Short communication

Rotavirus in Oysters, Lettuce, and feces in Children with diarrhea from Panama

ABSTRACT

Rotavirus infections are the most common causes of infectious diarrhea in young children worldwide. We performed a genotyping of rotavirus strains by RT-PCR for G (VP7) and P (VP4), in *Anadara tuberculosa*, *Lactuca sativa* and feces samples of children with diarrhea collected in Panama. Combinations of genotypes G (VP7) and P (VP4) were identified in *Anadara tuberculosa*: 30 (15%) corresponding to G12P[8], 26 (13%) to G12P[6], 28 (14%) to G1P[8], 7 (3.5%) to G2P [6]. *Lactuca sativa*: 42 (18%) corresponding to G12P[8], 33 (14%) to G1P[8]. In feces of children with diarrhea: 39 (13%) corresponding to G12P[8], 14 (4.6%) to G9P[8], 12 (4%) to G12P[6], 2 (0.6%) to G2P[x], 1(0.3%) to G3P[8], 6(2%) to G2P[4] were identified. This research represents the first report of rotavirus strains identified in *Anadara tuberculosa* and *Lactuca sativa* in Panama and the Central American region.

Keywords: rotavirus, diarrhea, *Anadara tuberculosa*, *Lactuca sativa*.

INTRODUCTION

Rotavirus is considered the main etiological agent in diarrhea in children and occasionally in adults throughout the world [1, 2].

Group A rotaviruses (RVA) are classified using a binary system, based on the genetic and antigenic variation of two surface proteins VP7 (G) and VP4 (P). As of May 2018, human and animal RVA has been classified into genotypes 36 G and 51 P. Globally, genotypes G1 P[8], G2P[4], G3P[8], G4P[8], G9P [8] and G12P[8] are the most commonly identified cause of human infections [3].

Bivalves *(Anadara tuberculosa, Bivalvia: Arcidae*) are one of the main mollusks of commercial importance and consumption in Panama, as well as many other countries in Latin America [4].

Enteric viruses are prevalent in the environment due to a variety of causes, but primarily due to human fecal contamination; when viral particles of the feces of affected individuals are released into the environment, they can reach shallow waters and become a health hazard [5]. There are few studies that directly evaluate the presence of RVA in food; of these, most relate to detection of RVA in shellfish and some relate to detection of RVA in vegetables such as lettuce and other foods [6, 7]. Studies have shown that shellfish and lettuce have caused many enteric disease outbreaks in various parts of the world [8,9]. Viruses that are ingested by bivalves are trapped in the gills mucosa and transported by ciliary movements to the mouth and then to the stomach [10]. In this way, it has been shown that *Anadara tuberculosa* and other bivalves can act as concentrators of rotavirus, and other viruses or bacteria; thus, increasing the chances of infection when consumed raw or poorly cooked [10]. Though rotaviruses detected in the aquatic milieu are usually insignificantly low, concentrating them in the edible tissue through filter-feeding potentiates the degree of infectiousness [11]. Studies on RVA genotypes in the environment are limited [6]. Using hospital studies, the highest prevalence rate of rotavirus gastroenteritis is found in children <5 years of age, and research has shown rotavirus infection also in adult patients with gastroenteritis [1,2].

Based on epidemiological data published by various researchers, rotaviruses are the main cause of diarrhea and a variety of unusual strains and strains have been detected as zoonotic potential in children in Panama , Central America and the Caribbean [12, 13, 14]; Due to the above, and because bivalves and different types of vegetables are generally consumed raw or lightly cooked in our countries, we consider this research important, which represents the first genotyping study of rotavirus strains G (VP7) and P (VP4) detected in *Anadara tuberculosa* and *Lactuca sativa* in Panama and in the Central American region.

Two hundred (200) Samples of *Anadara tuberculosa* were taken from January to April 2018 in the Chame mangroves, which are located 60 km west of the capital city; *Lactuca sativa* samples were purchased at the La Chorrera vegetable market (230 samples), also located west of the Capital, 37 km away, and 300 stool samples were collected from children with diarrhea under five years of age at the National Children's Hospital in Panama, which houses sick patients from all areas of the capital city of Panama. (Figure 1).

