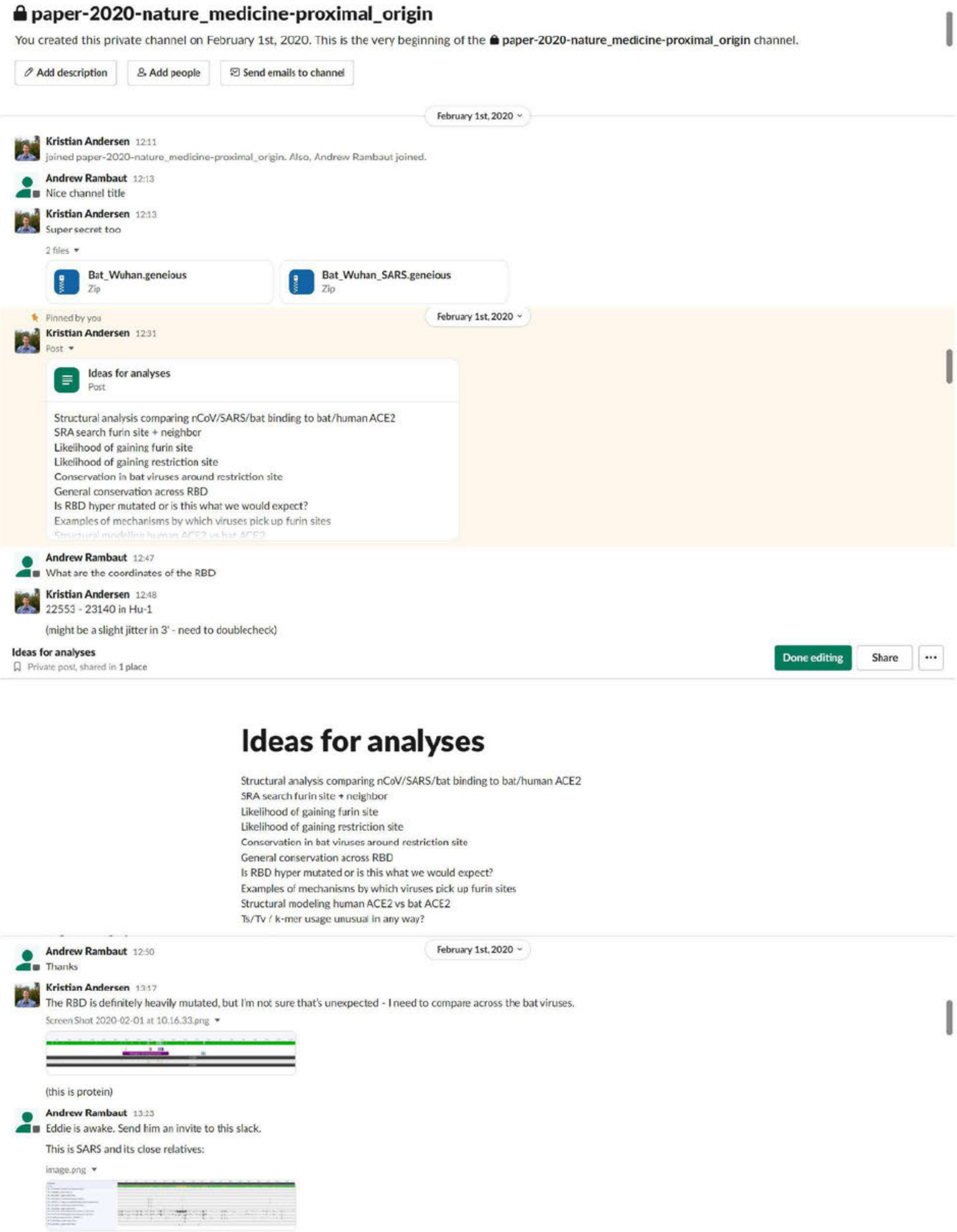


paper-2020-nature_medicine-proximal_origin

You created this private channel on February 1st, 2020. This is the very beginning of the **paper-2020-nature_medicine-proximal_origin** channel.

Kristian Andersen 12:11
joined paper-2020-nature_medicine-proximal_origin. Also, Andrew Rambaut joined.

Andrew Rambaut 12:13
Nice channel title

Kristian Andersen 12:13
Super secret too

2 files

Bat_Wuhan.geneious
Zip

Bat_Wuhan_SARS.geneious
Zip

Pinned by you

Kristian Andersen 12:31
Post

Ideas for analyses
Post

Structural analysis comparing nCoV/SARS/bat binding to bat/human ACE2
SRA search furin site + neighbor
Likelihood of gaining furin site
Likelihood of gaining restriction site
Conservation in bat viruses around restriction site
General conservation across RBD
Is RBD hyper mutated or is this what we would expect?
Examples of mechanisms by which viruses pick up furin sites
Structural modelling human ACE2 vs bat ACE2

Andrew Rambaut 12:47
What are the coordinates of the RBD

Kristian Andersen 12:48
22553 - 23140 in Hu-1

(might be a slight jitter in 3' - need to doublecheck)

Ideas for analyses
Private post, shared in 1 place

Ideas for analyses

Structural analysis comparing nCoV/SARS/bat binding to bat/human ACE2
SRA search furin site + neighbor
Likelihood of gaining furin site
Likelihood of gaining restriction site
Conservation in bat viruses around restriction site
General conservation across RBD
Is RBD hyper mutated or is this what we would expect?
Examples of mechanisms by which viruses pick up furin sites
Structural modeling human ACE2 vs bat ACE2
Ts/Tv / k-mer usage unusual in any way?

Andrew Rambaut 12:50
Thanks

Kristian Andersen 13:17
The RBD is definitely heavily mutated, but I'm not sure that's unexpected - I need to compare across the bat viruses.

Screen Shot 2020-02-01 at 10.16.33.png

(this is protein)

Andrew Rambaut 13:23
Eddie is awake. Send him an invite to this slack.

This is SARS and its close relatives:

image.png

The two bat ones are about as far away as RaTG13 is from Wuhan

February 1st, 2020

image.png ▾

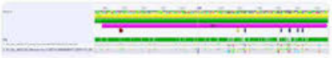
Kristian Andersen 13:28
Just invited Eddie

Eddie Holmes 13:30
joined paper-2020-nature_medicine-proximal_origin.

Eddie Holmes 13:30
Morning

Andrew Rambaut 13:30
nCoV vs RaTG13:

image.png ▾



Kristian Andersen 13:30

February 1st, 2020

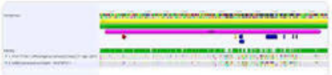
> The two bat ones are about as far away as RaTG13 is from Wuhan

Help me interpret. So distance between SARS and bat SARS-like is about the same as between RaTG13 and Wuhan?

Morning Eddie. Bright and early.

Do you have those comparisons just in protein space?

Andrew Rambaut 13:33
image.png ▾



Yes hold on a tick

Eddie Holmes 13:33
That's a great comparison!

Andrew Rambaut 13:33

February 1st, 2020

SARS:

image.png ▾

nCov:

image.png ▾

So not particularly heavily mutated.

Kristian Andersen 13:35
Good! These are very similar. What's the difference between SARS and that bat virus?

Andrew Rambaut 13:36
92.86% identity across spike for nCoV vs Bat, 92.03% for SARS vs bat

So I don't think the 'hypermutation' in RBS is a goer.

Kristian Andersen 13:37

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Agreed

It's hyper mutated, however, that region in general is hyper mutated - in other words, this is what we'd expect.

Andrew Rambaut 13:37
Yes.

Kristian Andersen 13:38
🍺

Andrew Rambaut 13:38
So cleavage site and restriction sites. Thoughts?

Kristian Andersen 13:38
I'm looking at cleavage site right now - lemme share alignment

