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Evidence Against GB virus C Infection in Dromedary Camels

Jack T. Stapleton[1,*], **Donald B. Smith**[2], and **Peter Simmonds**[2]

[1]Department of Internal Medicine, Veterans Administration Medical Center and the University of Iowa, Iowa City, IA, USA

[2]Centre for Infectious Diseases, University of Edinburgh, Edinburgh, UK

Abstract

A recent publication described finding GB virus C (GBV-C) RNA in four of twenty two dromedary camel sera, and sequence analysis found that these viruses were phylogenetically clustered within human GBV-C isolates. Since all other GB viruses to date form monophyletic groups according to their host species, the close relationship between the sequences generated from camel sera and human GBV-C isolates seemed implausible, leading us to conduct an independent analysis of the sequences. Our investigation found three lines of evidence arguing against GBV-C infection in dromedary camels. First, strong evidence of artifactual sequence generation was identified for some of the sequences. Secondly, the sequence diversity within individual camel sera was ten- to one-hundred fifty two-fold greater than that described for GBV-C within a human host. Finally, GBV-C sequences generated from each camel shared near complete identity with human isolates previously described by the same laboratory. Taken together, these data strongly suggest laboratory contamination. We suggest that additional validation experiments are needed before it is possible to conclude that camels are permissive for GBV-C infection.

Keywords

GB virus; Flavirirus; Dromedary Camel

Introduction

Five different GB viruses have been described: GB virus A (GBV-A) and GBV-B from New world primates, GBV-C from humans, GBV-D in bats, and a variant of GBV-C in chimpanzees (GBV-Ccpz) (Simons *et al.*, 1995, Bukh *et al*., 1997, Birkenmeyer et al., 1998, Adams *et al.*, 1998, Epstein *et al.*, 2010, Mohr and Stapleton, 2011, Stapleton *et al*., 2011). GBV-B is a hepatitis virus, and is proposed to be classified as a member of the *Hepacivirus* genus within the family *Flaviviridae*. GBV-A, GBV-C, GBV-Ccpz and GBV-D are also members of the *Flaviviridae*. However, they are phylogenetically distinct from the three currently assigned genera and have recently been proposed to be members of a newly designated *Pegivirus* genus (Stapleton *et al*., 2011). Analyses of all of the GB viruses have determined that they segregate into monophyletic groups according to their host species,

[*]Corresponding author address: Department of Internal Medicine, SW54,4 GH, The University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242, USA, Phone: 1-319-356-3168; Fax: 1-319-356-4600, jack-stapleton@uiowa.edu.

supporting a hypothesis for their evolutionary co-speciation with their hosts (Gonzalez-Perez,, 1997, Pavesi, 2001, Smith *et al.*, 1997, Smith *et al.*, 2000, Stapleton *et al.*, 2011).

A recent article reported the detection of GBV-C like viruses in dromedary camel sera (Odeh, 2011), a finding that contrasts markedly with the exclusive detection of GBV-C in humans and chimpanzees to date. The pattern of phylogenetic clustering of the sequences obtained from camel sera within the human GBV-C group was even more astonishing, with some sequences similar or identical to human-derived GBV-C variants and others divergent from human, chimpanzee and other camel-derived sequences. In this study, we report the results of our own analysis of the sequences recovered from the camel sera. Our findings cast considerable doubt on the conclusion that camels are permissive for GBV-C infection.

Methods

Nineteen published 5'untranslated region (UTR) sequences generated from four dromedary came sera were used for analysis (Odeh, 2011). The four camels were identified as H11, H12, H13, and H15, and the sequences were GQ352236 GQ352254 (GenBank accession numbers). These sequences were manually aligned with nine human GBV-C 5'UTR sequences reported by the same laboratory (GQ273923-GQ273932), and forty complete GBV-C genome sequences (AB003288, AB0032389, AB003290, AB003292, AB003293, AB008335, AB008336, AB008342, AB013500, AB013501, AB018667, AB021287, AF006500, AF031827, AF031828, AF031829, AF081782, AF104403, AF121950, AF309966, AY196904, AY949771, D87255, D87262, D87263, D87708 D87715, D90600, D90601, HGU35380, HGU45966, HGU63715, HGU75356, HGU94695). A phylogenetic tree was generated using the neighbor-joining method and bootstrap re-sampling as implemented in the MEGA4 package (Tamura *et al.*, 2007).

