Short communication

G and P Genotypes of Group A Rotavirus in Diarrheic Calves in Costa Rica

ABSTRACT

Rotavirus infections are the most common causes of infectious diarrhea in young children and animals worldwide. We performed a genotyping of rotavirus strains by RT-PCR for G (VP7) and P (VP4), in diarrhea samples from calves, in several locations in Costa Rica. Combinations of genotypes G (VP7) and P (VP4) were identified in cattle: 4 (26%) corresponding to G8 P [11], 4 (24%) to G6P [11], 3 (19%) to G8P [7], 2 (13%) to G10P [1], 3 (19%) to G10P [11], and 1 (6%) to G8P [1] and mixed infections. This research represents the first report of bovine rotavirus strains (calves) in Costa Rica and in the Central American region.

Key words: rotavirus, diarrhea, calves, VP7 and VP4.

Rotavirus is considered the main etiological agent in diarrhea in young animals and children around the world [1].

Group A Rotaviruses are classified using a binary system, according to the genetic and antigenic variation of two surface proteins (VP7 and VP4). So far, 36 types G (VP7) and at least 51 types P (VP4) of rotavirus group A circulating in humans and animals [2] have been reported. Particularly in cattle, 14 different G (VP7) genotypes (G1 -G6, G8, G10-G12, G15, G18, G21, and G24 and 11 P (VP4) genotypes (P [1], P [3], P [5] -P [7], P [11], P [14], P [17], P [21], P [29], and P [33]) [3, 4] have been identified. They affect animal populations causing great economic losses for livestock producers, due to vaccination expenses, treatment, weight loss of affected animals and mortality.

Risk factors that contribute to the appearance of diarrhea caused by rotavirus are failures in the transfer of passive immunity especially observed in heifers, presence of seasonal diarrhea, and lack of hygiene in artificial rearing systems [5].

Knowledge of the epidemiology of rotavirus 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 differences in climate [6].

In Costa Rica, in the Central American region and in the Dominican Republic we have carried out characterizations of rotavirus strains in children, where we reported common and unusual genotypes [7].

This research represents the first genotyping study of rotavirus G (VP7) and P (VP4) strains detected in cattle in Costa Rica and in the Central American region.

Samples of feces from calves under 1 month of age were taken from 17 dairy farms (bovine-calves) located in Río Cuarto de Grecia, Pital, Pos, Zarcero, Sucre and Pacayas in 2008 in Costa Rica. All calf stool samples were analyzed in the virology laboratory, at the School of Veterinary Medicine at the National University of Costa Rica (UNA), to determine the presence of rotavirus using the immunochromatography technique (CorisBioConcept, Belgium) and the RT-PCR technique (VP7) and (VP4) described by Genstch JR et al [8] and Gouvea V et al [9].

From 200 samples (calves feces), a total of 38 samples were ELISA positive for rotavirus and 25 samples were sent for characterization of the G (VP7) and P (VP4) genotype.

By using the RT-PCR technique, the combinations of genotypes G (VP7) and P (VP4) were identified in cattle: 4 (26%) corresponding to G8 P [11], 4 (24%) to G6 P [11], 3 (19%) to G8 P [7], 2 (13%) to G10 P [1], 3 (19%) to G10P [11], and 1 (6%) to G8P [1]. In 3 samples, it was not possible to identify the G (VP7) genotype with the primers used. Mixed infections were found in 6 samples (Table 1).

The distribution of genotypes G (VP7) and P (VP4) of Group A bovine rotavirus in the dairy farms of the different locations in Costa Rica, were different. Namely, G10 was the most prevalent genotype in the Poas, Zarcero and Sucre area, while the G6 genotype prevailed in the Río Cuarto de Grecia, Poas and Pital de San Carlos areas. Finally, G8 was identified mainly in the Pacayas, Poas, Cedral de San Carlos and Zarcero areas. In the P (VP4) types, P [11] was identified as the main genotype in Río Cuarto de Grecia, Pital de San Carlos, Poas, Cedral de San Carlos and Pacayas , P [7] was found only in Río Cuarto de Grecia, whereas P [1] was identified in Zarcero and Sucre. Mixed infections were identified in Pacayas and Poas (Figure 1) (Table 2).