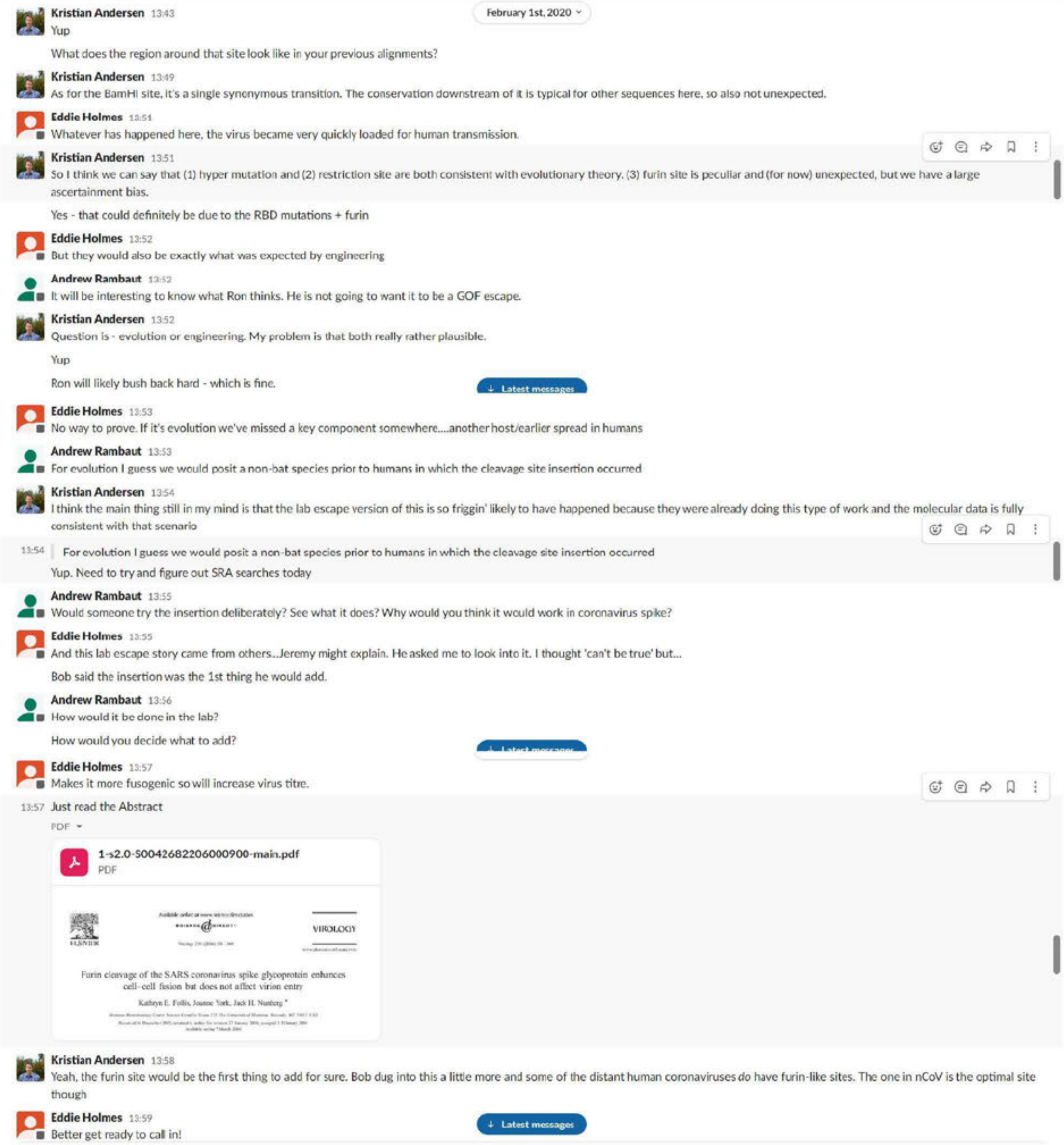
Zip ▾

Protein alignment.geneious
Zip

For this I took ~ 30 AAs flanking the furin site in nCoV and protein blasted it - then downloaded everything that came up and aligned everything. A lot of diversity around that site in general

Andrew Rambaut 13:42
RaTG13 is identical except for the 4 residue insertion.

[redacted]



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Kristian Andersen 13:43
Yup

What does the region around that site look like in your previous alignments?

Kristian Andersen 13:49
As for the BamHI site, it's a single synonymous transition. The conservation downstream of it is typical for other sequences here, so also not unexpected.

Eddie Holmes 13:51
Whatever has happened here, the virus became very quickly loaded for human transmission.

Kristian Andersen 13:51
So I think we can say that (1) hyper mutation and (2) restriction site are both consistent with evolutionary theory. (3) furin site is peculiar and (for now) unexpected, but we have a large ascertainment bias.

Yes - that could definitely be due to the RBD mutations + furin

Eddie Holmes 13:52
But they would also be exactly what was expected by engineering

Andrew Rambaut 13:52
It will be interesting to know what Ron thinks. He is not going to want it to be a GOF escape.

Kristian Andersen 13:52
Question is - evolution or engineering. My problem is that both really rather plausible.

Yup

Ron will likely bush back hard - which is fine.

Eddie Holmes 13:53
No way to prove. If it's evolution we've missed a key component somewhere....another host/earlier spread in humans

Andrew Rambaut 13:53
For evolution I guess we would posit a non-bat species prior to humans in which the cleavage site insertion occurred

Kristian Andersen 13:54
I think the main thing still in my mind is that the lab escape version of this is so friggin' likely to have happened because they were already doing this type of work and the molecular data is fully consistent with that scenario

13:54 > For evolution I guess we would posit a non-bat species prior to humans in which the cleavage site insertion occurred

Yup. Need to try and figure out SRA searches today

Andrew Rambaut 13:55
Would someone try the insertion deliberately? See what it does? Why would you think it would work in coronavirus spike?

Eddie Holmes 13:55
And this lab escape story came from others...Jeremy might explain. He asked me to look into it. I thought 'can't be true' but...

Bob said the insertion was the 1st thing he would add.

Andrew Rambaut 13:56
How would it be done in the lab?

How would you decide what to add?

Eddie Holmes 13:57
Makes it more fusogenic so will increase virus titre.

13:57 Just read the Abstract

PDF

1-s2.0-S0042682206000900-main.pdf
PDF

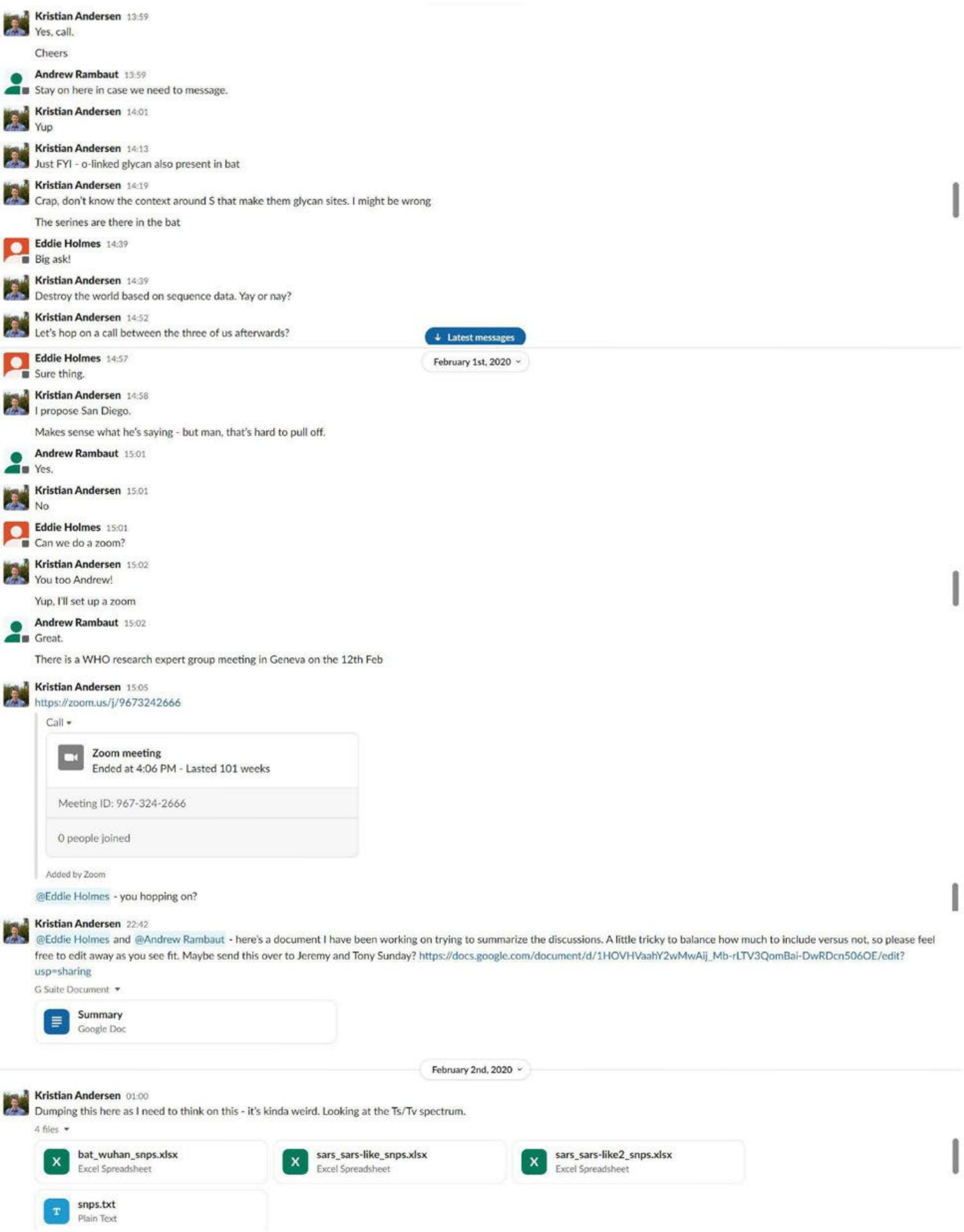
VIROLOGY

Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell–cell fusion but does not affect virion entry

Kathryn E. Follis, Joanne York, Jack H. Nunberg *

Kristian Andersen 13:58
Yeah, the furin site would be the first thing to add for sure. Bob dug into this a little more and some of the distant human coronaviruses *do* have furin-like sites. The one in nCoV is the optimal site though

Eddie Holmes 13:59
Better get ready to call in!



Kristian Andersen 13:59
Yes, call.

Cheers

Andrew Rambaut 13:59
Stay on here in case we need to message.

Kristian Andersen 14:01
Yup

Kristian Andersen 14:13
Just FYI - o-linked glycan also present in bat

Kristian Andersen 14:19
Crap, don't know the context around S that make them glycan sites. I might be wrong

The serines are there in the bat

Eddie Holmes 14:39
Big ask!

Kristian Andersen 14:39
Destroy the world based on sequence data. Yay or nay?

Kristian Andersen 14:52
Let's hop on a call between the three of us afterwards?

February 1st, 2020

Eddie Holmes 14:57
Sure thing.

Kristian Andersen 14:58
I propose San Diego.

Makes sense what he's saying - but man, that's hard to pull off.

Andrew Rambaut 15:01
Yes.

Kristian Andersen 15:01
No

Eddie Holmes 15:01
Can we do a zoom?

Kristian Andersen 15:02
You too Andrew!

Yup, I'll set up a zoom

Andrew Rambaut 15:02
Great.

There is a WHO research expert group meeting in Geneva on the 12th Feb

Kristian Andersen 15:05
https://zoom.us/j/9673242666

Call

Zoom meeting
Ended at 4:06 PM - Lasted 101 weeks

Meeting ID: 967-324-2666

0 people joined

Added by Zoom

@Eddie Holmes - you hopping on?

Kristian Andersen 22:42
@Eddie Holmes and @Andrew Rambaut - here's a document I have been working on trying to summarize the discussions. A little tricky to balance how much to include versus not, so please feel free to edit away as you see fit. Maybe send this over to Jeremy and Tony Sunday? https://docs.google.com/document/d/1HOVHVaahY2wMwAij_Mb-rLTV3QomBai-DwRDcn506OE/edit?usp=sharing

G Suite Document

Summary
Google Doc

February 2nd, 2020

Kristian Andersen 01:00
Dumping this here as I need to think on this - it's kinda weird. Looking at the Ts/Tv spectrum.