The presence of RVA was detected by the immunochromatography technique (Coris BioConcept , Belgium). Viral RNA was extracted from each sample using TRIzol LS (Invitrogen, Carlsbad, CA). Primers and procedures were performed, as previously described, as a modification of the original methods described by Bucardo F et al. [15].

Of the 200 *Anadara tuberculosa* samples, a total of 120 (60%) samples were ELISA positive for rotavirus and 91 samples were sent for characterization of the G (VP7) and P (VP4) genotypes: 30 (15%) corresponding to G12P[8], 26 (13%) to G12P[6], 28 (14%) to G1P[8], 7 (3.5%) to G2P[6]. Of the 230 *Lactuca sativa* samples, a total of 92 (40%) samples were ELISA positive for rotavirus and 75 samples were sent for characterization of the G (VP7) and P (VP4) genotype: 42 (18%) corresponding to G12P[8], 33 (14%) to G1P[8]. In feces of children with diarrhea, out of the 300 samples collected, 132 (44%) were positive for RVA, of which 74 were typed for G (VP7) and P (VP4), resulting in: 39 (13%) corresponding to G12P[8], 14 (4.6%) to G9P[8], 12 (4%) to G12P[6], 2 (0.6%) to G2P[x], 1(0.3%) to G3P[8], 6(2%) to G2P[4]. (Table 1).

The detection of rotavirus in the samples of *Anadara tuberculosa* resulted in a higher percentage of contamination (60%) with rotavirus than the samples of lettuce (40%) and the feces of children with diarrhea (44%). This may be since the oysters were collected in areas containing human wastewater in Chame, a region where many residents live and is an area that contains several vacation hotels. All the samples collected from bivalves, lettuce, and feces from children with diarrhea were collected during the dry season months in Panama, and this is the time when high peaks of diarrhea outbreaks, due to rotavirus, have been reported in our Central American countries [16]. Furthermore, bivalve shellfish, such as oysters and mussels, can accumulate rotavirus [10] and may be associated with an outbreak of gastroenteritis [6,7]. In addition, it is in this dry season where there is a shortage of water. Farmers use water from wells or rivers to irrigate vegetables and other agricultural crops in Panama. Studies have shown that many of the rivers and well waters in Panama have microbiological contamination [17]. Studies have shown that the irrigation of vegetables with untreated water leads to the greatest loss of sanitary quality of these foods [6]. Contamination in lettuce can also occur from sewage seepage into farm fields, the presence of farm animals on farmland, crop handling during harvesting and packaging, as well as distribution and commercialization [5,6]. The concern about microbial contamination of these foods becomes even greater when we consider the survival time in this case of rotaviruses, often weeks or months [18].

For some years, the G12 genotype was considered an emerging strain of RVA worldwide and some studies conducted in Latin America show the detection of the G12 genotype in feces of children with diarrhea [19, 20]. We are considerably struck by the fact that in this study all the samples analyzed presented the G12 P genotype [8]; and in the bivalves, as in the samples of children with diarrhea, the genotypes G12P[8] and G12 P[6] circulated, which is an indication that it is probably a transmission route. A study carried out in Africa identifies this G12P[6] genotype with high homology to strains of porcine origin researchers state that this may be due to genetic rearrangement events [21]. Interestingly, the (VP4) P [6] genotype, traditionally detected in animals, was therefore proposed to be a mechanism by which a new genotype could interfere into human populations [22]. Studies carried out in Central America and the Dominican Republic show the detection of unusual genotypes, and possible evidence of zoonotic transmission [12, 13, 14]. The genotypes G1P8, G9P[8], G3 P[8], G2P[4] and G2 P[x] that were detected in this study, in the feces of children with diarrhea, have previously been identified in Panama and Central America [12, 23].

