Minireview Article

Rotavirus in Food and Water in Latin American.

ABSTRACT

Rotavirus is considered a major public health problem worldwide because many children, adults, and animals die from gastroenteritis due to rotavirus; rotavirus contamination follows the fecal/oral route, and it is well supported that infection can also be achieved by consuming food and water that is contaminated with rotavirus. Research has shown that, in Latin American countries, unusual emerging strains of rotavirus are occurring in children and adults with gastroenteritis; which contain in their sequence genes from rotavirus genotypes detected in animals; Therefore, researchers consider it a virus with zoonotic potential. In the Latin American region, many farm animals and other wild animals share drinking water resources with the inhabitants, which proves to be an important vehicle for the transmission of rotavirus. Studies on the molecular characterization of rotavirus strains detected in food and water provide new insights into possible rotavirus genetic rearrangements and zoonoses. The emergence of strains derived from interspecies transmission has implicated and inspired the study of different vaccine strategies.

KEYWORDS: Rotavirus, food, waters, genotype.

INTRODUCTION

Foodborne diseases are currently a major public health problem; It is estimated that the consumption of contaminated food is responsible for 600 million cases of foodborne illness and approximately 420,000 deaths [1]. It is one of the main causes of infections due to water consumption, with 1,700 million cases registered each year [2], and 842,000 deaths of people [3]. Rotaviruses (RV) are the most important cause of childhood gastroenteritis worldwide, approximately 258 million cases of diarrhea occur worldwide in children under five years of age, which are attributed to RVs [4]. Between 2013 and 2017, it is estimated that 122,000 to 215,000 children die from diarrhea due to RVs annually [4]. RV is an endemic pathogen in many regions of the world [5]. It has been shown to cause deaths in mammals and birds [6]. The combination of high viral concentrations in environmental waters, RV's low infective dose required for infection, and its ability to remain stable and persist in the environment, has made it a successful agent that can remain in the aquatic environment, producing bad results for the health of the population by contaminating food [7]. In addition, it has been shown that it is an agent that has the capacity to produce many mutations in the genome and genetic rearrangements, for which research has shown that it is considered to have zoonotic potential, which leads to the need to maintain molecular surveillance of RV in the continent [8,9].

RV is classified as a genus of the Reoviridae family; it is a naked virus, and it has a segmented genome [10]. It belongs to group A; antigenically RVs are classified into serogroups, subgroups, and serotypes. To date, 8 serogroups are known, defined by the epitopes present in the VP6 protein, each of which has been assigned a letter: groups A, B, C and H have been isolated both in humans and in animals, while groups D, E, F and G have only been isolated in animals [6]. Most RV infections are caused by group A RV [10]. Serogroup A is typically associated with diarrhea in people and young animals [10]. The VP6 protein is the predominant group antigen, constituting 51% of the virion [10].

Currently, 32 genotypes of G (VP7) and 47 genotypes of P (VP4) respectively are known [11]. Recognizing genetic relationships between human and animal strains has been made possible by whole genome sequence analysis of RVs [6, 12].

ROTAVIRUS INFECTION

RV infection follows the fecal-oral route and also through contaminated food and water [10]. RVs are excreted in high concentrations in the feces of infected individuals, which are routinely discharged into the environment [8]. High concentrations of RV have been found in wastewater, even after secondary wastewater treatment that is only partially efficient in removing viral agents [13]. Studies have shown that the respiratory tract is also involved, as is the case with influenza and measles viruses [14]. The detection of RV in drinking water for human consumption [15, 16], contaminated water, swimming pools [17, 18], in food [19, 20] and in contaminated objects, is well documented [14]. This virus is very stable in the environment, being able to survive for long periods of time [10, 14].

Other observations that suggest environmental contamination as a source of infection are: the persistence of infections in day care centers and the high frequency of nosocomial infections in RVs [21].

There are reports in Latin America and the world on the role of animals as a source of infectious RVs for humans, which consider RVs as a possible zoonotic potential [6, 8, 9, 22].

