Acta Microbiologica et Immunologica Hungarica, 60 (1), pp. 21–28 (2013)
DOI: 10.1556/AMicr.60.2013.1.3

SHARED G12 VP7 GENE AMONG HUMAN AND BOVINE ROTAVIRUSES DETECTED IN CAMEROONIAN VILLAGES*

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(Received: 17 September 2012; accepted: 5 October 2012)

Group A rotaviruses (RVA) are an important enteric pathogen in humans and livestock animals. Transmission of animal RVA strains to humans has been documented on several occasions. A reverse route of transmission of RVA under natural circumstances is anticipated, although evidence is scarce. However, experimental studies indicated that animals can be infected with human RVAs. By screening the stool samples collected from 157 cattle during 2011 in two Cameroonian villages, four samples (2.5%) were found positive for RVA. Upon sequence analysis of a 410 bp fragment of the VP7 gene, the RVA strains shared up to 100% nt identity to each other and to G12 RVAs identified in human patients living in the same geographic regions. This finding provides evidence for a human-to-animal transmission of an epidemic human rotavirus strain.

Keywords: enteric zoonosis, interspecies transmission, Cameroon

* *Disclaimer:* The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the funding agency or the Centers for Disease Control and Prevention (CDC). This article did receive clearance through the appropriate channels at the CDC prior to submission.

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Introduction

Group A rotaviruses (RVAs) are the primary cause of acute dehydrating diarrhea in infants and children under 5 years of age [1]. Estimates of rotavirus-related mortality are about 453,000 children under 5 years of age die worldwide annually due to rotavirus [2]. Rotavirus is an important pathogen in domestic livestock, as well, although their economic impact may differ between areas or are unrecognized in some regions.

RVAs have been classified using a binomial nomenclature GxP[x] based upon type specificities of the outer capsid antigens, VP7 (G-type) and VP4 (P-type). Thus far, at least 27 G genotypes and 35 P genotypes have been detected from humans and animals [3]. G and P type distribution across diverse animal species indicates that there are host species barriers and restrictions. For instance, genotypes G1–G4, and more recently G9 and G12, collectively, have been identified in >90% of children hospitalized with RVA infection worldwide, while a variety of human rotaviruses with unusual G genotypes (such as G5, G6, G8, G10 and G11) have been sporadically identified [4]. In cattle, G6, G8 and G10 are the most commonly found genotypes, although additional VP7 genotypes have been also reported [5, 6].

The sharing of a serotype between human and animal RVAs suggests a possible zoonotic origin of such strains. However, sequencing studies of rotavirus strains with shared serotypes often reveals genetic divergence between animal and human strains. For example, the G1 type is very common in children worldwide, however, it is rarely identified in animals [6, 7]. In addition, the genetic lineages of G1 found in humans are different from those detected in calves. On the other hand, shared G6 VP7 types have been reported in children from Hungary and cattle from Slovenia and the Netherlands [8–11]. Many of the identified human RVA type specificities that are rare in humans, but common in animals, suggest the occurrence of natural transmission of heterologous rotaviruses from animals to humans through direct infections of whole virions or through a reassortment event [6, 12–14]. Others type specificities are more common in humans and infrequently detected in animals, which could suggest a reverse transmission route for human strains to animals [15, 16]. However, the ecological and epidemiological background of interspecies transmission events are not well described for RVA, a finding suggesting that simultaneous surveillance of RVAs in humans and animals is needed.

Materials and Methods

A pilot study on the occurrence of bovine RVA strains was conducted from January 2011 to April 2011 in Cameroon. Fecal samples were collected from diarrheic (n = 84) or healthy (n = 73) animals. All samples were screened individually by a VP6 gene specific one step RT-PCR assay [17]. The VP7 gene was amplified using primers published previously [18, 19]. Amplicons of the expected sizes were gel purified and then sequenced by using the sequencing primers. The cycle sequencing reaction was carried out with the ABI PRISM BigDye Terminator v1.1 Cycle Sequencing Reaction Kit (Applied Biosystems, Carlsbad, California) and sequence data were collected by means of an automated DNA analyzer ABI3500. Resulting sequences were subjected to Blast search to find highest similarities with reference sequences. Sequences were subsequently aligned and phylogenetic analyses were done with the MEGA5 software [20] using the neighbor-joining method and Kimura-2 parameter supported with bootstrapping (1000 replicates). Reference strains were obtained from GenBank.