Results

Three sequences from camel H12 shared less than 90% similarity with GBV-C genotype 2 as described (Odeh, 2011). Direct examination of these sequences show that all appear to be artifacts comprised of primer concatamers. In addition, a BLAST search of Genbank revealed that the sequence GQ352243 is identical to a human GBV-C isolate from Kuwait previously reported by the same laboratory (GenBank accession number GQ273932) (Odeh *et al.*, 2010). Both these sequences have the structure: inner sense primer (IS) followed by part of the outer sense primer (OS), followed by IS, OS, part of the inner antisense (IAS), part of the outer antisense (OAS), then IAS, IAS, IAS, OAS (part) and IAS. GQ352243 then continues with a further two copies of the IAS primer. The other two divergent H12 isolates (GQ352241, GQ352242) differed from each other, but both contained primer sequences in the order OS, IAS, OAS, IAS, IAS, and IAS. GQ273927, another human GBV-C sequence obtained from the serum of a Jordanian published by the same laboratory is also a primer concatamer (Odeh *et al*., 2010). Finally, another sequence generated from the camel sera (GQ352245) has two copies of the IAS primer at the end. Hence, all three divergent camel sequences, another reported sequence from the camel sera, and two previously reported human GBV-C sequences appear to contain PCR artifacts.

The remaining sequences reported from the four camel sera cluster with human GBV-C isolates. Analysis of these sequences reveals high degrees of similarity or identity to human-derived GBV-C sequences. A group of seven of the camel sequences cluster with a human (Kuwaiti) GBV-C isolate previously reported by the same laboratory (GQ273926) (Figure 1, arrow A). Another two camel-derived sequences (GQ352237, GQ352240; Figure 1, arrow B) are shown in (Odeh, 2011) as being nearly identical to two isolates obtained from humans from the United Arab Emirates (UAE3, UAE4 and UAE5) that were also reported

by the same laboratory (Odeh *et al.*, 2005). Unfortunately, these UAE sequences have not been provided in the original or subsequent publications (Odeh *et al.*, 2005, Odeh, 2011) and are not available on Genbank, which means that they can not be included in our phylogenetic analysis. A third closely related group of seven sequences derived from the camel sera are identical to a sequence from a human from Germany (GU440670), Burma (AB018662), and others (data not shown), and differ at a single nucleotide position from a French isolate (AF104403) (Figure 1, arrow C). Conservation of these human sequences from diverse geographic locations suggests that this sequence is highly conserved among human isolates.

The reported GBV-C diversity values within a single human were 0.23% (Shao *et al.*, 2007). In contrast, the nucleotide sequence diversity of the GBV-C sequences amplified within each of the four camel were 4.3% (H11), 35% (H12), 3% (H13), and 4.2% (H15) using the maximum composite likelihood distance model (MEGA4; Tamura *et al.*, 2007). Excluding the three divergent sequences and artifactual sequences reported for animal H12, the sequence diversity was 2.3%. Thus, within a given came sera, the GBV-C sequence diversity was 10- to 152-fold higher than described for GBV-C within an individual human host.

Discussion

All of the GB viruses reported to date show close phylogenetic relationships with other isolates obtained from the same host species, suggesting that GB viruses co-evolve with their host species (Gonzalez-Perez,, 1997, Pavesi, 2001, Smith *et al.*, 1997, Smith *et al.*, 2000). Thus the report that dromedary camels have GB virus isolates that cluster within GB viruses of humans (GBV-C) was quite unexpected. To address this we re-analyzed the sequences and compared them with additional human sequences. We found that the three most divergent sequences reported (Odeh, 2011) aligned with a human isolate reported by the same laboratory in 2010, and that all four of these sequences contained concatemers of PCR primer sequences, presumably artifacts generated during PCR amplification. All of the remaining putative camel isolate sequences were reported to phylogenetically cluster within GBV-C genotype 2 (Odeh, 2011). Seven of these sequences group closely with a human GBV-C sequence cloned and reported by the same laboratory, while another two isolates are closely related to three unpublished human GBV-C cloned sequences from the same laboratory. This clustering is consistent with contamination of PCR reactions. Finally, the sequence diversity of products amplified from all of the four camel sera is greater than normally seen in humans, and in several cases a sequence from one camel was more closely related to GB virus sequences from different camels than to sequences derived from the same camel.