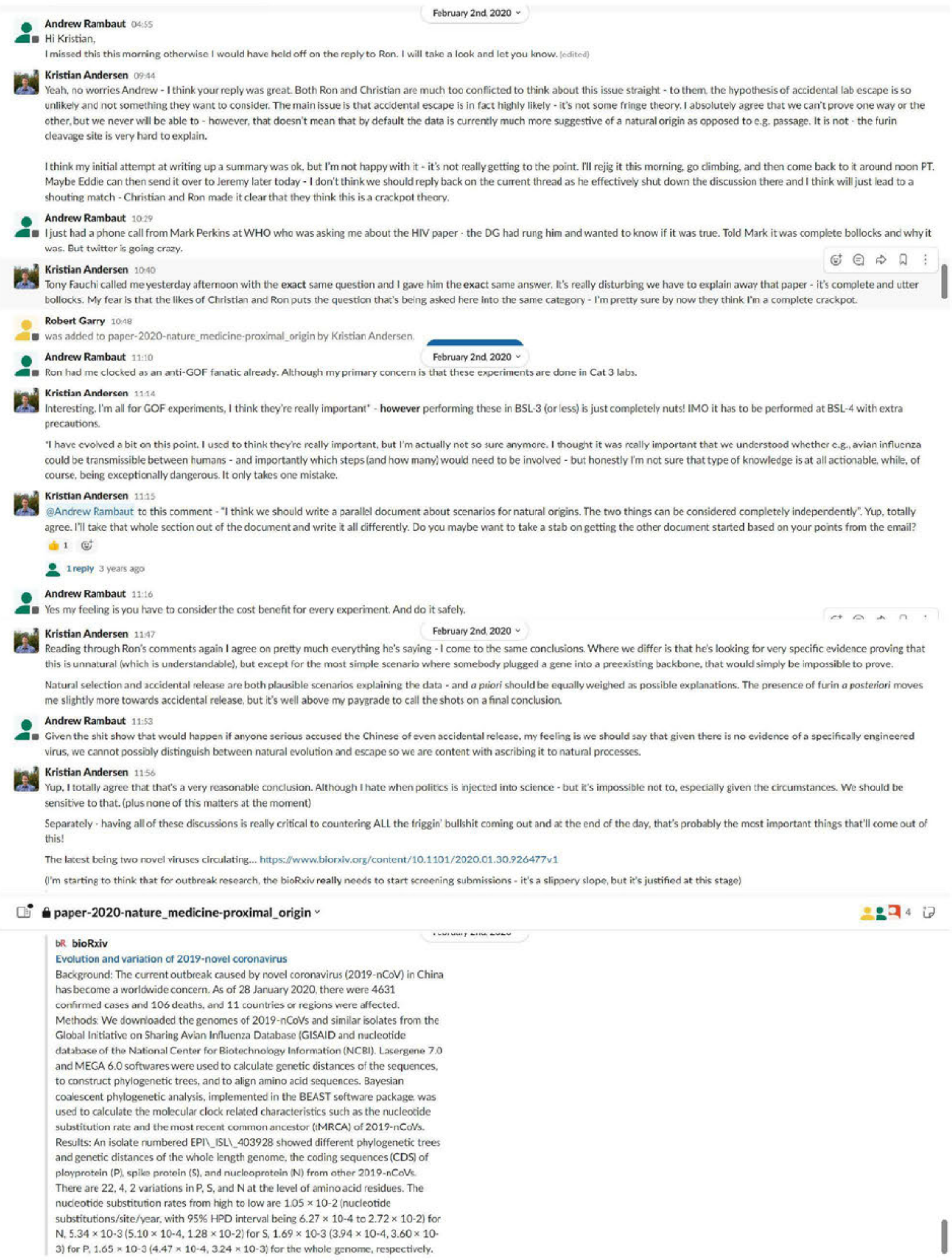
4 files

bat_wuhan_snps.xlsx
Excel Spreadsheet

sars_sars-like_snps.xlsx
Excel Spreadsheet

sars_sars-like2_snps.xlsx
Excel Spreadsheet

snps.txt
Plain Text



February 2nd, 2020

Andrew Rambaut 04:55
Hi Kristian,
I missed this this morning otherwise I would have held off on the reply to Ron. I will take a look and let you know. (edited)

Kristian Andersen 09:44
Yeah, no worries Andrew - I think your reply was great. Both Ron and Christian are much too conflicted to think about this issue straight - to them, the hypothesis of accidental lab escape is so unlikely and not something they want to consider. The main issue is that accidental escape is in fact highly likely - it's not some fringe theory. I absolutely agree that we can't prove one way or the other, but we never will be able to - however, that doesn't mean that by default the data is currently much more suggestive of a natural origin as opposed to e.g. passage. It is not - the furin cleavage site is very hard to explain.

I think my initial attempt at writing up a summary was ok, but I'm not happy with it - it's not really getting to the point. I'll rejig it this morning, go climbing, and then come back to it around noon PT. Maybe Eddie can then send it over to Jeremy later today - I don't think we should reply back on the current thread as he effectively shut down the discussion there and I think will just lead to a shouting match - Christian and Ron made it clear that they think this is a crackpot theory.

Andrew Rambaut 10:29
I just had a phone call from Mark Perkins at WHO who was asking me about the HIV paper - the DG had rung him and wanted to know if it was true. Told Mark it was complete bollocks and why it was. But twitter is going crazy.

Kristian Andersen 10:40
Tony Fauchi called me yesterday afternoon with the **exact** same question and I gave him the **exact** same answer. It's really disturbing we have to explain away that paper - it's complete and utter bollocks. My fear is that the likes of Christian and Ron puts the question that's being asked here into the same category - I'm pretty sure by now they think I'm a complete crackpot.

Robert Garry 10:48
was added to paper-2020-nature_medicine-proximal_origin by Kristian Andersen.

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Andrew Rambaut 11:10
Ron had me clocked as an anti-GOF fanatic already. Although my primary concern is that these experiments are done in Cat 3 labs.

Kristian Andersen 11:14
Interesting. I'm all for GOF experiments, I think they're really important* - **however** performing these in BSL-3 (or less) is just completely nuts! IMO it has to be performed at BSL-4 with extra precautions.

*I have evolved a bit on this point. I used to think they're really important, but I'm actually not so sure anymore. I thought it was really important that we understood whether e.g., avian influenza could be transmissible between humans - and importantly which steps (and how many) would need to be involved - but honestly I'm not sure that type of knowledge is at all actionable, while, of course, being exceptionally dangerous. It only takes one mistake.

Kristian Andersen 11:15
@Andrew Rambaut to this comment - "I think we should write a parallel document about scenarios for natural origins. The two things can be considered completely independently". Yup, totally agree. I'll take that whole section out of the document and write it all differently. Do you maybe want to take a stab on getting the other document started based on your points from the email?
👍 1

1 reply 3 years ago

Andrew Rambaut 11:16
Yes my feeling is you have to consider the cost benefit for every experiment. And do it safely.

February 2nd, 2020

Kristian Andersen 11:47
Reading through Ron's comments again I agree on pretty much everything he's saying - I come to the same conclusions. Where we differ is that he's looking for very specific evidence proving that this is unnatural (which is understandable), but except for the most simple scenario where somebody plugged a gene into a preexisting backbone, that would simply be impossible to prove.

Natural selection and accidental release are both plausible scenarios explaining the data - and *a priori* should be equally weighed as possible explanations. The presence of furin *a posteriori* moves me slightly more towards accidental release, but it's well above my paygrade to call the shots on a final conclusion.

Andrew Rambaut 11:53
Given the shit show that would happen if anyone serious accused the Chinese of even accidental release, my feeling is we should say that given there is no evidence of a specifically engineered virus, we cannot possibly distinguish between natural evolution and escape so we are content with ascribing it to natural processes.

Kristian Andersen 11:56
Yup, I totally agree that that's a very reasonable conclusion. Although I hate when politics is injected into science - but it's impossible not to, especially given the circumstances. We should be sensitive to that. (plus none of this matters at the moment)

Separately - having all of these discussions is really critical to countering ALL the friggin' bullshit coming out and at the end of the day, that's probably the most important things that'll come out of this!

The latest being two novel viruses circulating... https://www.biorxiv.org/content/10.1101/2020.01.30.926477v1

(I'm starting to think that for outbreak research, the bioRxiv **really** needs to start screening submissions - it's a slippery slope, but it's justified at this stage)

paper-2020-nature_medicine-proximal_origin

bR **bioRxiv**
Evolution and variation of 2019-novel coronavirus
Background: The current outbreak caused by novel coronavirus (2019-nCoV) in China has become a worldwide concern. As of 28 January 2020, there were 4631 confirmed cases and 106 deaths, and 11 countries or regions were affected. Methods: We downloaded the genomes of 2019-nCoVs and similar isolates from the Global Initiative on Sharing Avian Influenza Database (GISAID and nucleotide database of the National Center for Biotechnology Information (NCBI). Lasergene 7.0 and MEGA 6.0 softwares were used to calculate genetic distances of the sequences, to construct phylogenetic trees, and to align amino acid sequences. Bayesian coalescent phylogenetic analysis, implemented in the BEAST software package, was used to calculate the molecular clock related characteristics such as the nucleotide substitution rate and the most recent common ancestor (tMRCA) of 2019-nCoVs. Results: An isolate numbered EPI_ISL_403928 showed different phylogenetic trees and genetic distances of the whole length genome, the coding sequences (CDS) of ployprotein (P), spike protein (S), and nucleoprotein (N) from other 2019-nCoVs. There are 22, 4, 2 variations in P, S, and N at the level of amino acid residues. The nucleotide substitution rates from high to low are 1.05 × 10-2 (nucleotide substitutions/site/year, with 95% HPD interval being 6.27 × 10-4 to 2.72 × 10-2) for N, 5.34 × 10-3 (5.10 × 10-4, 1.28 × 10-2) for S, 1.69 × 10-3 (3.94 × 10-4, 3.60 × 10-3) for P, 1.65 × 10-3 (4.47 × 10-4, 3.24 × 10-3) for the whole genome, respectively.

