreaks, involving all age groups but particularly children or elderly people, in schools, hospitals, nursing homes, and care centers, as well as grandparents in the household. Despite mortality, particularly in the case of elderly or subjects with reduced health conditions, the direct costs for the society related to outbreaks in long-term care institutions may be high, and long-term residence in a closed community is a risk factor for rotavirus illness.

Particularly in waterborne outbreaks, subjects of all ages can be affected presenting severe symptoms [53]. Increase of symptoms in adults in these cases is thought to be caused by the high virus load, which is often present in water sources contaminated with sewage, as seemed to be the case during a rotavirus outbreak associated with drinking water in Finland, characterized by particularly severe cases in both young and older children [54]. Although this outbreak was apparently caused by a common human G1P[8] rotavirus, in other epidemic cases of disease affecting elderly communities or older children it seems that less common genotypes may be involved, such as genotypes G2 and G4 [54].

Also because the issue of a differential immunity to viral serotypes is still unclear, other factors may explain these observations. It is reasonable to assume that the infectious rotavirus dose transmitted from person-to-person contact is likely small in countries with a high level of hygiene. On the contrary, larger amounts of virus might be present in drinking or surface waters contaminated by sewage spillover, and a high virus load might justify both severity of disease and involvement of subjects outside the normal age range observed in some outbreaks [54].

In fact, several studies suggest that both surface waters and some foodstuff can be massively contaminated with a multiplicity of viruses of human and/or animal origin, also including rotavirus, thus posing the conditions for triggering an outbreak [50, 54-56]. In some cases, it may be difficult to identify a unique etiological agent since different virus strains or even species may be detected in patients and environmental samples. As an example, apart from a single human strain only swine or bovine rotavirus-specific VP7 gene sequences were detected in four home tap-water drinking water samples during a rotavirus diarrhea epidemic affecting 56 children in France. However, all of these were different from the sequences detected in stools of patients [56]. This outbreak study also highlights the role of water pipelines as a vehicle of rotavirus of both human and animal origin, possibly leading to co-infection of subjects with several rotavirus strains and consequent gene reassortment. As for other enteric viruses, similar conditions of risk may be present in filtering seafood, such as cockles, mussels and clams, which collect and may concentrate rotaviruses from contaminated waters, and are only lightly cooked or consumed raw [48, 57]. Rotavirus contamination of surface water may also end up in other food chains, including vegetables and soft fruit, correlated with irrigation. Although virus concentration may be lower on leaves and fruit skin, this type of food is a potential risk and has in fact been sporadically involved in outbreaks [32, 49]. Conditions for exceptional virus spread and large outbreaks may be generated by major natural events like flood and earthquakes [58].

**Poliomyelitis. Virus, disease and vaccination**

Poliovirus belongs to the genus Enterovirus within the family Picornaviridae, and strains are differentiated into three different serotypes. The genome is made of a single positive strand of RNA, which acts as a messenger RNA when released into the cytoplasm of susceptible cells, and is contained inside an icosahedral capsid made of 4 distinct proteins (VP1-4) [59].

The coding region of poliovirus genome is translated into a single polyprotein, which is then processed to generate the viral capsid (VP1 to VP4) and the nonstructural proteins. Surface-exposed loops in the capsid proteins VP1, VP2, and VP3 contain the antigenic determinants for poliovirus-neutralizing antibodies, and four main antigenic sites have been identified by the use of murine monoclonal antibodies [60].

Replication of poliovirus RNA is permitted by a virus encoded RNA polymerase with a high rate of error, which introduces frequent mutations in the genome at every replication cycle. Nucleotide substitutions may be selectively amplified during infections, ensuring an efficient mechanism for evolution of polioviruses through its progeny. Genetic recombination events between polioviruses and other clade C, non-polio enteroviruses (NPEVs) is another mechanism of evolution possibly leading to chimeric virus variants, exhibiting important phenotypic differences with respect to parental poliovirus [61].

Poliomyelitis is a very invalidating disease, although it develops in severe forms in a minority of...
infected subjects. Consequently, vaccination has soon appeared as the only tool to prevent occasional emergence of severe paralytic cases by halting the wide circulation of wild neurotropic viruses throughout an asymptomatic population.

After ravaging the global population for thousands of years, eradication of poliomyelitis (WHO resolution WHA41.28, 1988) has thus been achieved in most part of the world, but a few areas remain with low endemic circulation of wild polioviruses sometimes generating epidemic outbreaks. The massive efforts towards global eradication has required vaccination of over two billion children during the past decades, and has resulted in a decrease from several hundreds of thousand to fewer than 2000 annual cases in 2010, to 156 in the first 9 months of 2012 (Figure 3). Wild type 2 poliovirus was eradicated by 1999, and no further case due to this serotype has been reported thereafter. Most of the credit for breaking the endemic circulation of wild-type viruses worldwide goes to massive use of the Sabin’s oral live attenuated polio vaccine (OPV), which was able to elicit an immune barrier against the virus at the intestinal mucosa level, resulting in a strong limitation for pathogenic poliovirus to replicate in the human gut and be shed with feces into the environment [62, 63].

