Twitter Thread by Daoyu

Daoyu
@Daoyu15

https://t.co/hXlo8qgkD0

Look like that they got a classical case of PCR Cross-Contamination.
They had 2 fabricated samples (SRX9714436 and SRX9714921) on the same PCR run. Alongside with Lung07. They did not perform metagenomic sequencing on the "feces" and they did not get

A positive oral or anal swab from anywhere in their sampling. Feces came from anus and if these were positive the anal swabs must also be positive. Clearly it got there after the NA have been extracted and were from the very low-level degraded RNA which were mutagenized from

The Taq. https://t.co/yKXCgiT29w to see SRX9714921 and SRX9714436.
Human+Mouse in the positive SRA, human in both of them. Seeing human+mouse in identical proportions across 3 different sequencers (PRJNA573298, A22, SEX9714436) are pretty straight indication that the originals

Were already contaminated with Human and mouse from the very beginning, and that this contamination is due to dishonesty in the sample handling process which prescribe a spiking of samples in ACE2-HEK293T/A549, VERO E6 and Human lung xenograft mouse.

The "lineages" they claimed to have found aren't mutational lineages at all—all the mutations they see on these sequences were unique to that specific sequence, and are the result of RNA degradation and from the Taq polymerase errors accumulated from the nested PCR process

Where the extremely low error-ridden cross-contaminating molecule were amplified with mutagenesis happening on each step—especially between the initial RT-PCR and sequencing PCR process, which cross contamination have happened. Notice that any extra Pro or Cys on the highly

Conserved N protein sequences are lethal to the virus—especially the extra Cys which will become a nucleophile and a Ribonuclease in the budded virion in an extracellular environment, degrading the genome and inactivating the virus immediately. These Cys will also cause the N to

Dimerize and consequently lose it's ability to bind RNA or mediate virion assembly in the first place. While the Pro will break a conserved secondary structure in the C-terminal domain and prevent the N from folding correctly. These are not found anywhere else and are obviously

Sequencing errors. Especially since these samples came from cross-contamination without a corresponding positive oral or anal swab (they didn't get a single swab that is positive).

M1, they cite as having "hemorrhage" claimed to be Coronavirus symptoms, are anything BUT coronavirus symptoms. https://t.co/3a8nqY6rOz "Open in new tab
X-ray tests revealed a large area of shadow in the left lung of pangolin 2-Lishui, suggestive of pneumonia" but

"we have not found coronaviruses in these pangolins by meta-transcriptomic and PCR methods.". Notice the PLT- in the Pestivirus-infected pangolin samples. Pestivirus is seen in the SRA of M1, SRX7756769 (107267359 spots as in the Xiao et al article). The Pestivirus is seen

In all PRJNA573298 sample lungs, and it causes interstitial pneumonia and gives the same kind of CT scan and blood test result as in the 06/22 article. This sample also contained a locally circulating CCoV (Canine Coronavirus, often infects zoo/rescued solitary animals due to

It's ubiquitous nature in most human-associated environments) strain with reads that weren't rolling circles as the GD-1 reads and are mostly related to the Canine Coronavirus isolates circulating in dogs in GZ.

Coronavirus infections can not generate rolling circles as they does not encode RNA ligases, an enzyme that is necessary to create a true CircRNA. (What they claim to be CircRNA reads from CoV infections in other papers aren't true CircRNA but are Partially duplicated RNA due to

The Coronavirus RdRp template-switching to a location before it's current location. This RNA is still linear. However, the Rolling Circles corresponds to two hotspots of M13 primers seen in the amplicons for nsp3 and for the 3'-end of the genome. This imply that they were

Remnants of cDNA cloning, stemming from DNA that were circularized without a vector backbone.)

Canine Coronavirus and FIPV (alphacoronavirus 1) infections also generate very similar symptoms, and will result in positive cross-reactive NP in the immunofluorescence screening. Even the right GEO profile provide they remove it from the final SRA. Finally, the ACE2 expression

"Lowest in the lungs" claimed in this paper is at odd with the 06/22 paper claim that "ACE2 and TMPRSS2 is most highly expressed in the lungs". This suggest that the GEO profile of the 06/22 article associated with A22/SRX8582289 have been fabricated or were derived from hACE2

Mouse. The hemorrhage seen in M1 is consistent with DYPV(Flavivirus/pestivirus related to BVDV) which is seen in the metagenome, while the pneumonia could be either be the result of DYPV-related Pestivirus, from the Sendai (murine respirovirus), from the Canine Coronavirus or

From any of the non-Coronavirus (Colitivirus or Murine Respirovirus) agents found in PRJNA573298.
M1 have Murine Respirovirus.
https://t.co/ZJnLSxwe5o
SeV is in the same family as human Parainfluenza virus. It causes bilateral ground-glass opacity.
https://t.co/NeY2ajwsDo

The blood tests—pestivirus once again fully explains all the blood changed observed. When a Parainfluenza virus is also present, the blood test become indistinguishable.

Another snRNA-seq say ACE2 and TMPRSS2 are barely exiressed in the lungs and are never coexpressed.

Weaker than the kidneys—this is again at odds with the SCAU claim that even negative pangolins have lung>>kidney for ACE2. Both publication say that Kidney is the highest ACE2 expressing organ in all pangolins where lung expression is negligible. The 06/22 article is the only

One that claim that all pangolins including negative samples, have lung ACE2 >> Kidney. This indicate that they have Spiked all of them with K12-hACE2 mouse as it is in disagreement of all previous or later pangolin transcriptomes.

“(Individual number: L08) as a positive control”—and guess what L08 actually is—Human AND Mice!

L08: Lung08.