Short communication

Group A Rotavirus Detection in Calves and Piglets from Honduras and Panama.

ABSTRACT

The epidemiology of animal rotaviruses infection and their association with illnesses in humans remains unknown in Central America and the world. In this study, we performed a genotyping of rotavirus strains by RT-PCR for-G (VP7) and P (VP4), in samples of diarrhea in cattle and pigs, in Honduras and Panama. Mixed infections and combinations of genotypes G (VP7) and P (VP4) were identified in cattle in Honduras: 4 (5%) G4P [6], 3 (4%) G2P [6], 2 (2%) G2P [4], and in Panama: 63 (72%) G10 P [2], 12 (13%) G8 P [11], 9 (10%) G6 P [11] and 3 (3%) G10 P [1].This investigation represents the first report of bovine and porcine rotavirus strains in Honduras and Panama.

Key words: Rotavirus, calves, piglets, G (VP7) and P (VP4).

Introduction

Rotavirus (RV) is considered the main etiological agent in diarrhea in young animals and children worldwide [1]. Group A rotaviruses play an important role in human gastroenteritis and are common viral pathogens associated with neonatal diarrhea in numerous animal species [1].

Group A rotaviruses are classified using a binary system, based on the genetic and antigenic variation of two surface proteins (VP7 and VP4). So far, 36 G (VP7) types and at least 51 P (VP4) types of group A rotaviruses circulating in humans and animals have been reported [2]. Particularly in cattle and pigs, 14 different G (VP7) genotypes have been informed (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]) [2,3]. Diarrhea due rotavirus in neonatal mammals, such as calves and piglets [2]; occurs to increased morbidity and mortality, treatment costs, and reduced growth rates [4]. The epidemiology of animal rotaviruses infection and their association with illnesses in humans remains unknown in Latin American [5]. There is evidence that animal rotaviruses can infect humans, either by direct transmission of the virus or by contributing one or several RNA segments that can reassort with human strains [5]. Genotyping of RVs is important to assess, whether vaccine efficacy might be altered by the changing pattern of the distribution of different genotypes, since there is strong evidence for the emergence of new strains through natural reassortment between rotaviruses among different species [5, 6]. In Costa Rica, Honduras, Nicaragua, Panama and the Dominican Republic we have performed characterizations of rotavirus strains in children, reporting common and unusual genotypes [7,8]. While surveillance has been mainly focused on infections in humans, there is a need to include domestic and farm animals in these surveillance programs, due to their close contact with humans, especially in developing countries. There is a surge in reports of the detection of strains common to animals in human populations and the increasing spread and detection of strains with unusual G and P types and untypable strains [5].

This research represents the first genotyping study of rotavirus strains G (VP7) and P (VP4) detected in cattle in Honduras and Panama.

A total of 100 and 75 fecal specimens were collected from diarrheic piglets and calves less than 30 days old, from the same number of small farms respectively, located in the village of Siguáte, near Catacamas, Olancho in the central-east section of Honduras. Also, 157 feces samples were collected from bovines (calves) of 6 month of age in two farms located to the west of Colon in Panama.

All fecal samples from calves and piglets from Honduras and Panama were analyzed for the presence of rotavirus using the immunochromatography technique (Coris BioConcept, Belgium).

In samples from Honduras, consensus gene sequence related to external group A RV protein was detected with a pair of consensus primers for VP7 (9con1-9con2) or VP4 (4con2-4con3) genes [9, 10]. Reaction products of the first RV PCR were genotyped by heminested multiplex-PCR with two sets of specific primers for the G1-G5, G9 and P [4], P [6], P [8] and P [9] types described by Gentsch et al. [9] and Das et al. [10], with modification by Leite et al. [11]. The samples from Panama underwent the RT-PCR G (VP7) and P (VP4) technique described by [1].

In Honduras, Group A RV was found in 6 out of 100 (6%) fecal specimens collected from diarrheic piglets and in 9 out of 75 (12%) diarrheic calves. Among isolates of porcine and bovine group A rotaviruses, different genotypes: 3 (4%) G2P [6], and 2(2%) G2 P [4] were detected in calves and G4 P [6] 4(5%) were detected in piglets. In Panama, out of the 157 isolates of bovine (calves) samples, a total of 139 were ELISA positive for rotavirus an/d 87 samples were amplified for detection of G (VP7) and P (VP4) genotype, identifying the combinations: 63 (72%) corresponding to G10 P [2], 12 (13%) to G8 P [11], 9 (10%) to G6 P [11] and 3 (3%) to G10 P [1]. In 2 samples, it was not possible to identify the genotype with the P (VP4) “primers” used (Table 1).

