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Questions about Zhou et al. Addendum in Nature

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Sat, Nov 28, 2020 at 1:42 PM

To: zlshi@wh.iov.cn

Cc: Gary Ruskin <gary@usrtk.org>

Dear Dr. Shi,

I work with the public interest nonprofit [U.S. Right to Know](#) to help increase public awareness about what is known [about the origins of SARS-CoV-2](#). I am writing to ask for your response to concerns about the veracity and reliability of an ["Addendum"](#) published on November 17, 2020 by you and your co-authors (Zhou et al.). The addendum is linked to your [paper](#) published on February 3, 2020 titled "A pneumonia outbreak associated with a new coronavirus of probable bat origin."

By way of context, the addendum seems to address [concerns](#) that have been raised about [data discrepancies](#) in Zhou et al.'s original claims about RaTG13. In the February 3 paper, Zhou et al. from the Wuhan Institute of Virology (WIV) stated: "We then found that a short region of RNA-dependent RNA polymerase (RdRp) from a bat coronavirus (BatCoV RaTG13)—which was previously detected in *Rhinolophus affinis* from Yunnan province—showed high sequence identity to 2019-nCoV. We carried out full-length sequencing on this RNA sample (GISAID accession number EPI_ISL_402131)." This statement made it appear to readers that the authors conducted full sequencing of RaTG13 only after they had found a high sequence match between SARS-CoV-2 and a partial RdRp sequence of the bat coronavirus. More than 3 months after the publication of Zhou et al., when you and your co-authors finally released amplicon sequence data, it became clear that WIV had in fact fully sequenced the genome of RaTG13, which they called BtCoV 4991, in 2018.

Importantly, you and your WIV colleagues [reported](#) in 2016 that BtCoV 4991 was discovered "in an abandoned mineshaft in Mojiang County, Yunnan Province" in 2012-2013, and BtCoV 4991 was the only SARS-like bat coronavirus. Zhou et al. did not acknowledge, until they published their addendum, that RaTG13 was found in the very same mineshaft, where 6 miners had experienced acute respiratory distress syndrome in 2012 after exposure to bat feces, and 3 had died. Xu (2013) [reported](#) that WIV testing showed patient serum samples to be IgM positive—suggesting a viral infection. Huang (2016) [reported](#) that WIV blood test results showed 4 of the patients as testing positive for SARS virus IgG antibodies, which suggests a SARS-like virus infection.

After more than 9 months of controversy about the accuracy and veracity of Zhou et al.'s RaTG13 dataset, the public has been presented with an addendum that raises more questions about missing data and data discrepancies:

1. Did you and your co-authors provide data and associated methods to *Nature* to support the claims you made in the addendum? If so, why didn't *Nature* publish those along with the addendum?
2. Why did you not provide information in your original February 3 *Nature* publication about the link between RaTG13 and the Mojiang mineshaft where 6 miners had experienced severe respiratory disease after exposure to bat feces?
3. Zhou et al. claimed they took serum samples from the patients in question and tested these using PCR methods and antibody-binding methods for the presence of Ebola virus, Nipah virus and bat SARSr-CoV Rp3. What positive and negative controls were used to prove they were not getting false negative or false positive results? How were the patients' sera samples stored and how were they prepared for the PCR tests and antibody-binding? At what temperatures were the 13 serum samples stored and utilized for these various assays?
4. Zhou et al. claimed they used their own "validated enzyme-linked immunosorbent assay (ELSA)" to test for the presence of SARS-CoV-2 in the serum samples. What was the reference for the in-house validation of their ELSA? Where are the plots of the ELSA results?

5. Zhou et al. stated that they discovered 9 SARS-like betacoronaviruses from the abandoned mineshaft in Mojiang County, Yunnan Province. In the [2016 publication](#) Zhou et al. cite, the only SARS-like betacoronavirus reported was RaTG13 (BtCoV 4991). Why hasn't your research group released any information about the full genome sequences of the other 8 SARS-like betacoronaviruses?
6. Apart from the 13 serum samples, does the WIV store any other kinds of samples of the Mojiang shaft miners, and if so, did Zhou et al. conduct tests on any of those?
7. Why does the published sequence of RaTG13 not match the raw metagenomic and amplicon sequencing trace data?

Since Zhou et al. omitted key data about the history of RaTG13, failed to release amplicon data until 3 months later, and then proceeded to change the published genome sequence of RaTG13 without explanation, on what basis are the claims made by Zhou et al. in their addendum scientifically defensible and verifiable? **Zhou et al. should immediately publicly release all relevant supporting data for their addendum.**

I would appreciate your comments regarding Zhou et al.'s addendum.

Sincerely,

Sainath

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