The spectrum of RV infection varies from being asymptomatic, through mild-moderate clinical presentation, to producing profuse, watery diarrhea that can cause dehydration and death. After an incubation period of 1 to 3 days, the picture begins abruptly with vomiting and fever, followed by profuse watery diarrhea that leads to dehydration; the temperature drops rapidly, vomiting subsides within 24 to 48 hours, and diarrhea in 2 to 7 days. There is passive immunity, which comes via the placenta and by breastfeeding [10]; occasionally, fatal gastroenteritis occurs, because dehydration is not treated on time [1,10].

EPIDEMIOLOGICAL CHARACTERISTICS OF ROTAVIRUSES

Knowledge of the epidemiology of RV is essential for the control of this disease, especially since it has been observed that it presents differences between developed and developing countries, as well as between temperate and tropical regions [23]. These differences could be associated with risk factors that are not yet well established but have been linked to socioeconomic status and home overcrowding (23). RV diarrhea is much more severe in populations of low socioeconomic status; most RV deaths occur in less developed countries [23, 24]. RV transmission is mainly fecal-oral or/through ingestion of food and water that has been contaminated with animal and/or human waste [20, 25]. It has been identified that in poorer countries, many of them in Latin America, the appearance of unusual emerging genotypes of RVs is occurring in children and adults with gastroenteritis; which harbor in their sequence genes from RV genotypes detected in animals; Therefore, RV is considered to have zoonotic potential [8, 24]. Studies have shown that in Latin America many farm animals and other wild animals share drinking water resources with the inhabitants, which could be an important vehicle for RV transmission [8, 24]. In recent decades, this agent was responsible for 3.1% of foodborne outbreaks in Brazil [26]. Enteric viruses, such as RV, can be used as indicators of environmental contamination in the food handling process, and evaluate the sanitary quality of the water used in these procedures [27]. Thus, the spread of these pathogens can occur in food handling environments, mainly through food handlers or contaminated water [18,19, 20].

Some viruses can be resistant to cleaning and disinfection processes and can serve as a source of cross-contamination in commercial and domestic settings [13]; RVs exist extracellularly as small, non-enveloped virions; they are devoid of superficial lipid bilayers that form an envelope, while the outer protein layers, the so-called capsids, provide them with greater resistance to environmental conditions, disinfectants, and common sanitizers [28].

It is important to be aware that RV has a seasonal behavior that influences the age at which the first RV infection occurs [23]. In tropical countries, the age at which the first infection appears is very early (< 6 months of age), because all children born are exposed to the virus, regardless of date of birth; while, in countries with marked seasonality or temperate countries, the first infections occur at later ages (9-15 months of age) [23, 29].

ROTAVIRUS VACCINE

The following vaccines are approved against RV: RotaTeq®, Rotarix® Rotavac® and Rotasiil® [30]. In 2015, 1.31 million children died due to diarrhea, of which 500,000.00 were children under five years of age. From 2005 to 2015, the number of cases of diarrhea in children under five years of age decreased by approximately 10%. Deaths from diarrhea decreased by about 34% and deaths due to RV decreased by 44% [31]. The World Health Organization (WHO) recommends maintaining RV vaccines in all national immunization programs worldwide [32]. Currently, 19 countries in Latin America and the Caribbean include RV vaccines in their national immunization programs [33]. To date, more than one hundred countries in the world have introduced RV vaccination programs [34].