There are reports in the literature of infections in cattle caused by various genotypes of Group A Rotavirus such as G1, G2, G3, G6, G8, G10 and G11 [4, 10, 11, 12]. In the present study, the (VP7) G6, G8 and G10 genotypes were identified, with the G10 genotype prevailing, which was identified in 10 samples, followed by G6 and G8 identified in 8 and 7 fecal samples, respectively. It is important to clarify that the farms sampled in Costa Rica all belonged to the province of Alajuela except for Pacayas, which belongs to the province of Cartago, both provinces are part of the Central Valley Mountain Range of the country. Our findings coincide with reports in Brazil, Argentina, and other countries in the world where high frequencies of these genotypes were reported and considered common of epidemiological importance in cattle [10, 11, 12]. Regarding the genotypes P (VP4), we have the P [1], P [5] and P [11] as frequent as they reported worlwide [10, 11, 12]. In this work, the genotype P [11] was the most frequent, followed by P [1] that was identified in 2 positive samples and P [7]. In addition, mixed infections corresponding to P [11 + P1] and P [11 + P7] were identified. For example, Da Silva Medeiros et al [10], found P [11] as the most frequent genotype in calf diarrhea in Brazil. The finding of mixed infections is very important from the epidemiological point of view, since animals affected by one type of strain can be infected with another different strain or be affected by two different strains at the same time, which makes control of the illness even more difficult.

Among the findings from different locations in Costa Rica, a variation of P groups (VP4) was detected, but despite the variety of P groups found in different areas of the country, they are similar to those described in other parts of the world [11, 12, 13]. Additionally, as previously mentioned, the locations sampled belong to the Central Valley of Costa Rica, for which it would be important to carry out another study in which more samples are collected from other regions of the country.

Within the different combinations of genotypes G (VP7) and P (VP4) of Group A Rotavirus in bovines, G6 P [11] was identified as the most frequent detected in 4 of the 16 positive samples, G8 P [7] was the second most frequent combination identified in 3 of the 16 positive samples, followed by G8 P [11] and G10 P [11] found in 2 samples; G8 P [1] and G10 P [1] were identified in one sample. Our findings differ partially from the combinations of G6 P genotypes [5] reported as the most frequent in South America [10, 11]. Studies carried out in children have also revealed the presence of the G8, G10 genotype in America and other continents [14, 15, 16]. Although we also identified mixed infections in this investigation; studies have shown that rotaviruses produce “reassortants”. These genetic changes contribute to the evolution of rotaviruses, resulting in a diversity of genotypes and mixed infections, which is characteristic of rotavirus infections [1, 3, 13, 14]. A literature review reveals the emergence of new strains of rotavirus in the American continent

[14]. Studies made on the phylogenetics of rotavirus have demonstrated differences in the lineage and sub lineage of genotypes, highlighting the great genetic variability of rotavirus [1]. A small percentage of samples analyzed in this study could not be typed with the use of specific primers, which may be due to the accumulation of point mutations and diversity of reported lineages. We consider that additional experiments and sequencing analysis of these genotypes should be carried out in future investigations, because studies carried out in Nicaragua, Guatemala, Honduras, and the Dominican Republic have reported the presence of rotavirus strains of bovine origin, pigs, and bats in rotavirus samples, excreted from children with diarrhea, showing possible zoonosis [17, 18, 19, 20]. Furthermore, studies carried out in Uruguay have detected human rotavirus genes in calf diarrhea samples [13]. There is a report on the detection analysis of rotavirus strains in bats in Costa Rica. This investigation also included taking samples of bat feces from other countries in the world, revealing strains of rotavirus never reported before, even some of them have similarities with rotavirus strains found in other mammals [21]. Currently, commercial vaccines are available for the prevention of neonatal diarrhea in calves and children that have shown varying degrees of effectiveness. These vaccines are manufactured with the genotypes most frequently found and reported in different parts of the world. In this context, the immunogenicity and efficacy of rotavirus vaccines may be challenged by the evolution of the rotavirus viral genome.