However, disease has also been linked with infection with OPV vaccine-derived polioviruses (VDPVs), which can essentially arise by either person-to-person transmission among naïve, immune competent individuals (cVDPVs) or persistent infections of immune deficient individuals (iVDPVs) [64]. Infection with polio vaccine strains may quickly introduce substitutions that reverse neurovirulence attenuation into the progeny viruses. These viruses can evolve during replication in asymptomatic healthy persons or immunodeficient subjects, and give rise to vaccine-derived polioviruses (cVDPVs), which exhibit characteristics of transmissibility and virulence similar to wild viruses. Therefore, establishment of OPV persistent infections in immune deficient individuals constitutes a threat for the unimmunized subjects and for the continuous shedding of potentially pathogenic virus, particularly when this is related to asymptomatic anonymous individuals. To avoid similar problems, some countries have always used the killed poliovirus vaccine originally implemented by Salk (inactivated polio vaccine, IPV) and derivatives, which is now largely used throughout the world alone or in a combined schedule with OPV, and is recommended after eradication [63]. The main disadvantages of the killed vaccine are its scarce induction of a mucosal immune response and need for parenteral administration, which are however counterbalanced by IPV inability to start any replication in the vaccine, and by its genetic and chemico-physical stability.

**Eradication of polio and surveillance of poliovirus**

With the ultimate perspective of global eradication of polio, the WHO has proceeded for decades throughout regional and local eradication programs, and large areas of the world are now established to be polio-free, including Europe since 2002 [65]. Clearance of new polio cases due to wild strains in a country must be confirmed for at least three consecutive years, and eradication status shall be sustained by continued mass vaccination with IPV and by an active surveillance plan of AFP cases within all cases of paralysis for poliovirus etiology, as an indicator of poliovirus circulation (Polio laboratory manual, WHO/IVB/04.10). Additional measures encompass the identification, containment and destruction of all samples that might contain live wild or vaccine-
derived poliovirus or polio vaccine. In the case of endemic areas, universal vaccination of all children with OPV is still required in order to combat wild virus circulation.

As an example for European countries, Italy has adopted the mixed OPV-IPV schedule of vaccination in 1999, and a full IPV schedule in August 2002, maintaining approximately 96.5% coverage of complete vaccination in < 2 year-old children. The ongoing AFP surveillance program [66] indicates that Italy is polio-free, the last polio case due to indigenous wild virus having occurred in 1982 and the last imported wild polio case having come from Libya to receive specific medical care, in 1988. Because polio is no longer perceived as a frequent or emerging risk by the population and the physicians, AFP surveillance in Italy has requested more and more attention in order to fulfill the WHO performance indicators (Polio laboratory manual, WHO/IVB/04.10), whilst the geographical location of Italy, and the globalization and immigration dynamics have continued to put the country at increasing risk of importation of wild polioviruses or neurovirulent Sabin-derived polioviruses from areas with endemic poliomyelitis. The use of a full IPV vaccination has eliminated vaccine associated poliomyelitis cases (VAPP) in Italy, and has progressively decreased the circulation of Sabin poliovirus strains in both the population and the environment, but it has also limited passive immunization of contacts and the degree of herd immunity at the mucosal level.

It should be considered that the absence of clinical polio cases in a country does not necessarily imply the absence of poliovirus circulation. Acute flaccid paralysis (AFP) surveillance may thus conveniently be supplemented or replaced by enterovirus surveillance and/or environmental surveillance of sewage. Such supplementary surveillance, especially environmental surveillance, has proven in several instances to be a powerful tool for monitoring the importation and circulation of wild or vaccine-derived polioviruses before appearance of clinical cases, as well as for evaluating the effectiveness of control measures adopted in response [67].

The major obstacles to global polio eradication have been: a) the “failure to vaccinate” the susceptible cohorts particularly in countries characterized by poverty, war, and political or religious constraints; b) the “vaccination failures” in case of vaccine cold-chain breaches or immune depression linked to malnutrition; and c) the “emergence of VDPVs”. Whereas availability of current optimized live attenuated or inactivated vaccines in endemic areas can help overcome the first obstacle, the other difficulties require an integrated worldwide approach, to reduce poverty and to monitor poliovirus circulation via immigration in the global world.