ROTAVIRUS DETECTED IN FOOD AND WATER IN LATIN AMERICA

Acording to the WHO, after the development and application of the Rotarix® vaccine, it should be noted that the figures for deaths in children under five years of age have decreased from 500,000.00 to 215,000.00 deaths per year, between 2013 and 2016, worldwide [33]. In Latin American countries, diarrheal diseases continue to be one of the most important public health problems [35]. An investigation published by Santos et al. [36] illustrates that in low- and middle-income countries in Latin America, the early and widespread use of the RV vaccine has resulted in a significant reduction in deaths and hospitalizations due to RV. Meta-analysis of the many studies on the impact of Rotarix™ and RotaTeq® vaccines estimated that an overall vaccine effectiveness of 53% was observed against RV infections, 73% against RV-related hospitalizations, and 74% against severe RV disease [36]. Foodborne viruses, such as RV, are a common and probably the least recognized cause of gastroenteritis outbreaks [19]. Research has shown that the main foods implicated in the transmission of human enteric viruses are mollusks, fruits and vegetables irrigated with wastewater and/or washed with non-potable or contaminated water; or these foods can be contaminated by contact with surfaces or hands of infected personnel during their preparation [37]. In addition to causing acute illness, they are of public health concern because low infectious doses are needed; to cause an infection such as gastroenteritis [2]. RV transmission is primarily through ingestion of food and water that has been contaminated with animal and/or human waste [19, 20, 24].

RV has been detected in perishable foods for consumption such as shellfish and vegetables in countries like Mexico, where this virus has been detected together with other viruses such as norovirus and hepatitis [13]. RV detection studies have been carried out in drinking water for consumption in the Central Valley

of Costa Rica, where the presence of RV has been identified [38]. In Leon, Nicaragua, RV was identified in wastewater, in low prevalence, after vaccination [25]. In a study carried out in Panama, the presence of RV was determined in lettuce and mollusks (*Anadara tuberculosa*) in the capital and in the western region of that country, where the same genotype was identified that circulated in both foods [20]. In Colombia, the presence of RV has been detected in drinking water for consumption [39, 40]. In Uruguay, RV have been detected in groundwater and wastewater [41, 42]. Studies have shown the presence of RV in berries, vegetables, and oysters (*Crassostrea gigas*) in Argentina [43, 44, 45]. In Chile, a pilot study was used for the first time in Latin America by studying the RNA virosphere using a single wastewater sample in Santiago de Chile using viral metagenomics, demonstrating high RV content [46]. In a poor marginal neighborhood in Quito, Ecuador, the use of reused water for hand washing and the washing of raw vegetables are applied, detecting the presence of RV [47]. Several studies of RV in food and water have been carried out in Brazil locations, such as Manghinhos in Rio de Janeiro, detected the presence of RV in drinking water for human consumption [48]. RVs have been detected in rivers that are located within forest reserves. Other studies carried out in Rio Negro in Brazil during a flood showed that the increase in the presence of RVs was possibly related to the increases in diarrheas that occurred in the neighboring population [49]. RV has also been detected in recreational waters (swimming pools) in Brazil [50]. RV have been detected in mussels and oysters in Brazil [51, 52, 53, 54]. In another study in Brazil, they have shown the presence of RV in pork, beef and chicken, which shows failures in the food handling process [55]; as well as in fresh vegetables, lettuce [56], cheeses and jellies in that country. As far as it is known, it is the only report of a study of this type developed in that country and in Latin America [57].
I do not omit to state that 80% of the investigations where RV presence has been found in food and water in Latin America have also detected norovirus. This is demonstrated by the investigations that have revealed that norovirus is also considered one of the most important in the production of gastroenteritis in young people and adults, and its main source of contamination is through contaminated water and food [58].

According to the WHO and the United Nations Children's Emergency Fund (UNICEF) 2015 reports, untreated water is used by 663 million people worldwide. People living in poor or rural areas and developing regions are affected by a lack of access to safe drinking water, including people living in developed countries with advanced water and wastewater treatment facilities, which studies indicate that are not safe from diseases transmitted by viral contamination in water [59]. Furthermore, it has been estimated that by the year 2030, approximately 1.6 billion people (19% of the world's population) will lack clean water, leading to more outbreaks of waterborne diseases [60].

RV transmission through food and water is concerning from a public health perspective due to the low infectious dose required to establish an infection, and the high load of viral excretion through feces, even in asymptomatic cases, facilitating thus the spread in the environment [61]. Despite the public health relevance of foodborne illnesses caused by RVs and other viruses, only few studies have been conducted in Latin America to assess the presence of RVs and other pathogenic viruses in meat cuts of animal origin, mollusks, vegetables, and waters [55]. While RV infections are usually self-limited, studies of foodborne viruses in animal products are relevant and beneficial in the One Health concept to clarify epidemiological aspects and molecular characteristics [20, 55]. The presence of these pathogens in food may indicate a public health problem since RVs have the ability to survive on different surfaces [55], at low temperatures, and during food storage [20]. Considering that RV is considered an important pathogen associated with neonatal diarrhea and children worldwide, it should be noted that, in recent decades, this agent was responsible for 3.1% of foodborne outbreaks in Brazil [26].