CONCLUSION

Therefore, and adding that research reveals that rotaviruses cause deaths in children, cattle and pigs, we consider urgent the need to maintain an epidemiological surveillance of rotavirus strains in children, domestic animals, wild animals, cattle and pigs in the country, as well as the reinforcement in the maintenance and consolidation in the unification of detection and characterization protocols of rotavirus in laboratories in Central America.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1. Distribution of G (VP7) P (VP4) of rotavirus strains isolated from bovine in Costa Rica.

Genotype	P [11]	P[11]+P[7]	P[7]	P[1]	P[11]+P[1]	Total
G8	2(13)	2(13)	3(19)	1 (6)	0	8(51)
G6	4(24)	0	0	0	0	4(24)
G10	2(13)	0	0	1(6)	1(6)	4(25)
Total	8(50)	2(13)	3(19)	2 (12)	1(6)	16 (100)

No. (%) positive (n=16)

Figure 1

Distribution of Genotypes G (VP7) and P (VP4) in Sampled Localities of Costa Rica.

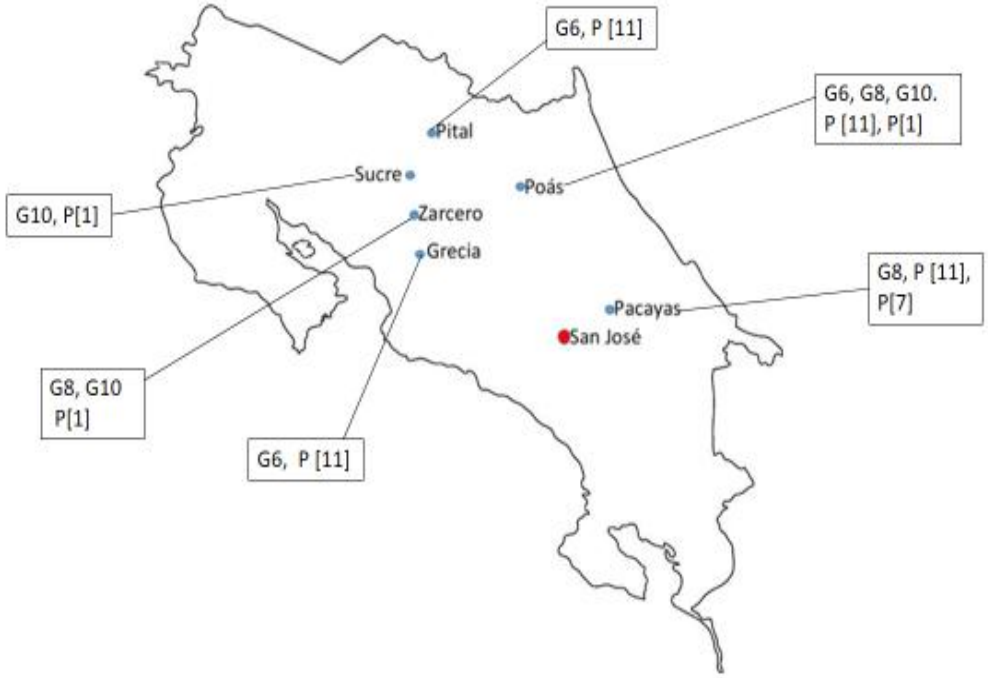

Table 2. Distribution of genotypes G (VP7) and P (VP4) of Group A Bovine Rotavirus in 17 dairy farms sampled in the year 2008.

Farm	Location	SamplesCollected	Positive Samples	G (VP7)	P(VP4)
1	Río Cuarto de Grecia	22	6	G6	P[11]
2	Poas	21	7	G6 , G10	P[11]
3	Pital San Carlos	11	2	G6	P[11]
4	Poas	13	0		
5	Poas	8	1	G8	
6	Poas	7	0		
7	Poas	9	0		
8	Poas	8	0		
9	Poas	7	2	G10	P[11], P[1]
10	Poas	10	1		P[11], P[1]
11	Poas	8	0		
12	Pacayas, Cartago	18	4	G8	P[11], P[7]
13	Cedral, San Carlos	5	1	G8	P[11]
14	Zarcero	10	1	G8	P[1]
15	Sucre	16	1	G10	P[1]
16	Zarcero	12	1	G10	
17	Sucre	15	1	G10	
Total		200	28		

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