Environmental polio surveillance

As in the case of rotavirus and other viruses excreted with feces, also polioviruses can be spread in the environment and reach surface waters prompting environmental and food-borne transmission circuits, which in the end can return hazardous strains to susceptible humans. Besides being a risk source for poliovirus dissemination, sewage can be used to supply useful indicators of risk, independent of the current presence of diagnosed polio cases in the population.

Sewage surveillance is performed according to standard procedures recommended by the WHO (Guidelines for environmental surveillance of poliovirus circulation, WHO/V&B/03.03), and sometimes represents the only means to promptly identify introduction of wild or neurovirulent vaccine-derived polioviruses from endemic areas, particularly in countries declared polio-free using the IPV. The increased immigration from areas with persistence of pathogenic poliovirus circulation is a present public health threat for Mediterranean countries such
as Italy but also for other developed countries in Europe and elsewhere, which can be monitored by environmental surveillance strategies. Also, environmental surveillance may permit to detect the presence of VDPV excretors in the community, even in the absence of clinical cases or outbreaks, a fact that may be ascribed to a level of herd immunity across the population sufficient to contain but not terminate transmission of neurovirulent polio. This implies that VDPVs shall be considered a potential source for outbreaks and for reemergence of polio even after eradication.

The distribution of VDPV excretors worldwide is in large part unknown, and the identification of asymptomatic shedders of VDPV in any specific area is a very difficult task. Countries with VDPV excretors are required to maintain a high herd immunity level by vaccine coverage, until shedding is extinguished. It shall also be considered that asymptomatic shedders might move from an area to another during their lives, changing the geographical risk conditions. If not recognized and properly contained, persistently infected VDPV excretors, particularly if immunodeficient, might shed poliovirus for years or even decades. Considering that the IPV vaccine is less effective than OPV at inducing mucosal immunity, possible circulation of poliovirus in IPV-immunized children might pass undetected despite active AFP surveillance is in place [65]. All of this makes the rationale for implementing environmental surveillance of sewage and surface waters in countries with risk of polio reintroduction.

Environmental polio surveillance virtually samples the entire population independent of cases of paralysis, as far as sampling sites are properly selected and investigation is performed using standardized methods (Guidelines for environmental surveillance of poliovirus circulation, WHO/V&B/03.03) [65]. With this in mind, a network of laboratories has also been built in Italy, using standardized sample collection methods on Italian wastewater treatment plants (WWTP), complementing the nationwide AFP surveillance activity, to gain evidence in support of the maintenance of polio-free status of Italy [66, 67]. No wild polioviruses were isolated from environmental samples after the surveillance started in seven cities in 2005, supporting the outcomes of AFP surveillance, but Sabin-like polioviruses were detected in sewage, although rarely and presenting a low mutation rate, in agreement with modest circulation of vaccine-derived strains in Italy. These findings are per se a confirmation that polio immunization is effective in Italy and generates high protection versus polio. However, since OPV vaccination was replaced with IPV in 2002, the polio Sabin-like strains found in the environment were most likely excreted by children immunized with the OPV in countries that still use Sabin vaccine, and shall thus be considered as imported cases from abroad, related to immigration flows. The possible risks of further amplification and genetic drift of these polioviruses especially in subjects with compromised immunity shall not be neglected. Surveillance on WWTPs yields relevant amounts of non-polio enteroviruses, which confirms the validity of the approach, but also requires detailed characterization to be performed on viral strains by both sensitive and specific molecular and cell culture methods (Guidelines for environmental surveillance of poliovirus circulation, WHO/V&B/03.03) [65-67].

### Residual polioviruses in the 2000's

Risks associated with environmental spread of viral strains derived from the oral poliovirus vaccine (OPV) have emerged with particular high severity in the Dominican Republic and Haiti in the 2000's, where significant outbreaks of poliomyelitis have occurred among unvaccinated children [68]. A single type 1 vaccine-like strain was involved, presenting a highly mutated genome, that is suggestive of prolonged replication in the intestine of non-immune subjects [69], and neurovirulence and transmissibility characters similar to wild type poliovirus. These and other more recent findings on polio outbreaks in Africa and Asia [70] highlight the necessity that eradication of wild poliovirus from a country is promptly followed and sustained by adoption of vaccination strategies to prevent possible resurgence of the disease and continuing circulation of potentially pathogenic polioviruses.

The occurrence of sporadic outbreaks of poliomyelitis due to mutated vaccine-derived strains is also related to the presence of asymptomatic “long term excretors” of polioviruses in the healthy community. Studies performed on immunodeficient subjects in Europe (UK and Germany) and in US have revealed the presence of otherwise healthy subjects shedding vaccine-derived polioviruses presenting a marked rate of mutation toward the neurovirulent phenotype, who represent an actual risk of dissemination of highly pathogenic poliovirus strains in the environment and a threat for other unvaccinated or immunodeficient members of the same population.