The distribution of genotypes G (VP7) and P (VP4) of bovine group A rotavirus in Honduras and Panama were different.

At a global level, rotavirus group AG (VP7) G1, G2, G3, G6, G8, G10 and G11 have been detected in bovines. Regarding P (VP4) bovine genotypes reported frequently worldwide, there are: P [1], P[5] and P[11] [1,2,3];on the other hand, twelve G genotypes (G1 to G6, G8 to G12, and G26) and 16 P genotypes (P [1] to P [8], P [11], P [13], P[19], P[23], P[26], P[27], P[32], and P[34]) of RVA, have been associated with pigs [12]. However, G3, G4, G5, G9 and G11 were historically considered the most common G genotypes in swine and were usually associated with P [5], P [6], P [7], P [13] and P [28] [12]. In the present study, the bovine genotypes (VP7) G2, G6, G8, G10, and P (VP4) P [1], P [2], P [4], P [6], and P [11] were identified. These results coincide with findings detected in Costa Rica [1], Brazil [13], Uruguay [14] Argentina [15], and other countries of the world [12,16], where high frequencies of these genotypes are reported, considered, as well as, frequent and of epidemiological importance in cattle and pigs.

Concerning the G10 P [1] genotype detected in Panama, it differs from that reported for bovine rotavirus in Latin America; however, regarding bovine rotavirus infections G6, G8, and G10 together with P[1], P[5], and P[11], respectively, are the G and P genotypes most commonly found [17]. Other investigations show detection of this same combination in goats and lambs in Bangladesh [18; 19]; genotype P [1] has also been detected in cattle in Japan [16]. It is important to note that the genotypes detected in Honduras were amplified with the primers used to detect group A rotavirus in humans. Other studies carried out in Honduras reveal evidence of detection of unusual strains [7] and possible zoonotic transmissions [20]; therefore, it is probable that due to the presence of genetic rearrangements, the primers used to amplify human group A rotavirus have amplified animal rotavirus. Genomic sequencing is necessary to clarify this argument. It is worth mentioning that genotypeG2P [4] detected in Honduras was the major genotype circulating in children during that same year (Annabelle Ferrera unpublished data). Studies carried out in children have also revealed the presence of genotypes G2, G4, G8 and G10, which are common in bovines in America and other continents [21, 22, 23]. Research shows that rotaviruses produce “reassorting” and these genetic changes contribute to rotavirus evolution, resulting in a diversity of genotypes and mixed infections [2, 4, 5, 8]. We believe that additional experiments should be performed, because studies carried out in Central America and the Dominican Republic have reported the presence of unusual strains of rotavirus in children with diarrhea. In addition, rotavirus strains that share high homology with rotavirus genes of bovine and pig origin have been identified, revealing possible zoonotic transmission [5, 6, 8]. Although interspecies transmission has not been documented to occur directly, there have been reports in Latin America, for example, of an increased number of reports of unusual rotavirus strains in humans that are apparently derived from transmission between farm animals, domestic and wild [5]. Specifically, there are studies carried out in bats where it is shown that many of the rotavirus strains that circulate in children at a global level share high homology with rotavirus genes detected in bats [6]. For example, in Costa Rica, fecal samples were collected from the bat *Carollia perspicillata* to carry out rotavirus studies, demonstrating a surprising genetic diversity of rotaviruses, with a close genetic relationship between rotaviruses and other mammals; the researchers emphasized that this strain diversity will continue to grow genetically and geographically [6]. It is interesting to note that the rotavirus strain detected in this bat *C. perspicillata* (RVA/ Bat-wt /CRC/KCR10-93/2010/ G20P47) has nucleotide similarities grouping phylogenetically with the VP6, VP1, VP2, VP3, NSP2 and NSP4 proteins with rotaviruses identified in children with diarrhea from Ecuador, Suriname, and Brazil [6]. It is important to highlight, that the transmission of a virus from bats to humans requires certain conditions that connect the distribution of hosts, the viral infection within these hosts and the exposure and susceptibility of the recipient hosts; however, before it reaches man, it must be transmitted to other hosts such as cattle, pigs, alpacas or others, including domestic animals, a phenomenon that is increasing due to the rapid fragmentation of forests. The risk of such transmission is also present in the contamination of water or food reservoirs by feces of infected animals [6].