Results and Discussion

Out of 157 bovine fecal specimens, four (2.5%) tested positive for RVA by the diagnostic RT-PCR assay, each identified in asymptomatic animals [17]. All four bovine RVA strains, had G12 VP7 gene specificity, and shared up to 100% nucleotide identity. The nucleotide identity values along a ~410 nt fragment of the VP7 gene between the four bovine strains and numerous international human strains whose gene sequences were available in GenBank was >99%. Essentially equivalent nucleotide similarity values were found with the VP7 gene sequences of human G12 RVA strains detected in the same period and areas in Cameroon (Fig. 1). In contrast, lower similarities were seen with an early human G12 isolate (L26; nt 88.9%–89.7%) and a porcine G12 rotavirus (RU172; nt 90.9%–91.2%%). Phylogenetic analysis placed all four bovine strains into the widespread modern lineage of G12. In addition, the Cameroonian bovine G12 RVA strains formed a common cluster with Cameroonian human G12 strains, which was the epidemiologically major RVA serotype in the North West and Far north regions of the country during 2010–2011 [21].

From a historical perspective, detection of G12 strains dates back to the 1980–1990s. The human strain, L26, was the first G12 strain detected in the Philippines in 1987 [22]. Subsequently, modern human G12 strains have been found

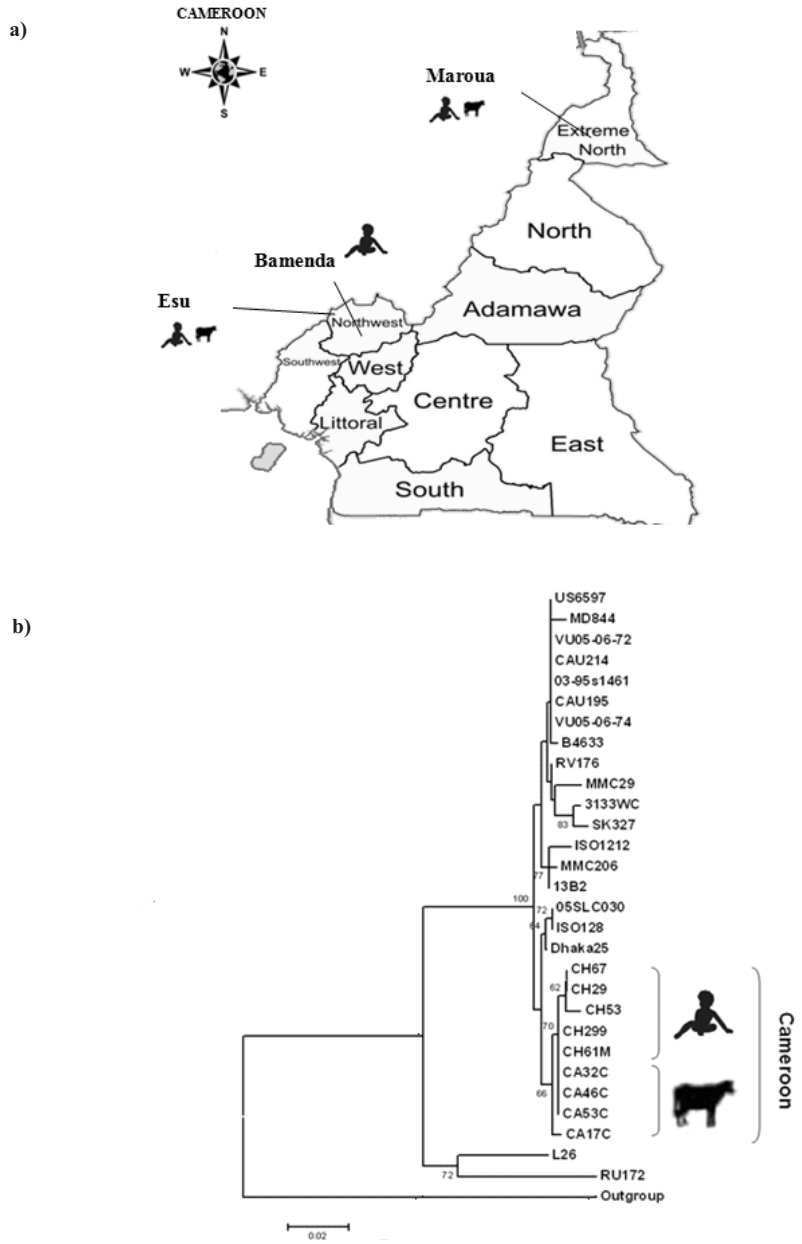

Figure 1. (a) Map of Cameroon showing human and bovine rotavirus genotype G12 distribution (extreme left), and (b) phylogenetic tree generated by neighbor joining using ~410 nt fragment of VP7 of the four G12 bovine RVA (CA32C, CA46C, CA53C and CA17C) showing the close relationship with the representative modern human G12 RVA (CH61M, CH299, CH53, CH29, CH67) in Esu, Bamenda and Maroua (extreme right). Scale bar at the bottom of tree is proportional to the genetic distance

circulating in numerous countries worldwide [21, 23–31]. In contrast, the number of countries reporting the occurrence of G12 in animals remained low. A porcine strain, RU172, was detected in India during 2002, although this strain was found genetically divergent from the modern human G12 lineage. Furthermore, G12 strains were detected by genotyping PCR assay in pigs and cattle in Spain and Denmark using primer sets designed to detect and genotype modern lineages of human G12 VP7 genes [32]. However, sequence data were not collected from these European G12 strains [32] and the species origin of the modern lineage of the G12 VP7 remains unknown [33].