> At this nucleotide substitution rate, the most recent common ancestor (tMRCA) of 2019-nCoVs appeared about 0.253-0.594 year before the epidemic. Conclusion: Our analysis suggests that at least two different viral strains of 2019-nCoV are involved in this outbreak that might occur a few months earlier before it was officially reported.
> Show less
> Jan 30th, 2020

Robert Garry 13:18

This new sequence EPI_ISL_403928 essentially has three consecutive mutations in what we would say is the fusion peptide, although that's "controversial."

Just saying- if I was going to do gain of function or loss of function research I might mutatethe fusion peptide (right after adding the furin site). So this is - at the very least going to pour gas on the fire. Jeremy is absolutely right this needs to be discussed in the light of day. And, ASAP.

Andrew Rambaut 13:25

EPI_ISL_403928 was one of the ones which originally had 50 SNPs which were sequencing errors. The lab then updated it (silently) and it is now only 1 SNP different from other Wuhan ones.

This paper is entirely an artefact of that.

Robert Garry 13:30

In the bioRxiv pdf they say: "When compared with the other 2019-nCoVs, EPI_ISL_403928 has four variations in S protein (T572I, G799V, F800C and N801K) and two variations in N protein (A414C and D415I)." I can totally buy that that's's still an artfact.

Here is the alignment of BatG13 vs nCoV.

PDF ▾

LALIGN results Bat RatG13 vs nCoV .pdf
PDF

LALIGN

Home | Contact

lalign output for WURS vs. BATRT13

These are very similar Spike proteins except for the RBD that looks like it was human adapted and the insertion of the PRRA, that concerts the site to an optimal furin-like cleavage site and potentially creates O-linked glycan sites.

To convert an low pathogenicity avian flu v to a high pathogenicity virus what happens is the insertion of two arginines - Duan 2007.

2 files ▾

Alexander and Brown.pdf
PDF

DUan2007 LPAI vs HPAI.pdf
PDF

Aleander and Brown teach that: "All the current evidence indicates that HPAI viruses arise by mutation after LPAI viruses of the H5 or H7 subtype have been introduced into poultry. Several mechanisms may be responsible for this mutation. For most HPAI viruses, there appears to have been spontaneous duplication of purine triplets, which results in the insertion of basic amino acids at the HA0 cleavage site, and this seems to occur due to a transcription error by the polymerase complex (76)."

This is what Andrew stated last night -it can happen in poultry. But its and insertion of two amino acids not four at once.

H9 flu viruses optimize a minimal furin cleavage site to an optimal one.

PDF ▾

H9 and furin site.pdf
PDF

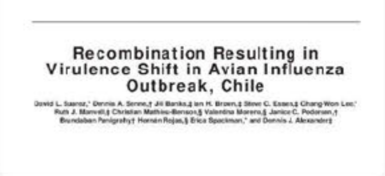
JVI

A Novel Activation Mechanism of Avian Influenza Virus H9N2 by Furin

13:43 H7 viruses appear to make new polybasic furin like cleavage sites by recombining in longish stretchs of nucleotides.

PDF ▾

H7 recombination.pdf
PDF



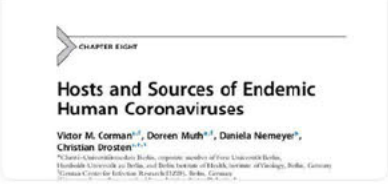
Recombination Resulting in Virulence Shift in Avian Influenza Outbreak, Chile

David L. Suarez,* Dennis A. Senne,† Jill Banks,‡ Ian H. Brown,‡ Steve C. Essen,‡ Chang-Won Lee,* Ruth J. Manvell,‡ Christian Mathieu-Benson,§ Valentina Moreno,§ Janice C. Pedersen,† Brundaban Panigrahy,† Hernán Rojas,§ Erica Spackman,* and Dennis J. Alexander‡

A very good review by Drosten.

PDF ▾

DRosten - source of CoVs.pdf
PDF



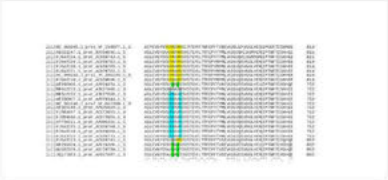
Robert Garry 13:51

New analysis: Some strains of murine hepatitis viruses have a super-optimal furan-like cleavage site (with predicted O-liked glycans), some just have an optimal site and some have no site at all. Just based on the spike phylogeny this seems to have evolved with the spike protein more or less but this is out of my wheelhouse. Not sure if spike evolution in MHV follows evolution,tMCRA etc of other proteins but all are relevant questions given the current issues being discussed IMO

Word Document ▾

February 2nd, 2020 ⌄

MHV spike evolution.docx
Word Document



And a first look at the HKU-1 spike -is a close relative of MHV.

Word Document ▾

And a first look at the HKU-1 spike -is a close relative of MHV.

Word Document ▾

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HKU-1 Spike.docx
Word Document

Robert Garry 13:58

Two pattens seen here (i think there is a third variant as well). There is an insert of three serines right next to the already super optimal furin like cleavage betwee S1 and S2. And, this creates predicted o-linked glycans at and around the site. THere is another mucin-like domain in 5i08 the prefusion structure on the pdb batabase. tHese presence of this mucin like domain expains why the authors were unsuccessful in determining the structure of the top of the trimer, but they didn't know why.

Robert Garry 14:07

Bottom line on all this analysis - mechanisms exist in flu as Andrew stated to make insertions at the junction where the two subunits are cleaved - enhancing virulence and human infectivity. CoV apparently do this as well or potentially cam do this. This is an important message from this discussion and need to be talked about in light of the furin like cleavage site being noticed.

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Robert Garry 14:16

I still don't know if the nCoV was the results of a deliberate manipulation or not. If nCov was not engineered then RatG13 or a very closely related Bat virus somehow ended up in a situation in nature like the poultry farms for H5 etc, as Andrew stated. That's very scary and perhaps engineered would be better - at least that can be regulated so it doesn't happen again.

Robert Garry 14:42

So,

Of nCoV developed that optimal furin cleavage site with the o-linked glycans (which I now suspect are important because they are present elsewhere) then:

1. THe insertion mechanisms is different tan flu H5 in that it's longer and doesn't just involve purines.
2. The generation of the site is different than H7 and MHV because it involves an insertion, not just mutating existing codons.
3. the generation of the furin site is different than H9 because the insertion is a perfect 12 nucleotides, not a rather non-specific recombination.

Robert Garry 14:58

It would be important IMO to get a estimate on the timing on how long ago the MHC mutations and the HKU-1 SSS insertion took place.

Kristian Andersen 15:04

Thanks Bob, these are really good points. Can you please share the sequences from your analysis or the alignment? I'll then take a closer look at overall divergence, etc. I looked at these yesterday, but I wasn't very successful at getting meaningful alignments.

As for the recent bioRxiv paper - as Andrew stated, that can be ignored - the sequence is wrong and that's where they're getting their signal.

Robert Garry 15:20

KHU-1

sequence (6).txt ▾

```
>lcl|DQ437619.1_prot_ABD96198.1_1 [gene=S] [protein=spike glycoprotein] [protein_id=ABD96198.1] [location=1..4071] [gbkey=CDS]
MLLIIFILPTTLAVIGDFNCTNFAINDKNTTVPRISEYVVDVSYGLGTYYILDRVYLNTTILFTGYFPKS
GANFRDLSLKGTTYLSTLWYQKPFLSDFNNGIFSRVKNTKLYVNKTLYSEFSTIVIGSVFINNSYTIVVQ
PHNGVLEITACQYTMCEYPHTICKSKGSSRNESWHFDKSEPLCLFKKNFTYNVSTDWLYFHFYQERGTFY
AYYADSGMPTTFLFSLYLGTLLSHYYVLPLTCNAISSNTDNETLQYWVTPLSKRQYLLKFDNRGVITNAV
```

MHV

sequence just s.txt ▾

```

>lcl|MF618252.1_prot_ATN37888.1_3 [protein=spike glycoprotein] [protein_id=ATN37888.1] [location=22720..26694] [gbkey=CDS]
MLFVFILFLPSCLGYIGDFRCIQLVNSNGANVSAPSISTETVEVSQGLGTYYVLDRVYLNATLLLTGYYP
VDGSKFRNLALTGTNSVSLSWFQPPYLSQFNDGIFAKVQNLKTSTPSGATAYFPTIVIGSLFGYNSYTVV
IEPYNGVIMASVCQYTICQLPYTDCKPNTNGNKLIGFWHTDVKPPICVLKRNFTLNVNADAFYFHFYQHG
```

Here are the clustal alignments for the entire spike proteins.

2 files ▾

Kristian Andersen 15:33

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Thanks Bob - I'll take a look

Andrew Rambaut 18:21

If you want to look here is a bunch of cleavage sites in high-path avian influenza H5 and H7.

Zip ▾

Kristian Andersen 18:34

Do we have any location information on the bat SARS-like viruses? I'm reading through papers and I found this particular sentence from one of Shi's papers interesting - "Interestingly, all the SARSr-CoVs that are capable of using human ACE2 were found in R. sinicus in Yunnan Province".

I believe RaTG13 is from Yunnan, which is about as far away from Wuhan as you can be and still be in China. What are the chances of finding a viruses that are 96% identical given that distance? Seems strange given how many SARS-like viruses we have in bats (which is what Eddie has been telling us for a while...). (edited)

Andrew Rambaut 18:37

Ebola got from Middle Africa to West Africa in 10-20 years.

Kristian Andersen 18:37

Yup, that's true

Yup, that's true

February 2nd, 2020 ⌄

Andrew Rambaut 18:42

I personally think we should get away from all the strange coincidence stuff. I agree it smells really fishy but without a smoking gun it will not do us any good. The truth is never going to come out (if escape is the truth). Would need to be irrefutable evidence. My position is that the natural evolution is entirely plausible and we will have to leave it at that. Lab passaging might also generate this mutation but we have no evidence that that happened.

Not that discussing it isn't fun.