Taken together, these data strongly suggest that the findings of Odeh are most consistent with laboratory contamination of PCR reactions with plasmids containing cloned GBV-C sequences, some of which are artifacts generated by concatenation of primers during PCR. Prevention of nucleic acid contamination generated by nested RT-PCR assays performed in the same laboratory is extremely difficult (Wolk *et al.*, 2001). We would suggest that additional validation experiments are needed before one can reach the conclusion that camels are permissive for GBV-C infection.

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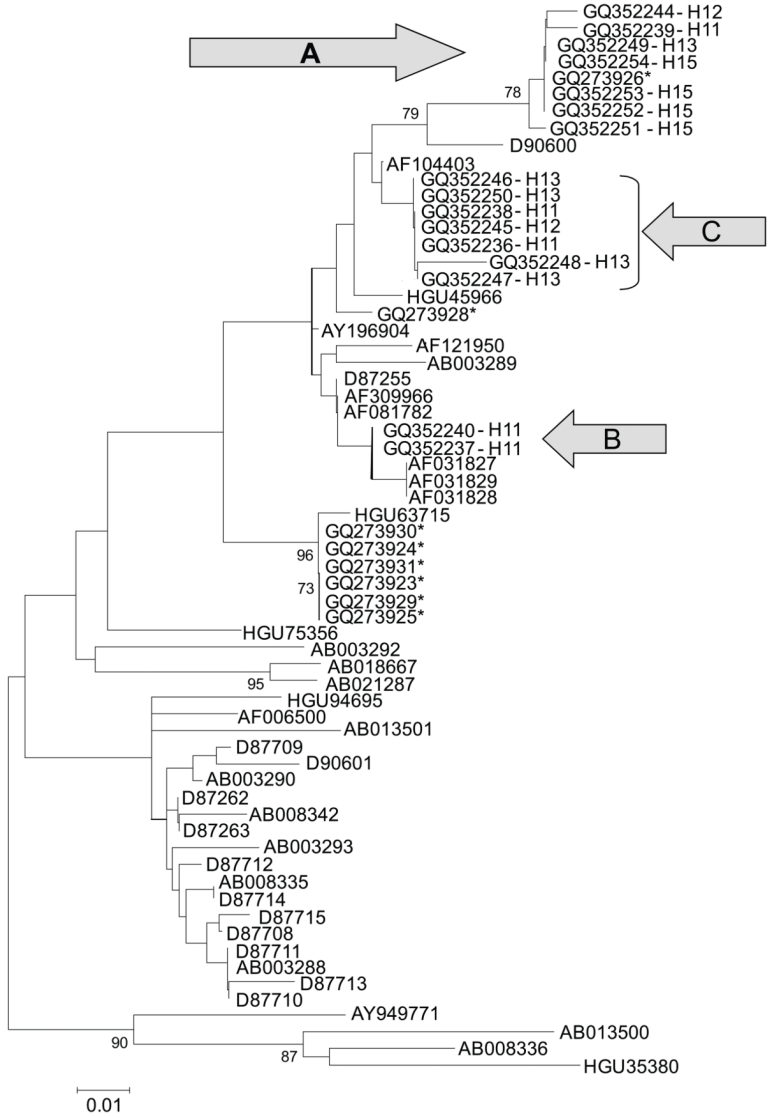

Figure 1. Phylogenetic analysis of putative GB virus sequences from dromedary camels

Phylogenetic analysis of sixteen 5'untranslated sequences (160 nt) generated from four camel sera (H11, H12, H13, H15), forty GBV-C complete genome sequences obtained from human sera, and eight human GBV-C sequences previously reported by Odeh et al. (noted by asterix [*]). GenBank accession numbers are provided and the isolates generated from camel sera show the camel number from which they were derived. Bootstrap support is indicated (1000 replicates).