JVI Journals.ASM.org

Novel Hybrid Parvovirus-Like Virus, NIH-CQV/PHV, Contaminants in Silica Column-Based Nucleic Acid Extraction Kits

Heidi Smuts,[a,b] Michael Kew,[c] Aabida Khan,[a,b] Stephen Korsman[a,b]

Division of Medical Virology, University of Cape Town,[a] National Health Laboratory Service, Groote Schuur Hospital, University of Cape Town,[b] and Department of Medicine, University of Cape Town,[c] Cape Town, South Africa

The discovery of a novel hybrid parvovirus-like virus, NIH-CQV, in non-A to -E (non-A-E) hepatitis virus samples by Xu et al. (1) as a possible causative agent of seronegative hepatitis prompted our investigation of samples collected from patients with a range of liver diseases, including acute hepatitis, chronic hepatitis, cirrhosis, hepatocellular carcinoma, and amoebic liver disease ($n = 48$) as well as 40 healthy individuals. Nucleic acid from these 88 samples was extracted in 2009 using the silica column-based QIAamp viral RNA Mini Kit (Qiagen). A further 112 samples from patients with non-A-C hepatitis, with nucleic acid extracted in 2012-2013 using the NucliSENS easyMag platform (bioMérieux), were also screened. A nested PCR, designed with primers targeting the rep gene of NIH-CQV (1), generated a 176-bp fragment. Inner primers used were those reported by Xu et al. (1); the outer primers used were OF (5′-GTCTTTTAGAAAG TTTCCACG) and OR (5′-CGATCGAAGAATCGGTTCTC).

Screening of the liver disease samples showed that 46 of 48 (96%) were strongly positive for NIH-CQV, while 80% of the healthy controls (32 of 40) also showed evidence of the novel virus. In contrast, only 4 of 112 non-A-C hepatitis samples (3.7%) were weakly positive. These results suggested that the samples extracted using the Qiagen kit were contaminated, which was confirmed when parallel extraction in 2013 of 12 random samples with Qiagen and easyMag kits showed that only samples extracted using the silica column-based method were positive. Reagents from three Qiagen kits were examined, and only water spun through the silica column was positive. Sequencing of PCR products showed 100% identity to NIH-CQV.

Testing of an additional four silica-based magnetic platforms—MagNA Pure LC (Roche), MagNA Pure Compact (Roche), COBAS AmpliPrep/COBAS TaqMan (Roche), and Abbott m2000sp (Abbott)—failed to amplify NIV-CQV from 10 replicates (each) of water.

Naccache et al. (2), identifying a similar parvovirus-like hybrid virus, PHV, confirmed our suspicions that the Qiagen silica columns were the source of the contamination. However, in contrast to the results from these authors, our results show that the Qiagen kits manufactured before 2012 were contaminated, as the nucleic acid used in our study had been extracted in 2009. Further, we screened stored nucleic acid from respiratory samples ($n = 24$) extracted in 2004-2005 using the silica column-based viral RNA Seek kit manufactured by Talent in Trieste, Italy. These samples showed a similar contamination with NIH-CQV/PHV, with 88% of samples testing positive (21 of 24).

The detection of NIH CQV/PHV sequences only in metagenomic databases of ocean waters off the coast of North America led Naccache et al. (2) to suggest that the silica contamination may be time dependent and geographically specific. Our results suggest that this inadvertent contamination is not time dependent, as samples extracted in 2004, 2005, 2009, and 2013 showed contamination. The source of the silica in the manufacture of the Talent kits is not known; thus, geographical specificity of this possible virus of diatoms may be plausible. Our study further confirms that when a novel virus is discovered, caution about making specific associations with clinical disease needs to be taken. Laboratory and reagent contamination must be considered.

REFERENCES

1. **Xu B, Zhi N, Hu G, Wan Z, Zheng X, Liu X, Wong S, Kajigaya S, Zhao K, Mao Q, Young N.** 2013. Hybrid DNA virus in Chinese patients with seronegative hepatitis discovered by deep sequencing. Proc. Natl. Acad. Sci. U. S. A. **110:**10264–10269. http://dx.doi.org/10.1073/pnas.1303744110.
2. **Naccache SM, Greninger AL, Lee D, Coffey LL, Phan T, Rein-Weston A, Aronsohn A, Hackett J, Jr, Delwart EL, Chiu CY.** 2013. The perils of pathogen discovery: origin of a novel parvovirus-like hybrid genome traced to nucleic acid extraction spin columns. J. Virol. **87:**11966–11977. http://dx.doi.org/10.1128/JVI.02323-13.

Published ahead of print 11 December 2013

Address correspondence to Heidi Smuts, Heidi.Smuts@uct.ac.za.

doi:10.1128/JVI.03206-13