Kristian Andersen 18:48

Agreed. However, I do think some of these points could be important - e.g., would it be impossible to see a bat virus 96% identical that far away? Answer to that, no - we might expect that.

The main concern coming up reading through all these papers is the kind of stuff that is being done - getting MERS-like viruses to infect humans, getting SARS-like viruses to cause disease in mice and infect humans, etc. There's a very strong focus on the spike protein for all of that work.

But I do agree with you - the mind can do amazing things and it's easy to get sucked in with confirmation bias.

One important thing I came across though - for the SARS GoF studies they created a reverse genetics system for their bat virus on a whim. So Ron's and Christian's argument (which I found to be the strongest) about that not being feasible is not true - they were already creating those.

Andrew Rambaut 19:19

I think it would be good idea to lay out these arguments for limited dissemination. And quite frankly so we can learn from it even if it wasn't an escape - it easily could have h

Kristian Andersen 19:28

February 2nd, 2020 ⌄

Yeah, I'm conflicted - I honestly don't know if any of this information is useful without having read all the various papers. Personally, it's useful for context, but even though there's some strange research going on here, there's no smoking gun. Not quite sure what such a gun would look like though.

Bob said it well though - I'd prefer this thing being a lab escape so we have less reason to believe other coronas might do this again in the future 😉.

What is useful is to summarize the main points considered and discussed. I'll get back on that document tomorrow - for now I still need to read more and also want to take a closer look at the alignments. Bottom line is that we can't prove whether this is natural or escape - leaving it to others to make that decision, but hopefully we can ensure they're more informed.

Andrew Rambaut 19:31

I suggest we write this report erring on the side of extreme caution. Also I think the natural evolutionary story may be a interesting one as well. Then we can give all the curious coincidences and dodgy goings on to Marc Lipsitch to have fun with.

Kristian Andersen 19:31

Agreed.

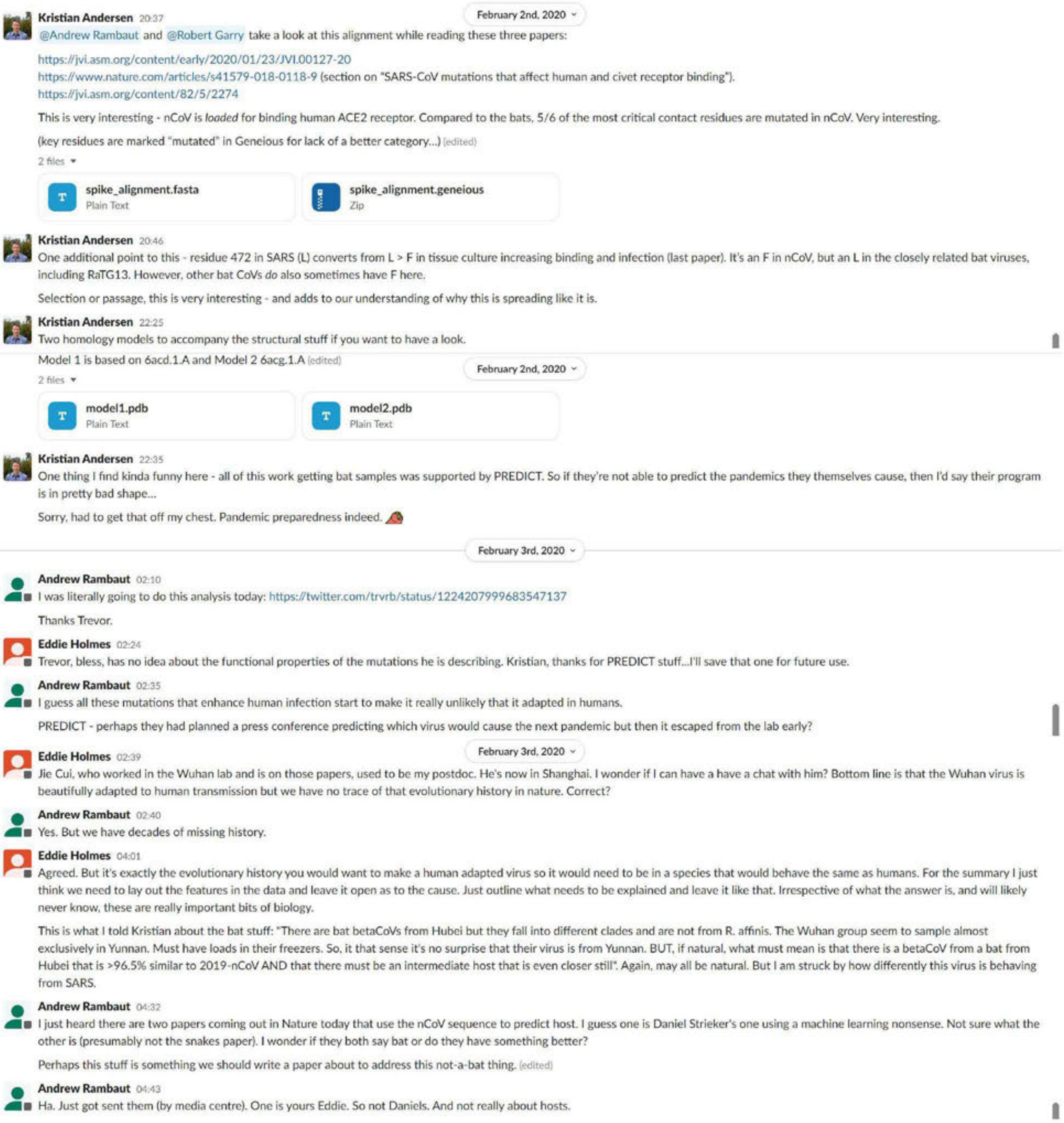
Andrew Rambaut 19:32

If nothing else - the fact that we are discussing this shows how plausible it is.

Kristian Andersen 19:33

And yeah - would love to go down the natural selection rabbit hole 😉

And yes, all of this is **highly** useful and absolutely required - taking a very close look at the different scenarios. Gives some really good ammo to shoot down all the fringe theories and bad studies going on as well.



February 2nd, 2020

Kristian Andersen 20:37
@Andrew Rambaut and @Robert Garry take a look at this alignment while reading these three papers:

https://jvi.asm.org/content/early/2020/01/23/JVI.00127-20
https://www.nature.com/articles/s41579-018-0118-9 (section on "SARS-CoV mutations that affect human and civet receptor binding").
https://jvi.asm.org/content/82/5/2274

This is very interesting - nCoV is *loaded* for binding human ACE2 receptor. Compared to the bats, 5/6 of the most critical contact residues are mutated in nCoV. Very interesting.

(key residues are marked "mutated" in Geneious for lack of a better category...) (edited)

2 files

Kristian Andersen 20:46
One additional point to this - residue 472 in SARS (L) converts from L > F in tissue culture increasing binding and infection (last paper). It's an F in nCoV, but an L in the closely related bat viruses, including RaTG13. However, other bat CoVs *do* also sometimes have F here.

Selection or passage, this is very interesting - and adds to our understanding of why this is spreading like it is.

Kristian Andersen 22:25
Two homology models to accompany the structural stuff if you want to have a look.

Model 1 is based on 6acd.1.A and Model 2 6acg.1.A (edited)

February 2nd, 2020

2 files

Kristian Andersen 22:35
One thing I find kinda funny here - all of this work getting bat samples was supported by PREDICT. So if they're not able to predict the pandemics they themselves cause, then I'd say their program is in pretty bad shape...

Sorry, had to get that off my chest. Pandemic preparedness indeed.

February 3rd, 2020

Andrew Rambaut 02:10
I was literally going to do this analysis today: https://twitter.com/trvrb/status/1224207999683547137

Thanks Trevor.

Eddie Holmes 02:24
Trevor, bless, has no idea about the functional properties of the mutations he is describing. Kristian, thanks for PREDICT stuff...I'll save that one for future use.

Andrew Rambaut 02:35
I guess all these mutations that enhance human infection start to make it really unlikely that it adapted in humans.

PREDICT - perhaps they had planned a press conference predicting which virus would cause the next pandemic but then it escaped from the lab early?

February 3rd, 2020

Eddie Holmes 02:39
Jie Cui, who worked in the Wuhan lab and is on those papers, used to be my postdoc. He's now in Shanghai. I wonder if I can have a have a chat with him? Bottom line is that the Wuhan virus is beautifully adapted to human transmission but we have no trace of that evolutionary history in nature. Correct?

Andrew Rambaut 02:40
Yes. But we have decades of missing history.

