LETTER

Concerns over the origin of NIH-CQV, a novel virus discovered in Chinese patients with seronegative hepatitis

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We read with great interest the PNAS article by Xu et al. reporting the discovery of National Institutes of Health–Chongqing virus (NIH-CQV), a novel circovirus-parvovirus hybrid DNA virus found in patients with seronegative hepatitis by next-generation sequencing (NGS) (1). Around the same time, we had also used NGS to discover and sequence the full genome of a similar hybrid DNA virus from clinical samples that we named "parvo-like hybrid virus" (PHV). However, on further investigation, we eventually tracked the origin of PHV to nucleic acid extraction spin columns produced by a single manufacturer (Qiagen). When the genome sequence of NIH-CQV was publicly released, we found, to our surprise, that all 12 PHV strains that we had previously identified were the same as NIH-CQV, sharing >98% nucleotide and 100% amino acid sequence identity.

As described in detail in Naccache et al. (2), published ahead of print in *Journal of Virology* on September 11, 2013, our data strongly indicate that PHV and NIH-CQV are viral contaminants of spin columns used for nucleic acid extraction and not bona fide infectious agents of humans. PHV/NIH-CQV sequences were unexpectedly detected in multiple clinical cohorts and sample types from two independent laboratories from as early as 2008, raising suspicion that this novel virus was a laboratory-derived contaminant. This was subsequently confirmed by PCR and NGS analysis of mock water controls eluted through contaminated spin columns.

How did the spin columns became contaminated with PHV/NIH-CQV? Interestingly, data mining of environmental sequence databases found PHV/NIH-CQV sequences in the coastal waters of the Pacific Ocean. The silica used in nearly all commercial spin columns is derived from the cell walls of diatoms (algae). We postulate that PHV may be a diatom virus that had inadvertently contaminated the silica-based spin columns during manufacture.

NIH-CQV was only found in 63 of 90 Chinese patients with seronegative hepatitis and was absent in 45 healthy controls in the study by Xu et al. (1). These PCR results may be due to lot-to-lot variability in the degree of spin column contamination by NIH-CQV or the use of different nucleic acid extraction methods for cases and controls. The discordance between 0% PCR positivity for NIH-CQV in healthy controls yet comparable rates of IgG antibody positivity to hepatitis patients is another striking yet unexpected finding. The serological data suggesting reactivity to a single NIH-CQV antigenic epitope in Chinese hepatitis may potentially be explained by cross-reactivity in the assay.

Given the high sensitivity of NGS for microbial detection, rigorous measures are required to minimize the impact of contamination, which can confound interpretation of results. These include (*i*) blinded processing of cases and controls, (*ii*) liberal use of negative water and reagent controls, (*iii*) analysis of multiple sample datasets distributed over time and space, (*iv*) replication studies in independent laboratories, and (*v*) development of nucleic acid-free reagents. The identification of a novel virus with the potential for blood-borne transmission such as NIH-CQV also has significant clinical and public health implications. Thus, it is critical that investigative studies on the validity of newly discovered pathogens be performed and reported in a timely fashion (1, 3).

Note Added in Proof. In an independent research study (4) published in December 2013, Smuts et al. reported contamination from NIH-CQV in silica column-based kits from a different manufacturer (Talent Srl.) in Trieste, Italy, supporting the finding that NIH-CQV is indeed a laboratory contaminant.

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1 Xu B, et al. (2013) Hybrid DNA virus in Chinese patients with seronegative hepatitis discovered by deep sequencing. *Proc Natl Acad Sci USA* 110(25):10264–10269.
2 Naccache SN, et al. (2013) The perils of pathogen discovery: Origin of a novel parvovirus-like hybrid genome traced to nucleic acid extraction spin columns. *J Virol* 87(22): 11966–11977.
3 Lee D, et al. (2012) In-depth investigation of archival and prospectively collected samples reveals no evidence for XMRV infection in prostate cancer. *PLoS ONE* 7(9):e44954.
4 Smuts H, Kew M, Khan A, Korsman S (2014) Novel hybrid parvovirus-like virus, NIH-CQV/PHV, contaminants in silica column-based nucleic acid extraction kits. *J Virol* 88(2):1398.

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The authors declare no conflict of interest.

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