Bidawid et al. [62] explains that food can be contaminated with viruses in the pre- or post-harvest stages; In the post-harvest stage the main source of viral contamination can be attributed to infected food handlers who could be the contaminating source of the virus during handling and packaging. They emphasize that 9.2% of infectious virus particles on contaminated hands can be transferred to lettuce during handling [62].

Indicators of fecal bacteria are among the most widely used indicators for microbial water quality, even though most evidence indicates that a direct correlation with pathogenic viruses is not observed and should not be expected, because enteric viruses are generally more resistant than bacteria to wastewater treatment procedures [63]. In turn, enteric viruses can enter environmental waters through a direct discharge route of treated wastewater; currently, there is no regulation that determines the control of enteric viruses in aqueous matrices in Central America and some South American countries. Some studies show that the pattern of viral genotypes circulating in the community is similar to that observed in contaminated water used for irrigation [64]. This situation suggests an implication of the discharge of untreated or poorly treated wastewater premises to environmental waters [64]. Unfortunately, this situation seems to be a common occurrence in different regions of the world [64]. Research reports the appearance of viruses in different aquatic environments, following the route of contamination from untreated wastewater to surface waters that receive other wastewater discharges [65].

Other studies indicate that some RV genotypes circulating in the community are also detected in green leafy vegetables, suggesting that irrigation water contaminated by fecal contamination could be a source of plant contamination with viral particles [20]. It should be noted that RV has been detected frequently in green vegetables compared to the frequency detected in wastewater samples, irrigation water, which could be related to the binding of RV to plants [45]. Another explanation could be that the vegetables are fumigated with the same irrigation water before they are put on the market [45]. Some researchers consider that perhaps the combination of the two factors mentioned above could contribute to a greater contamination of vegetables with RV [56]. Also, in the pre-harvest stage, food can be contaminated on the farm during the growth stage by contact with contaminated fertilizers, wastewater or the use of irrigation water contaminated with feces [7]. This is particularly relevant when the food is green leafy vegetables that are eaten raw [7].

Frozen and refrigerated foods are similar in their sowing and harvesting process, and only differ in their preservation; in frozen foods that are placed in bags, moisture forms causing a layer of frost and ice crystals that can damage the surface of the food causing the penetration of agents capable of causing diseases; it is known that RV can remain viable for up to several months at a temperature of 4 °C [66].