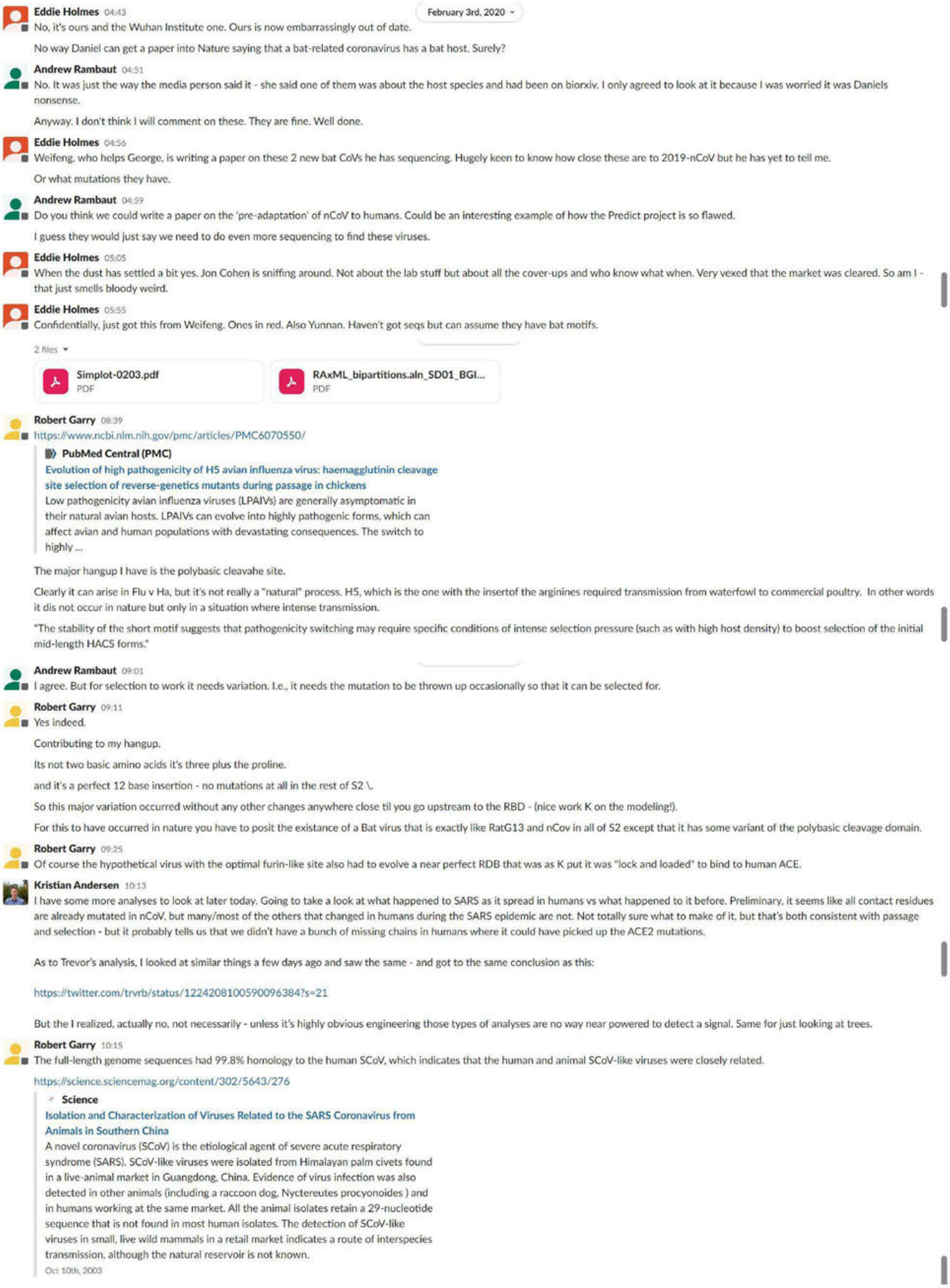
Eddie Holmes 04:01
Agreed. But it's exactly the evolutionary history you would want to make a human adapted virus so it would need to be in a species that would behave the same as humans. For the summary I just think we need to lay out the features in the data and leave it open as to the cause. Just outline what needs to be explained and leave it like that. Irrespective of what the answer is, and will likely never know, these are really important bits of biology.

This is what I told Kristian about the bat stuff: "There are bat betaCoVs from Hubei but they fall into different clades and are not from R. affinis. The Wuhan group seem to sample almost exclusively in Yunnan. Must have loads in their freezers. So, it that sense it's no surprise that their virus is from Yunnan. BUT, if natural, what must mean is that there is a betaCoV from a bat from Hubei that is >96.5% similar to 2019-nCoV AND that there must be an intermediate host that is even closer still". Again, may all be natural. But I am struck by how differently this virus is behaving from SARS.

Andrew Rambaut 04:32
I just heard there are two papers coming out in Nature today that use the nCoV sequence to predict host. I guess one is Daniel Strieker's one using a machine learning nonsense. Not sure what the other is (presumably not the snakes paper). I wonder if they both say bat or do they have something better?

Perhaps this stuff is something we should write a paper about to address this not-a-bat thing. (edited)

Andrew Rambaut 04:43
Ha. Just got sent them (by media centre). One is yours Eddie. So not Daniels. And not really about hosts.



Eddie Holmes 04:43

February 3rd, 2020

No, it's ours and the Wuhan Institute one. Ours is now embarrassingly out of date.

No way Daniel can get a paper into Nature saying that a bat-related coronavirus has a bat host. Surely?

Andrew Rambaut 04:51

No. It was just the way the media person said it - she said one of them was about the host species and had been on biorxiv. I only agreed to look at it because I was worried it was Daniels nonsense.

Anyway. I don't think I will comment on these. They are fine. Well done.

Eddie Holmes 04:56

Weifeng, who helps George, is writing a paper on these 2 new bat CoVs he has sequencing. Hugely keen to know how close these are to 2019-nCoV but he has yet to tell me.

Or what mutations they have.

Andrew Rambaut 04:59

Do you think we could write a paper on the 'pre-adaptation' of nCoV to humans. Could be an interesting example of how the Predict project is so flawed.

I guess they would just say we need to do even more sequencing to find these viruses.

Eddie Holmes 05:05

When the dust has settled a bit yes. Jon Cohen is sniffing around. Not about the lab stuff but about all the cover-ups and who know what when. Very vexed that the market was cleared. So am I - that just smells bloody weird.

Eddie Holmes 05:55

Confidentially, just got this from Weifeng. Ones in red. Also Yunnan. Haven't got seqs but can assume they have bat motifs.

Robert Garry 08:39

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6070550/

> **PubMed Central (PMC)**
> Evolution of high pathogenicity of H5 avian influenza virus: haemagglutinin cleavage site selection of reverse-genetics mutants during passage in chickens
> Low pathogenicity avian influenza viruses (LPAIVs) are generally asymptomatic in their natural avian hosts. LPAIVs can evolve into highly pathogenic forms, which can affect avian and human populations with devastating consequences. The switch to highly ...

The major hangup I have is the polybasic cleavahe site.

Clearly it can arise in Flu v Ha, but it's not really a "natural" process. H5, which is the one with the insertof the arginines required transmission from waterfowl to commercial poultry. In other words it dis not occur in nature but only in a situation where intense transmission.

"The stability of the short motif suggests that pathogenicity switching may require specific conditions of intense selection pressure (such as with high host density) to boost selection of the initial mid-length HACS forms."

Andrew Rambaut 09:01

I agree. But for selection to work it needs variation. I.e., it needs the mutation to be thrown up occasionally so that it can be selected for.

Robert Garry 09:11

Yes indeed.

Contributing to my hangup.

Its not two basic amino acids it's three plus the proline.

and it's a perfect 12 base insertion - no mutations at all in the rest of S2 \.

So this major variation occurred without any other changes anywhere close til you go upstream to the RBD - (nice work K on the modeling!).

For this to have occurred in nature you have to posit the existance of a Bat virus that is exactly like RatG13 and nCov in all of S2 except that it has some variant of the polybasic cleavage domain.

Robert Garry 09:25

Of course the hypothetical virus with the optimal furin-like site also had to evolve a near perfect RDB that was as K put it was "lock and loaded" to bind to human ACE.

Kristian Andersen 10:13

I have some more analyses to look at later today. Going to take a look at what happened to SARS as it spread in humans vs what happened to it before. Preliminary, it seems like all contact residues are already mutated in nCoV, but many/most of the others that changed in humans during the SARS epidemic are not. Not totally sure what to make of it, but that's both consistent with passage and selection - but it probably tells us that we didn't have a bunch of missing chains in humans where it could have picked up the ACE2 mutations.

As to Trevor's analysis, I looked at similar things a few days ago and saw the same - and got to the same conclusion as this:

https://twitter.com/trvrb/status/1224208100590096384?s=21

But the I realized, actually no, not necessarily - unless it's highly obvious engineering those types of analyses are no way near powered to detect a signal. Same for just looking at trees.

Robert Garry 10:15

The full-length genome sequences had 99.8% homology to the human SCoV, which indicates that the human and animal SCoV-like viruses were closely related.

https://science.sciencemag.org/content/302/5643/276

> **Science**
> Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China
> A novel coronavirus (SCoV) is the etiological agent of severe acute respiratory syndrome (SARS). SCoV-like viruses were isolated from Himalayan palm civets found in a live-animal market in Guangdong, China. Evidence of virus infection was also detected in other animals (including a raccoon dog, Nyctereutes procyonoides) and in humans working at the same market. All the animal isolates retain a 29-nucleotide sequence that is not found in most human isolates. The detection of SCoV-like viruses in small, live wild mammals in a retail market indicates a route of interspecies transmission, although the natural reservoir is not known.
> Oct 10th, 2003

February 3rd, 2020

Robert Garry 10:22
In the case of sars the isolation of a very close progenitor virus from three palm civets, a raccoon dog, and a Chinese ferret badger happened quickly . A similar virus was circulating amongst several animals in the wild - or they all got infected at the market.

Robert Garry 10:27
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698 i think this is the paper you want

> **journals.plos.org**
> Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus
> Author summary Increasing evidence has been gathered to support the bat origin of SARS coronavirus (SARS-CoV) in the past decade. However, none of the currently known bat SARSr-CoVs is thought to be the direct ancestor of SARS-CoV. Herein, we report the identification of a diverse group of bat SARSr-CoVs in a single cave in Yunnan, China. Importantly, all of the building blocks of SARS-CoV genome, including the highly variable S gene, ORF8 and ORF3, could be found in the genomes of different SARSr-CoV strains from this single location. Based on the analysis of full-length genome sequences of the newly identified bat SARSr-CoVs, we speculate that the direct ancestor of SARS-CoV may have arise... Show more

February 3rd, 2020

Kristian Andersen 10:31
Yeah, SARS seemed to have a significantly more widespread reservoir - later on in the epidemic, additional spillovers also occurred. That may still be the case with nCoV too, since it's a little early to tell - no additional spillovers into humans for now though.

Interestingly, in the structure paper on nCoV from Baric, they look at compatibility of the ACE2 interacting mutations with a set of potential (intermediate) host species - rats, mice, and civets are out, and probably bats too. Ferrets is a maybe.

I think it might be Hela though/

Robert Garry 10:40
"I'm pretty sure by now they think I'm a complete crackpot."

I think we're disproving this hypothesis. Lots of red flags and no it wont be possible to prove "natural" transmission until you find several closely related animal viruses (>99%). I pretty sure were not going to find the progenitor in humans.

Obviously not possible to prove escape.