It is evident that in Panama bivalve shellfish and lettuce may possibly be associated with an outbreak of gastroenteritis; as revealed by other researchers [7,10]. In addition, the presence of viral genomes and infectious particles in foods that, in general, undergo minimal treatment before consumption, underlines that green crop can act as potential sources of transmission of enteric viruses [6,7]. We believe that public intervention is necessary in Panama in the use of river water and wells as a source of irrigation, since it is evident that it represents a health problem. Unfortunately, the study of enteric viruses in our country is limited as it is in many countries of our region and currently, there is no regulation that determines the control of enteric viruses in waters in Panama.

Studies show that food can be contaminated with viruses in the pre-harvest and/or post-harvest stages [6]. In the post-harvest stage, the main source of viral contamination can be attributed to derived food handlers [6].

Studies made on the phylogenetics of RVA have demonstrated differences in the lineage and sub lineage of genotypes, highlighting the great genetic variability of rotavirus [13]. A small percentage of samples analyzed in this study for the P[X] groups could not be typed with the use of specific primers; this may be due to the accumulation of point mutations and diversity of reported lineages.

This research represents the first report of rotavirus strains identified in food in Panama and in the Central American region.

CONCLUSION

Due to the above, and in addition to the fact that research reveals that rotaviruses cause deaths in children and diarrheal outbreaks in adults, we consider the urgent need to maintain epidemiological surveillance of rotavirus strains in food. This preliminary study has highlighted the need for deeper surveillance of enteric viruses in aquatic environments and the link with contamination in bivalves and vegetables in Panama. Our findings reveal the need for public intervention in the use of river water as a source of irrigation for vegetables. As well as the development of a sanitary surveillance control on the consumption of bivalve mollusks that are consumed raw, as well as, lightly cooked in

Panama. We believe additional experiments and sequencing analysis of these rotavirus genotypes should be performed in future research and explore whether rotavirus genotypes, identified in bivalves and green vegetables, present genetic rearrangement events, which would be very interesting, since it would be a great contribution, considering there are reports of studies that show events of genetic rearrangement and zoonotic potential of rotavirus in Latin America. In this context, the immunogenicity and efficacy of rotavirus vaccines may be challenged by the evolution of the rotavirus viral genome.

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Figure 1. Locations of Panama where the samples were Taken

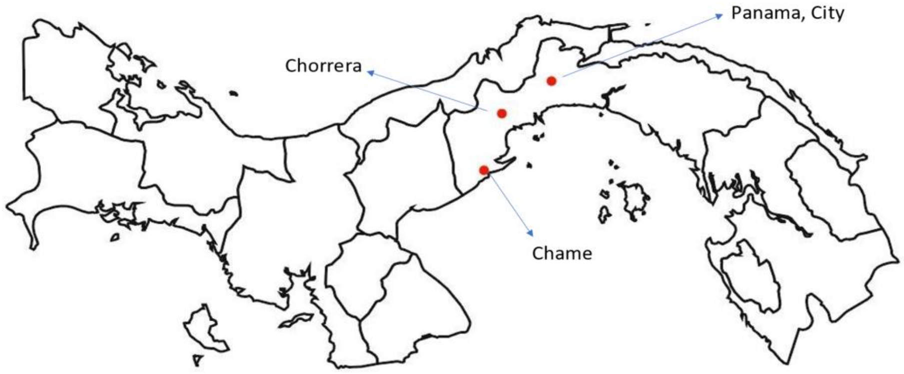

Table 1. G (VP7) P (VP4) of Rotavirus Strains isolated in Oysters (*Anadara tuberculosa*), Lettuce (*Lactuca sativa*), and Feces of children with diarrhea in Panama

Genotypes VP7 (G) and VP4 (P)	***Anadara Tuberculosa***	***Lactuca sativa***	**feces of children with diarrhea**
Locations	**chame**	**jabot**	**Panama City**
G12P[8]	30 (15%)	42(18%)	39(13%)
G12P [6]	26(13%)		12(4%)
G1P [8]	28(14%)	33(14%)	
G2P [6]	7(3.5%)		
G9P[8]			14(4.6%)
G2P[x]			2(0.6%)
G3P [8]			1(0.3%)
G2P [4]			6(2%)
Total	91	75	74

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