### Rotavirus and poliovirus vaccine strains in the environment, a problem or an asset?

During natural infection with wild pathogenic strains, rotavirus is shed at very high titers with human feces, often reaching 1010 infectious particles per gram lasting for several days or weeks [71]. This study was conducted on severely affected children, and highlights the chance for large amounts of infectious virus to be released into sewage, and possibly enter the environment and food routes of spread and transmission. Several other reports during decades of rotavirus investigation confirm this assumption, also using modern molecular detection approaches [72].

On the contrary, an early study with rotavirus vaccines for human use found limited vaccine rotavirus shedding by either vaccinated children or adults, but a possible effect of low level antibodies due
to previous unreported infection with wild virus could not be excluded. Conversely, studies following the introduction of either Rotarix or RotaTeq vaccines have confirmed measurable, although reduced, shedding of vaccine virus. Rotavirus antigen was detected in the stools of infants for more than a week after their first dose of pentavalent rotavirus vaccine [73]. A recent study in Australia, where the pentavalent RotaTeq is in use since 2007 [74], has shown some circulation of parental as well reassortant vaccine strains in both vaccinated children and subjects sampled in the community in the course of routine surveillance. Also, transmission of vaccine-derived rotavirus (RotaTeq) has been documented in a vaccinee sibling in US, resulting into symptomatic infection that the authors hypothesize may have involved reassortment with a co-infecting wild-type rotavirus [75]. Similarly, development of diarrhea appeared to be related to a double bovine-human reassortment occurred just after vaccine administration in 3 infants in Finland, concluding that this virulent vaccine-derived strain may have been release into the environment [76].

These and other reports are suggestive of an actual introduction of vaccine viruses, and vaccine-derived reassortants into the community, which may also involve environmental transmission.

Accidental ingestion of vaccine viruses via contacts with vaccinated children or contaminated fomites may pose a serious problem for immunocompromised subjects, where attenuated rotaviruses might start a serious symptomatic infection [77]. However, given the lower replication of last generation vaccine viruses compared to earlier vaccine strains or wild-type strains, this possible risk should not be taken as a reason for discouraging vaccination of susceptible cohorts.

After introduction of mass vaccination with the Rotarix vaccine in Brasil in 2006, environmental surveillance of rotavirus was performed on a major Wastewater Treatment Plant for along a year using RT-PCR and nucleotide sequencing [78]. Although all sewage samples examined were found to be positive for rotavirus, only wild-type genotypes were detected whereas no NSP4 or NSP3 sequences specific for vaccine-like strains were identified in any sewage sample. These data suggest that vaccine-derived rotavirus strains present in sewage are probably a minor part compared to the wild-type viral repertoire shed from the community as a consequence of natural infection. In different studies performed in Chile and Nicaragua after introduction of rotavirus vaccination, lower rates of rotavirus positive sewage samples were determined compared to other enteric viruses [79, 80], that suggests a possible reduction of cases of natural infection as a consequence of vaccine administration. For these reasons, environmental surveillance, particularly on wastewater, may represent an interesting approach to evaluate the potential impact of rotavirus vaccination on viral circulation in the community.

A final aspect concerns possible aids to susceptible children immunization via transmission of rotavirus vaccine strains excreted by vaccinees. A “passive vaccine passage” to unvaccinated contacts was considered a milestone in the fight to poliomyelitis using the live attenuated Sabin vaccines administered orally, but has lately been considered a risk of vaccine-related polio [69]. To date, several complete transmission dynamic studies on rotavirus vaccination have been published [81] exploring a variety of possible scenarios, but no study has approached the possibility of “passive contact immunization” for rotavirus vaccine. If any, this is however likely to be of minimal impact, considering both the reduced replication and shedding of vaccine strains by immunized subjects and the simultaneous co-circulation of more aggressive viral strains in the communities and the environment.

CONCLUSIONS

In conclusion, the environment and particularly surface water can play an important role in transmission of rotavirus as in the case of other enteric viruses, not excluding polioviruses. Waterborne disease outbreaks or cases should be investigated by molecular characterization methods, in order to identify risk factors, possible spread of novel emerging viruses or reassortants, and apply control measures. Environmental surveillance on sewage treatment plants can help monitor shedding of uncommon viruses by a specific population, to promptly identify threats due to emerging viral strains in the community, and finally to assess ongoing vaccine programs since sewage screening may provide a rapid and economical overview of the circulating rotavirus genotypes. In the case of poliovirus, detection and characterization of polio and other enterovirus strains in environmental samples will supply more and more important information as the course towards global eradication progresses.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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