Twitter Thread by Elisabeth Bik ■ 2/2

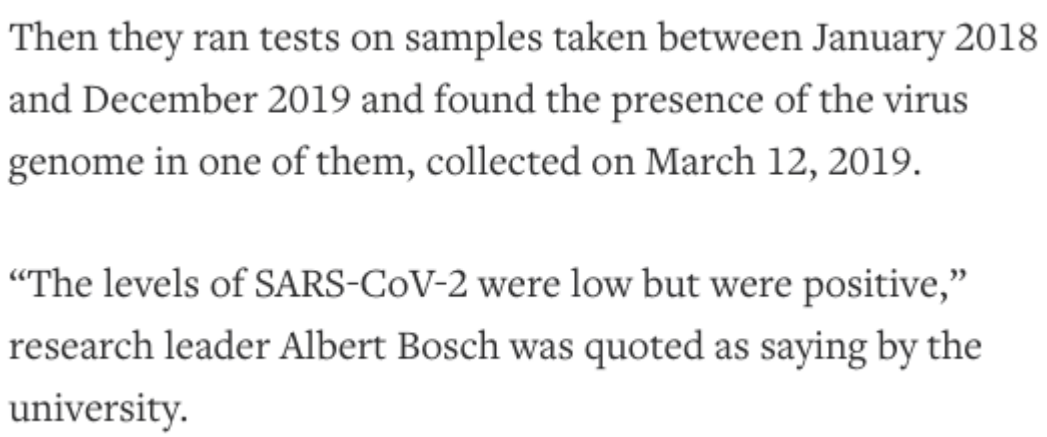
Elisabeth Bik ■ 2/2
@MicrobiomDigest

Several people asked me to say something about this rather fantastical claim, that SARS-CoV-2 might already have been lurking in Barcelona sewers in March 2019. TLDR: There is no solid evidence for this, just a possible false-positive/cross reactive RT-PCR. /

> Spanish virologists have found traces of the novel coronavirus in a sample of Barcelona waste water collected in March 2019, nine months before the COVID-19 disease was identified in China, the University of Barcelona says\xa0https://t.co/Ksl0slUnh5 pic.twitter.com/8XHm97rLV2
>
> — Reuters World (@ReutersWorld) June 26, 2020

The link that Reuters provided in their tweet is not working, but here is the correct link:
https://t.co/kY4CWLkytG
It is not a simple press release, but appears to have involved several journalists doing an investigation.

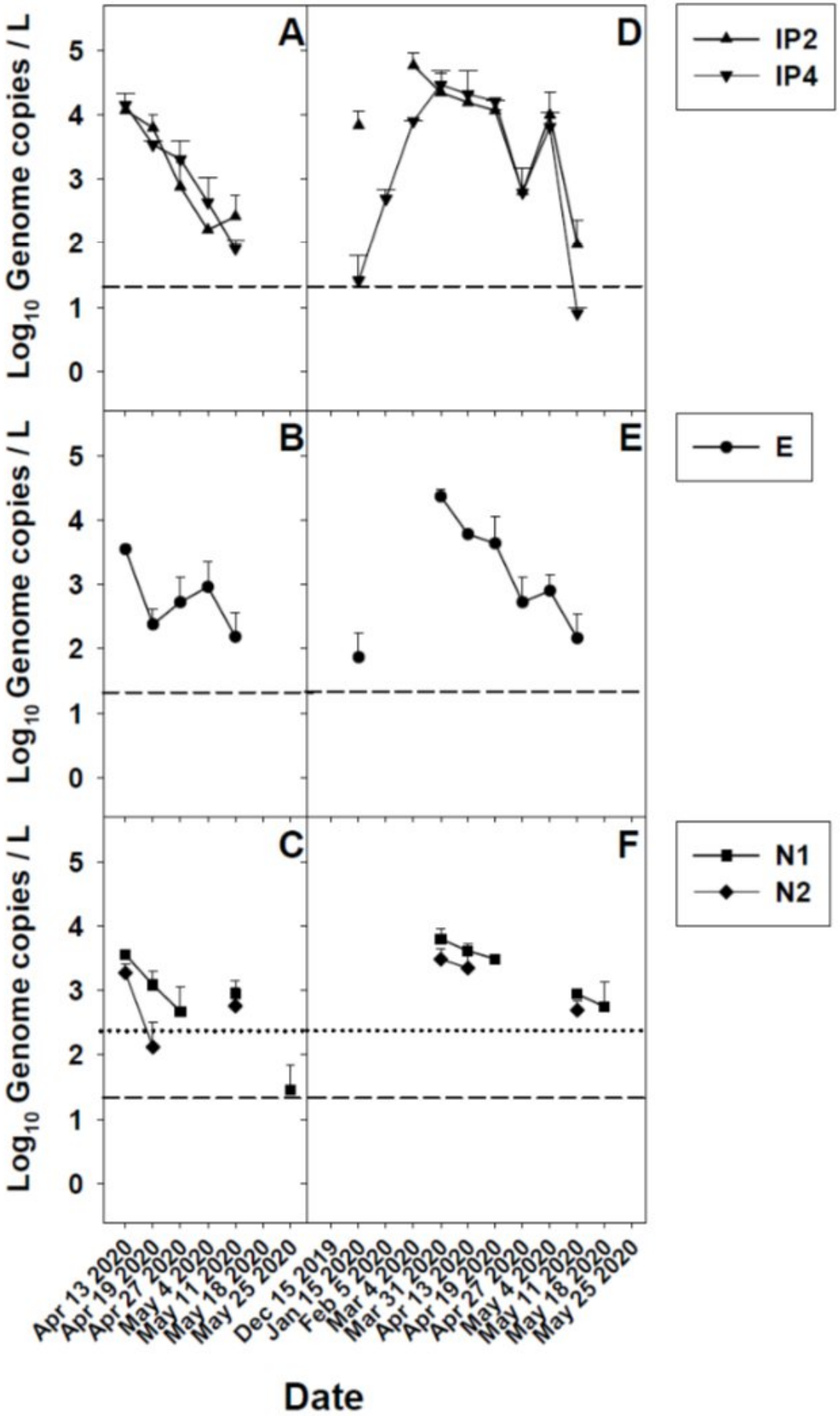
The Reuters article does mention that the results might be a bit preliminary. A scientist not involved with the study is quoted saying that it was "early to draw definitive conclusions". The authors however appear convinced that the finding was true.