This study reports some intriguing findings. Our sequencing based genotyping strategy allowed unambiguous typing of four bovine RVA strains into G12. This is particularly important given that a G12 VP7 specific primer was shown to misidentify genotype G6 RVA strains in human fecal samples collected from Hungarian children, indicating that confirmation of genotyping PCR results by sequencing is needed [34]. The Cameroonian bovine G12 VP7 gene was genetically related to the globally spread variant of human G12 strains, including human strains co-circulating in the area where these animal strains were identified during 2010–2011. Given that the modern lineage of G12 VP7 is considered a true human genotype specificity and there are no confirmed cases of natural infection of cattle with this particular genetic variant, we hypothesize that in Cameroon we found evidence for human-to-animal transmission of the modern lineage of genotype G12 RVAs. Cattle in parts of Cameroon appear not only susceptible to infection with G12 strains but also may serve as reservoirs for human infection.

In summary, more recent molecular evolutionary analyses have demonstrated that RVA strains carrying the modern genetic lineage of human G12 VP7 gene emerged during late 1990s and early 2000s as a result of interspecies transmission. Our data suggests that such interspecies transmission events of G12 RVA strains may be bi- or multidirectional and may occur more frequently in areas where humans and domesticated animals live in close contact. More structured surveillance is needed to see interspecies transmission dynamics of RVAs in Cameroon.

Acknowledgements

We acknowledge the technical assistance of Anett Horváth at the Pathogen Discovery unit, Institute for Veterinary Medical Research, Budapest, Hungary. We also acknowledge our colleagues in Cameroon for helping in sample collection (Tang Wilfred and Zang Magdaleine at the Esu Intergreted Health Center,

Ngu Felix at the Regional Hospital, Bamenda, and Dr Konyuy Emmanuel at the ELBA, range Esu). S.L.F. was supported by the János Bolyai Research Scholarship. K.B. was supported by the Hungarian Academy of Sciences (Momentum program, OTKA-PD76364, OTKA-K100727).

Conflict of interest statement

The authors report no financial and personal relationships with other people or organizations that could influence the content of the paper.

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