The worldwide distribution of RV G (VP7) and P (VP4) genotypes shows a number of diarrhea-associated combinations: G1P [8]; G2P [4]; G3P [8]; G4P [8] being these considered the most common in the history of the study of VR worldwide [24, 67]. Studies carried out in contaminated water, fresh fruits and vegetables in Latin America have detected the genotypes (G1, G2, G3; G4, G6, G8 and G9) [42, 45]. Studies carried out on mussels and oysters in Brazil in Arraial, in Rio de Janeiro, reveal in the sequencing of the VP6 gene of RV the identification of the I2 genotype, which presented high homology with human strains of the G1P [8] and G3P [8] genotypes isolated in Brazil, Taiwan, Australia and the Dominican Republic; Another investigation carried out in the coasts of the Brazilian Amazon identified the I2 genotype of RV in mussels and cultured oysters [53]. In Argentina, this I2 genotype was detected in oyster samples, associated with G8-P [1]-I2, which has a genome similar to that of bovine RV [44]. Investigations carried out in Brazil have detected the VP6 gene of RV genotype I2 in stool samples from children in that country [68, 69]. Previous investigations show that genetic rearrangement and zoonosis transmission of RV possibly occurs through transmission of RV to water, food, animals and from there to man (Fig 1). It is important to highlight; that, the latest investigations have revealed in Latin America the appearance of emerging strains of RV in humans and that also share genes of animal origin, for which, after carrying out an analysis of the results published in the region, they reveal the possibility of zoonoses [8, 24]. In Latin America, in some countries in poor conditions, part of their population lives close to animals and uses water contaminated with fecal remains to irrigate many crops, thus causing an increase in food contamination and new infections with RV strains. Considering that many farm animals share water for consumption by many residents, this also allows the increase and outbreaks of infections in our Latin American countries, thus causing a rapid increase in the detection of unusual new strains with zoonotic potential [8], emerging from RV in association with genetic heterogeneity, which raises interesting questions about the evolution of RV in the region. Another interesting finding discovered worldwide is that many fruit and insectivorous bats harbor large numbers of RV strains, demonstrating that bats appear to serve as reservoirs for multiple RV genotypes, which could pose a veterinary and public health risk [9]. However, several studies conclude that there is the possibility of RV zoonosis from bats to some wild animals, other farm animals and from there to man. Research carried out in Latin America reveals the possibility that the bat *Carollia perspicillata* is the reservoir (KCR10-93) and transmitter of the G20 genotype to children in Surinam (SUR/2014735512) and Ecuador (Ecu534). [9, 22]. In some Asian and Latin American countries where sanitary conditions are poor, some bats consume water and defecate in areas where this water is shared with animals and humans [70].

Bats such as *Eidolon helvum* have been observed skimming bodies of water in Africa, which researchers believe is probably done by collecting water to drink [70, 71]. In Asia and Africa, human excrement is sometimes used as fertilizer on farmland [70]. Viable human RV genotypes have been detected in surface water, reservoirs, wastewater, and drinking water [72, 73]; contact with human feces during drinking or feeding provides a mechanism by which fruit bats can likely ingest human RVs, which would serve as a source of RV genetic rearrangement. Hence the probable explanation of why RV strains of human origin were identified in the feces of bats in Bangladesh [70] and in other countries, as is the case of the G1 genotype that has also been detected in environmental water samples [74]. and bivalve shellfish samples [75], supporting the hypothesis that human RVA strains may contaminate local water sources. On the other hand, other studies have shown in Latin American countries that farm animals and other types of wild animals share water sources with humans [24] (Fig 1).

Although it is difficult to have exact estimates of epidemiological data and RV disease burden in Latin American children, it is clear that RV diarrhea is common in Latin America and the Caribbean. After vaccination programs, deaths, and hospitalizations due to diarrhea in children under five years of age have decreased considerably [76].

Further studies are needed to determine the seasonal profile of viruses in fresh products using metagenomics and, further sequencing studies on circulating foodborne RV strains are needed. Studies have shown that some pathogenic viruses can be internalized in lettuce once the product is contaminated, which represents a potential risk for consumer safety, since fresh vegetables are generally consumed without the use of preparation methods that would eliminate viruses. associates [77]. Studies carried out in Japan show that RVs infecting patients with either symptomatic or asymptomatic manifestations can accumulate in bivalve mollusks and can persist for a long time in them; moreover, research shows the accumulation of vaccine strains in bivalve mollusks that represent a threat of unexpected infections and RV genomic rearrangements [78].

It is important to stress that the Codex Alimentarius examines the types of risk management tools that can be created to help countries protect the health of consumers from foodborne viral illnesses. The Codex Alimentarius Commission has recently proposed a regulation to promote guidance on the control of viruses in food [79].

CONCLUSION

Further studies are required to understand the feasibility and whether this RV bioaccumulation and bioconcentration in bivalve mollusks has any stimulatory role in genetic rearrangement. A better knowledge about which viruses may be present in our food, even if they are not associated with human disease, may be valuable for surveillance of potential new public health threats.

The results of the molecular characterization of these RV strains detected in food and water would provide new knowledge about possible RV genetic rearrangements in the future. The emergence of strains derived from interspecies transmission has implicated and inspired the study of different vaccine strategies. Vaccine selection pressure could increase the circulation pressure of rare strains and consequently reduce the effectiveness of the current vaccine.