So let's take a look at the preprint. This study has not been peer reviewed. It is a preprint on MedRxiv, so it needs to be interpreted this with extreme caution.
Reuters does not provide the link (why not?) but I did the searching for you. Here:
https://t.co/IIURYwkXbM

The study's initial intent was to see if the virus could be detected in Barcelona wastewater during the COVID-19 outbreak in early 2020, and it was indeed found starting in Jan 2020. That is cool, because wastewater screening is a nice surveillance method to sample a whole city.

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But then, the authors looked at some older samples, and one of these, from March 2019 (a year before the first COVID-19 cases in the city) appeared to be positive.

A

	IP2	IP4	E	N1	N2
January 16, 2018	No Ct	No Ct	No Ct	No Ct	No Ct
February 6, 2018	No Ct	No Ct	No Ct	No Ct	No Ct
March 6, 2018	No Ct	No Ct	No Ct	No Ct	No Ct
January 15, 2019	No Ct	No Ct	No Ct	No Ct	No Ct
March 12, 2019	**$6.4x10^2$ ***	**$8.3x10^2$ ***	No Ct	No Ct	No Ct
September 10, 2019	No Ct	No Ct	No Ct	No Ct	No Ct
October 2, 2019	No Ct	No Ct	No Ct	No Ct	No Ct
November 6, 2019	No Ct	No Ct	No Ct	No Ct	No Ct
December 11, 2019	No Ct	No Ct	No Ct	No Ct	No Ct

* Genome copies / L

It was just one sample, though, and it does not appear that the authors repeated the test at all. No replicates as far as I can tell.
But also note that only 2 of the 5 RT-PCR tests were positive, i.e. on the IP2 and IP4 genes.

Now, I am not a viral gene specialist, but I do know that some SARS-CoV-2 PCRs can give false positives. Even the WHO protocol, followed by the Barcelona group, states that IP2/IP4 cross-reactivity with other viruses can occur.
https://t.co/QliEutF5Ga

SPECIFICITY

Cross-reactivity with other respiratory viruses was tested with specimens known to be positive for a panel of respiratory viruses (influenza A(H1N1)pdm09, A(H3N2), B-Victoria, B-Yamagata; influenza C; RSV A, B; hBoV; hPIV; hMPV; HRV/enterovirus; adenovirus; hCoV (HKU1, OC43, 229E and NL63); MERS-CoV. None of the tested viruses showed reactivity with PCR2 and PCR4.

But the authors did not do any other target PCRs, or (even better) genomic sequencing. They did not do any confirmation to rule out cross-reactivity with some other virus.

This is why peer review is so important. Yes, peer review has many flaws, but it is better than nothing.
Without peer review, researchers might draw preliminary or incorrect conclusions. We all love our own data. But other researchers' critical reading is needed to find flaws.

A paper that makes a fantastic claim has to have fantastic data.
This is not the case here.

The authors need much more than just 2 positive PCRs to prove their claim. They need to provide e.g. a genomic sequence and absolutely rule out a contamination with a 2020 sample.