Robert Garry 10:50
Transmitting a bat virus like RatG13 in HeLa cells and then asking your graduate student to insert a furin site (she would have had to be taken literally not change 4 amino acids but literally insert 4) would get you there. It's not crackpot to suggest this could have happened given the GoF research we know is happening.

Robert Garry 10:58
For me proving "natural" evolution of the furin site would require finding some animal CoV with a highly similar (identical) S2 and some version of the furin site insert - preferally at least a minimal cleavage site R-X-X-R.

Kristian Andersen 11:51
Yeah, agreed on all accounts. I think we can't prove either way, we can only lay out what we have learned about the virus and its evolution. Making the decision on what seems to be the most likely scenario would have to be done by others - we just need to lay out the science. And boy, is this virus interesting!

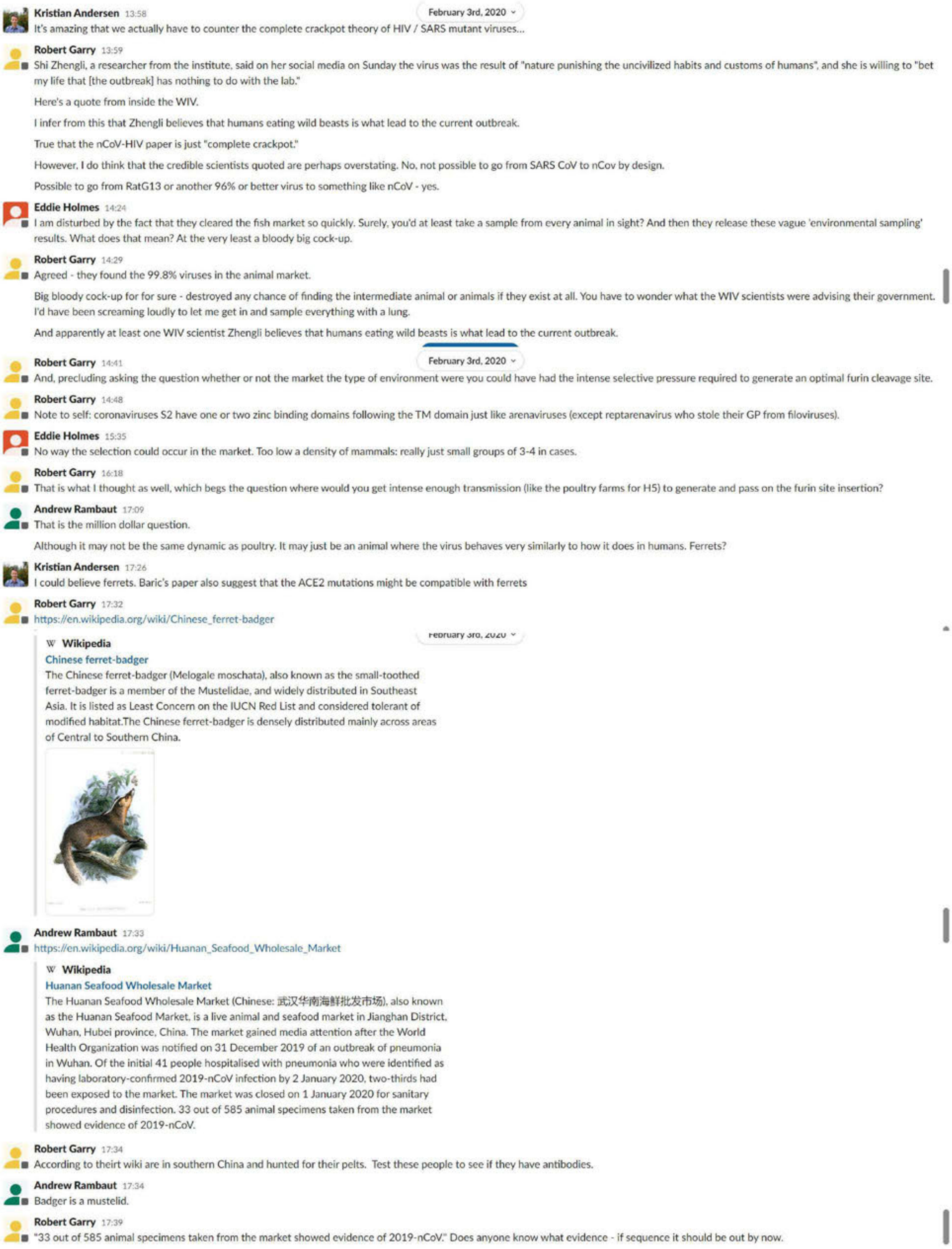
Robert Garry 13:53
https://www.globaltimes.cn/content/1178363.shtml

> **globaltimes.cn**
> Not possible novel coronavirus engineered in lab: experts
> The claim that the novel coronavirus was engineered in a lab has been refuted
> (350 kB)

https://www.forbes.com/sites/victoriaforster/2020/02/02/no-coronavirus-was-not ↓ Latest messages t-pieces-of-hiv-in-it/#3c291bec56cb

http://global.chinadaily.com.cn/a/202002/02/WS5e36b2b7a31012821727432e.html

> **global.chinadaily.com.cn**
> Coronavirus conspiracy debunked by Wuhan researcher - Chinadaily.com.cn
> A scientist from the Wuhan Institute of Virology of the Chinese Academy of Sciences has debunked a recent conspiracy which claimed the novel coronavirus was manufactured and escaped from the institute's most advanced biocontainment facility.
> (71 kB)



Kristian Andersen 13:58
It's amazing that we actually have to counter the complete crackpot theory of HIV / SARS mutant viruses...

Robert Garry 13:59
Shi Zhengli, a researcher from the institute, said on her social media on Sunday the virus was the result of "nature punishing the uncivilized habits and customs of humans", and she is willing to "bet my life that [the outbreak] has nothing to do with the lab."

Here's a quote from inside the WIV.

I infer from this that Zhengli believes that humans eating wild beasts is what lead to the current outbreak.

True that the nCoV-HIV paper is just "complete crackpot."

However, I do think that the credible scientists quoted are perhaps overstating. No, not possible to go from SARS CoV to nCov by design.

Possible to go from RatG13 or another 96% or better virus to something like nCoV - yes.

Eddie Holmes 14:24
I am disturbed by the fact that they cleared the fish market so quickly. Surely, you'd at least take a sample from every animal in sight? And then they release these vague 'environmental sampling' results. What does that mean? At the very least a bloody big cock-up.

Robert Garry 14:29
Agreed - they found the 99.8% viruses in the animal market.

Big bloody cock-up for for sure - destroyed any chance of finding the intermediate animal or animals if they exist at all. You have to wonder what the WIV scientists were advising their government. I'd have been screaming loudly to let me get in and sample everything with a lung.

And apparently at least one WIV scientist Zhengli believes that humans eating wild beasts is what lead to the current outbreak.

Robert Garry 14:41
And, precluding asking the question whether or not the market the type of environment were you could have had the intense selective pressure required to generate an optimal furin cleavage site.

Robert Garry 14:48
Note to self: coronaviruses S2 have one or two zinc binding domains following the TM domain just like arenaviruses (except reptarenavirus who stole their GP from filoviruses).

Eddie Holmes 15:35
No way the selection could occur in the market. Too low a density of mammals: really just small groups of 3-4 in cases.

Robert Garry 16:18
That is what I thought as well, which begs the question where would you get intense enough transmission (like the poultry farms for H5) to generate and pass on the furin site insertion?

Andrew Rambaut 17:09
That is the million dollar question.

Although it may not be the same dynamic as poultry. It may just be an animal where the virus behaves very similarly to how it does in humans. Ferrets?

Kristian Andersen 17:26
I could believe ferrets. Baric's paper also suggest that the ACE2 mutations might be compatible with ferrets

Robert Garry 17:32
https://en.wikipedia.org/wiki/Chinese_ferret-badger

> **Wikipedia**
> Chinese ferret-badger
> The Chinese ferret-badger (Melogale moschata), also known as the small-toothed ferret-badger is a member of the Mustelidae, and widely distributed in Southeast Asia. It is listed as Least Concern on the IUCN Red List and considered tolerant of modified habitat.The Chinese ferret-badger is densely distributed mainly across areas of Central to Southern China.

Andrew Rambaut 17:33
https://en.wikipedia.org/wiki/Huanan_Seafood_Wholesale_Market

> **Wikipedia**
> Huanan Seafood Wholesale Market
> The Huanan Seafood Wholesale Market (Chinese: 武汉华南海鲜批发市场), also known as the Huanan Seafood Market, is a live animal and seafood market in Jianghan District, Wuhan, Hubei province, China. The market gained media attention after the World Health Organization was notified on 31 December 2019 of an outbreak of pneumonia in Wuhan. Of the initial 41 people hospitalised with pneumonia who were identified as having laboratory-confirmed 2019-nCoV infection by 2 January 2020, two-thirds had been exposed to the market. The market was closed on 1 January 2020 for sanitary procedures and disinfection. 33 out of 585 animal specimens taken from the market showed evidence of 2019-nCoV.

Robert Garry 17:34
According to theirt wiki are in southern China and hunted for their pelts. Test these people to see if they have antibodies.

Andrew Rambaut 17:34
Badger is a mustelid.

Robert Garry 17:39
"33 out of 585 animal specimens taken from the market showed evidence of 2019-nCoV." Does anyone know what evidence - if sequence it should be out by now.

Andrew Rambaut 17:39
Runny noses?
😂 1

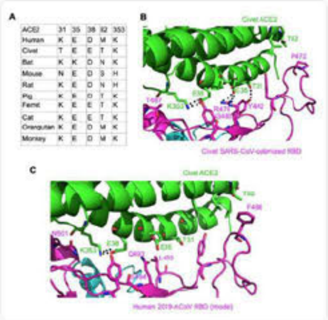
Robert Garry 17:40
Could be - ferrets with the flu look "just" like humans with the flu.

https://www.nature.com/articles/425915a"Serological and virological studies have indicated that Chinese ferret badgers (*Melogale moschata*), masked palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*) can be infected with a virus that is very similar to SCV (ref. 3). Domestic cats living in the Amoy Gardens apartment block in Hong Kong, where more than 100 residents contracted SARS last year, were also found to be infected with SCV."