We consider important to report theses preliminary findings. though, we also consider urgent to carry out sequencing studies of the genomes that we identified, expand larger studies that cover more sites with a greater number of samples that include domestic, farm and wild animals. Studies on rotavirus zoonosis have a limitation because there are very few sequencing studies of rotavirus strains in animals in Latin America, where there is an information bank of these circulating strains [5]. It is mandatory to mention that funds for research in Central America is scarce [25]. We have tried to join our efforts together to try to maintain our research and unify our protocols for the characterization of rotavirus strains in Central America.

It is important to note that commercial vaccines are being applied for the prevention of neonatal diarrhea in calves, as well as in children, which have demonstrated safety and efficacy.

This work represents the first documentation of group A rotavirus strains identified in piglets and cattle in Honduras and Panama.

CONCLUSION

This research reinforces the need for the characterization of rotavirus strains through genetic analysis to provide a new view of the ecology and evolution of rotaviruses in Central America, including studies of samples from domestic, farmed, and wild animals.

Global rotavirus studies have shown that some strains of rotavirus, isolated from a variety of species, are atypical for that species and, therefore, over time can produce genetic rearrangement and evolve into new species strains to be transmitted. In this context, the immunogenicity and efficacy of rotavirus vaccines may be challenged by the evolution of the rotavirus viral genome.

We consider it urgent to strengthen the maintenance and consolidation of the protocols for the detection and characterization of rotavirus in Central American laboratories.

Table 1. Distribution of G (VP7) P (VP4) of Rotavirus Strains Isolated from Honduras and Panama

Genotypes VP7 (G) and VP4 (P)	Honduras	Panama	Calves	Piglets (Honduras)
G4P [6]	4(5%)			6(6%)
G2P [6]	3(4%)		x	
G2P [4]	2(2%)		x	
G10P [2]		63(72%)	x	
G8P [11]		12(13%)	x	
G6P [11]		9(10%)	x	
G10P [1]		3(3%)	x	
Total, samples analyzed	75	87		100

References:

1. Bonilla-Espinoza J, Bourdett-Stanziola L, Jiménez C. G and P Genotypes of group A rotavirus in calves with diarrhea in Costa Rica. Asian Journal of Veterinary and Animal Science Research. 2021; 8(4), 177-182. Retrieved from https://www.journalajravs.com/index.php/AJRAVS/article/view/30183.
2. Luchs A, Timenetsky Mdo C. Group A rotavirus gastroenteritis: post-vaccine era, genotypes and zoonotic transmission. Einstein (Sao Paulo). 2016; 14(2):278-87.
3. RCWG, Rotavirus Classification Working Group, Newly Assigned Genotypes - Update May 29th 2018, (2018) (Accessed 2 July 2018), https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg .

4. Martella V, Bányai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. vet Microbiol. 2010; 140(3-4):246-55.
5. Bourdett-Stanziola L, Centeno E, Cuevas- Abrego M, Durant- Archibold AA, Ortega-Barría E, Bucardo F. The Emergence of New Rotavirus Strains in America. Review. South Asian Journal of Research in Microbiology. 2021; 11(1), 46-62. doi.org/10.9734/sajrm/2021/v11i130244 .
6. Bourdett-Stanziola, L. Genetic Diversity of Bat Rotaviruses. Microbiology Research International Journal. 2022; 32 (1), 17-31. https://doi.org/10.9734/mrji/2022/v32i130365
7. Bourdett-Stanziola L, Ortega-Barria E, Espinoza F, Bucardo F, Jimenez C, Ferrera A. Rotavirus genotypes in Costa Rica, Nicaragua, Honduras and the Dominican Republic. Intervirology. 2011; 54(1):49-52.
8. Bourdett-Stanziola L, Centeno E, Nordgren J, Durant- Archibold A, Ortega- Barría E and Bucardo F. Potential Bat-like rotavirus in Hospitalized Children with Diarrhea from the Dominican Republic. As J Res Infect Dis. 2021; 8(1):1-7. doi.org/10.9734/ajrid/2021/v8i130225 .
9. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol. 1992; 30(6):1365-73.
10. Das BK, Gentsch JR, Cicirello HG, Woods PA, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI. Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol. 1994; 32(7):1820-2.
11. Leite JP, Alfieri AA, Woods PA, Glass RI, Gentsch JR. Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization and sequence analysis. Arch Virol. 1996; 141: 2365-2374.
12. Vlasova AN, Amimo JO, Saif LJ. Porcine Rotaviruses: Epidemiology, Immune Responses and Control Strategies. Viruses. 2017; 9(3):48.
13. da Silva Medeiros TN, Lorenzetti E, Alfieri AF, Alfieri AA. G and P genotype profiles of rotavirus A field strains circulating in beef and dairy cattle herds in Brazil, 2006-2015. Comp Immunol Microbiol Infect Dis. 2019; 64:90-98.
14. Castells M, Caffarena RD, Casaux ML, Schild C, Miño S, Castells F, et al. Phylogenetic Analyzes of Rotavirus A from Cattle in Uruguay Reveal the Circulation of Common and Uncommon Genotypes and Suggest Interspecies Transmission. Pathogens. 2020; 9(7):570.
15. Bertoni E, Aduriz M, Bok M, Vega C, Saif L, Aguirre D, Cimino RO, Miño S, Parreño V. First report of group A rotavirus and bovine coronavirus associated with neonatal calf diarrhea in the northwest of Argentina. Trop Anime Health Prod. 2020; 52(5):2761-2768.
16. Hasan MA, Kabir MH, Miyaoka Y, Yamaguchi M, Takehara K. G and P genotype profiles of rotavirus A field strains circulating in a vaccinated bovine farm as parameters for assessing biosecurity level. J Vet Med Sci. 2022; May 4.
17. Alkan F, Ozkul A, Oguzoglu TC, Timurkan MO, Caliskan E, Martella V, and Burgu I. Distribution of G (VP7) and P (VP4) genotypes of group A rotaviruses from Turkish calves with diarrhea, 1997-2008. Veterinary Microbiology. 2010; 141, 231–237.
18. Hossain MB, Rahman MS, Watson OJ, Islam A, Rahman S, Hasan R, Kafi MAH, Osmani MG, Epstein JH, Daszak P, Haider N. Epidemiology and genotypes of group A rotaviruses in cattle and goats of Bangladesh, 2009-2010 . Infect Genet Evol. 2020; 79: 104170 .
19. Fitzgerald TA, Munoz M, Wood AR, Snodgrass DR. Serological and genomic characterization of group A rotaviruses from lambs. ArchVirol. 1995;140(9):1541-8.

20. Quaye O, Roy S, Rungsrisuriyachai K, Esona MD, Xu Z, Tam KI, et al. Characterization of a rare, reassortant human G10 P [14] rotavirus strain detected in Honduras. Mem Inst Oswaldo Cruz. 2018; 113(1):9-16.
21. Gautam R, Mijatovic-Rustempasic S, Roy S, Esona MD, Lopez B, Mencos Y, et al. Full genomic characterization and phylogenetic analysis of a zoonotic human G8P [14] rotavirus strain detected in a sample from Guatemala. Infect Genet Evol. 2015; 33:206-11.
22. Esona MD, Page NA, Akran VA, Armah GE, Steele AD. Characterization of 2 human genotype G10 rotavirus strains, 3008CM and 1784/CI/1999, isolated in Cameroon and Cote d'Ivoire during the 1999-2000 rotavirus season. J Infect Dis. 2010; 202 Suppl: S212-9.
23. Luchs A, Timenetsky Mdo C. Unexpected detection of bovine G10 rotavirus in a Brazilian child with diarrhea. J ClinVirol. 2014; 59(1):74-6.
24. Bourdett-Stanziola L, Cuevas-Abrego M, Ferrera A, Durant-Archibold AA. Rotavirus in Oysters, Lettuce, and Feces in Children with Diarrhea from Panama. Journal of Advances in Microbiology. 2022; 22(5), 16-21. https://doi.org/10.9734/jamb/2022/v22i530459 .
25. Moreno E, Gutiérrez JM, Chaves-Olarte E. 2011. The struggle of neglected scientific groups: ten years of NeTropica efforts to promote research in tropical diseases in Central America. PLoS Negl Trop Dis. 2011; 5(7): e 1055.