In response to the surveillance reports of unusual strains with zoonotic potential carried out in the Latin American Region, little information has been found. Therefore, we consider the urgent need to maintain molecular surveillance of RV strains in the region; with the intention of evaluating the impact that the vaccine will have in the future.

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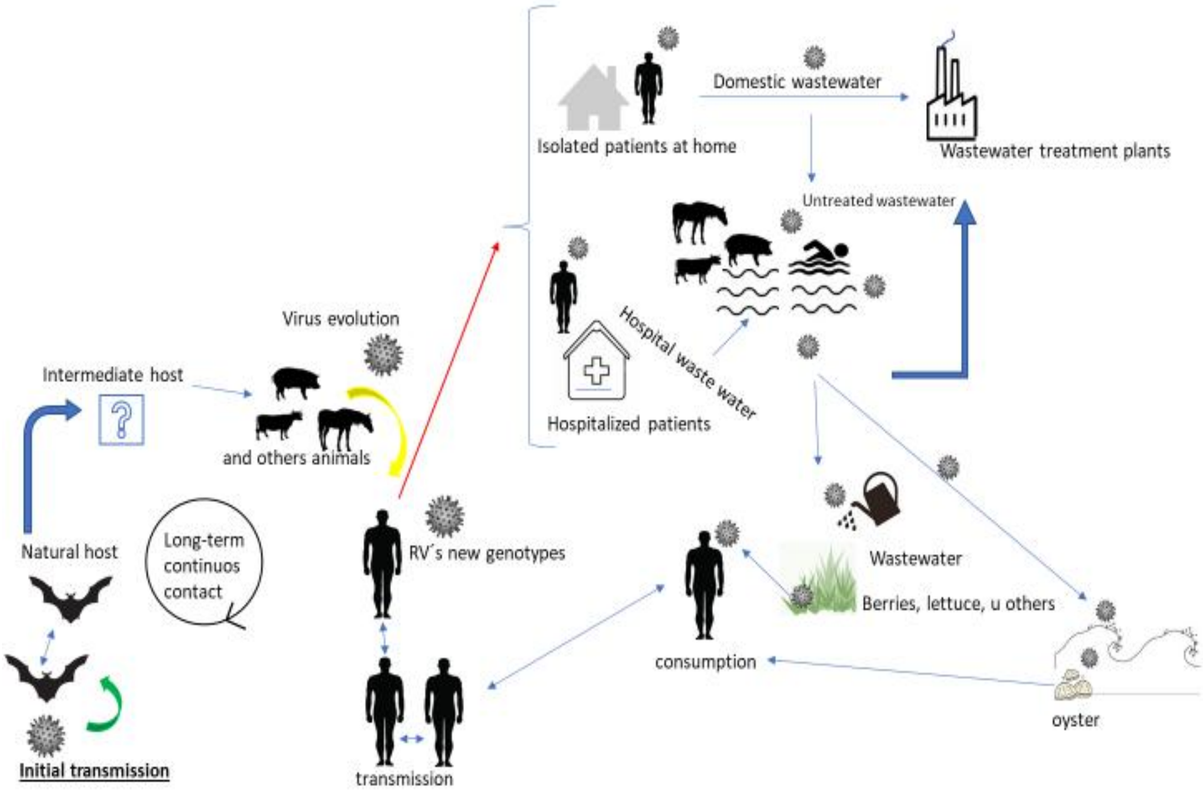
Figure 1 : Proposal for Transmission Mechanisms and Genetic Rearrangement of Rotavirus in Environments



Proposal for the transmission and genetic re-arrangement of the RV of animals, water, food until it reaches man. Research shows that there is a high probability that bats are the reservoirs of various viruses including RVs. Research carried out in Latin America reveals the probability that the bat *Carollia perspicillata* is the reservoir (KCR10-93) and transmitter of the G20 genotype to children in Surinam (SUR/2014735512) and Ecuador (Ecu534). Furthermore, it could be the probable explanation for the transmission of the VP6, I2 gene detected in water, oysters, animals and in children with diarrhea in Brazil and Argentina.