> **Nature**
> SARS virus infection of cats and ferrets
> There is now a choice of animal models for testing therapies against the human virus.

> **Nature**
> SARS virus infection of cats and ferrets
> There is now a choice of animal models for testing therapies against the human virus.

Kristian Andersen 17:46
Baric has this interesting table with the contact residues for the various species. I need to look at compatibility of nCoV
Screen Shot 2020-02-03 at 14.45.11.png ▾

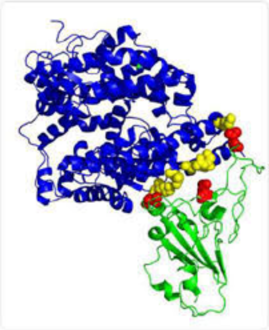


A

ACE2	31	35	38	82	353
Human	K	E	D	M	K
Civet	T	E	E	T	K
Bat	K	K	D	N	K
Mouse	N	E	D	S	H
Rat	K	E	D	N	H
Pig	K	E	D	T	K
Ferret	K	E	E	T	K
Cat	K	E	E	T	K
Orangutan	K	E	D	M	K
Monkey	K	E	D	M	K

Robert Garry 18:11
This is what that interaction with sars v rbd looks like.
image.png ▾

February 3rd, 2020 ⌄



THe yellow spheres are ACE 31, 53, 38, 82 and 353.

THe red spheres are SARS V 472, 479 and 487

the pdb is 2AJF.

Possible to model in nCoV - worth doing.

↓ Latest messages

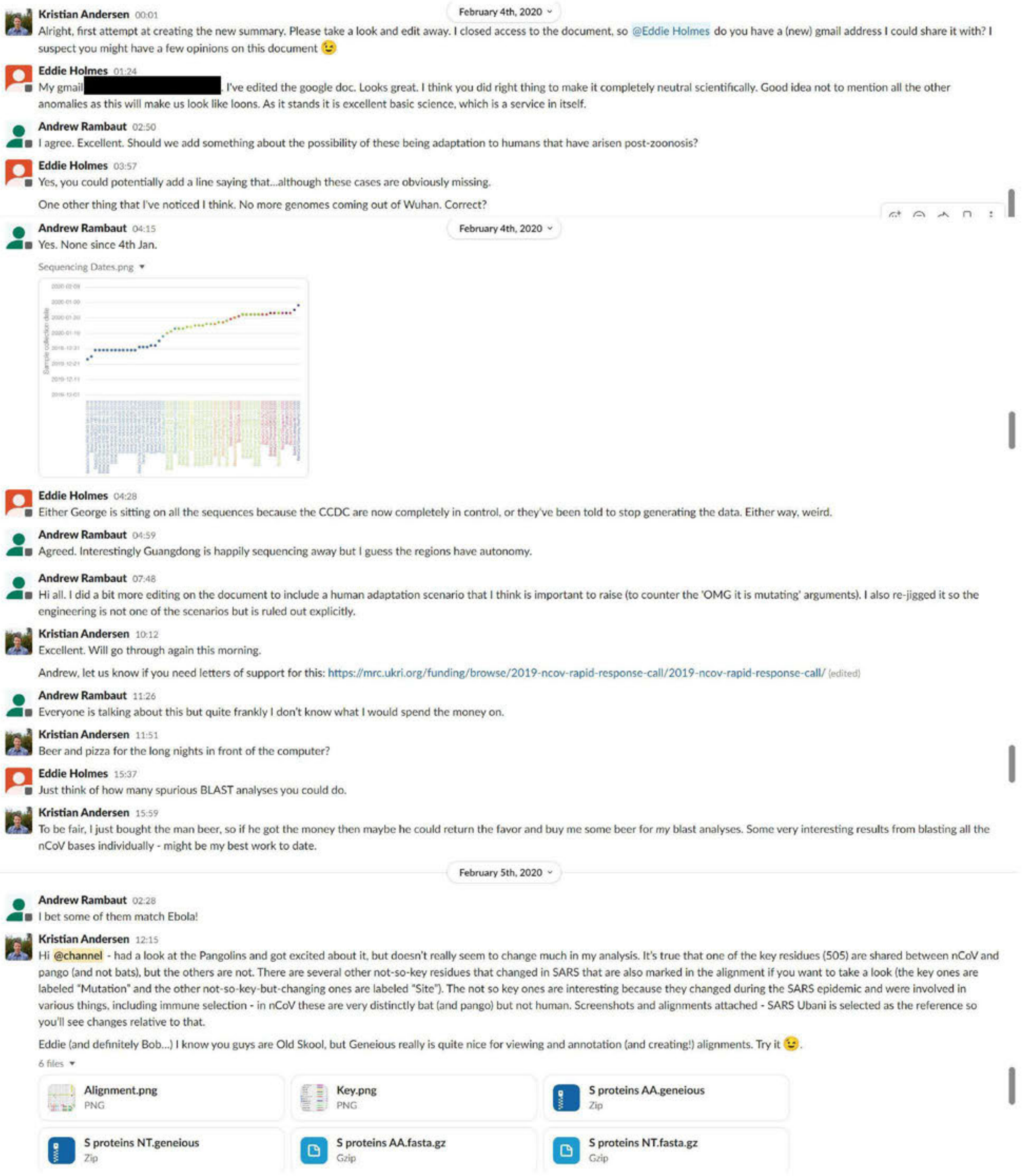
Kristian Andersen 18:26
Yeah, I'd be interested in seeing nCoV and RaTG13 binding to ACE2 from e.g., humans and bats. Might get to it later in the week - definitely a fair bit of work to do...

Eddie Holmes 18:28
The wiki info is wrong I believe. According to the official news agency report in English & Chinese it 33 environmental samples that tested positive, not animals. All were from one particular part of the market. Hard to know quite what this means.

Robert Garry 18:32
https://science.sciencemag.org/content/sci/309/5742/1864.full.pdf
👍 1

This has another binding table.

Robert Garry 18:40
Not testing the animals is definitely a crime against science, if not humanity.



February 4th, 2020

Kristian Andersen 00:01
Alright, first attempt at creating the new summary. Please take a look and edit away. I closed access to the document, so @Eddie Holmes do you have a (new) gmail address I could share it with? I suspect you might have a few opinions on this document 😉

Eddie Holmes 01:24
My gmail [redacted]. I've edited the google doc. Looks great. I think you did right thing to make it completely neutral scientifically. Good idea not to mention all the other anomalies as this will make us look like loons. As it stands it is excellent basic science, which is a service in itself.

Andrew Rambaut 02:50
I agree. Excellent. Should we add something about the possibility of these being adaptation to humans that have arisen post-zoonosis?

Eddie Holmes 03:57
Yes, you could potentially add a line saying that...although these cases are obviously missing.

One other thing that I've noticed I think. No more genomes coming out of Wuhan. Correct?

February 4th, 2020

Andrew Rambaut 04:15
Yes. None since 4th Jan.

Sequencing Dates.png ▾

Eddie Holmes 04:28
Either George is sitting on all the sequences because the CCDC are now completely in control, or they've been told to stop generating the data. Either way, weird.

Andrew Rambaut 04:59
Agreed. Interestingly Guangdong is happily sequencing away but I guess the regions have autonomy.

Andrew Rambaut 07:48
Hi all. I did a bit more editing on the document to include a human adaptation scenario that I think is important to raise (to counter the 'OMG it is mutating' arguments). I also re-jigged it so the engineering is not one of the scenarios but is ruled out explicitly.

Kristian Andersen 10:12
Excellent. Will go through again this morning.

Andrew, let us know if you need letters of support for this: https://mrc.ukri.org/funding/browse/2019-ncov-rapid-response-call/2019-ncov-rapid-response-call/ (edited)

Andrew Rambaut 11:26
Everyone is talking about this but quite frankly I don't know what I would spend the money on.

Kristian Andersen 11:51
Beer and pizza for the long nights in front of the computer?

Eddie Holmes 15:37
Just think of how many spurious BLAST analyses you could do.

Kristian Andersen 15:59
To be fair, I just bought the man beer, so if he got the money then maybe he could return the favor and buy me some beer for *my* blast analyses. Some very interesting results from blasting all the nCoV bases individually - might be my best work to date.

February 5th, 2020

Andrew Rambaut 02:28
I bet some of them match Ebola!

Kristian Andersen 12:15
Hi @channel - had a look at the Pangolins and got excited about it, but doesn't really seem to change much in my analysis. It's true that one of the key residues (505) are shared between nCoV and pango (and not bats), but the others are not. There are several other not-so-key residues that changed in SARS that are also marked in the alignment if you want to take a look (the key ones are labeled "Mutation" and the other not-so-key-but-changing ones are labeled "Site"). The not so key ones are interesting because they changed during the SARS epidemic and were involved in various things, including immune selection - in nCoV these are very distinctly bat (and pango) but not human. Screenshots and alignments attached - SARS Ubani is selected as the reference so you'll see changes relative to that.

Eddie (and definitely Bob...) I know you guys are Old Skool, but Geneious really is quite nice for viewing and annotation (and creating!) alignments. Try it 😉.

6 files ▾

Alignment.png PNG
Key.png PNG
S proteins AA.geneious Zip
S proteins NT.geneious Zip
S proteins AA.fasta.gz Gzip
S proteins NT.fasta.gz Gzip

February 5th, 2020

Andrew Rambaut 12:19
This kind of looks like convergence to me (nCoV shares with RaT13 as much as with the pangolins).

Kristian Andersen 12:28
Agreed. Do we know anything about these Pango sequences? Any cell culture involved? I was really hoping these guys would disprove the cell hypothesis by being (a) highly similar to nCoV, and (b) not from culture.

Robert Garry 12:28
Agree. It's interesting that the Pangolin sequences were detected (and in dead animals). Shows that there is a reservoir of previously undetected circulation of Bat-like CoVs in mammals. But, no more of a smoking gun than RaTG13 as far as nCoV goes - not close enough to be the progenitor nor locally close enough to make a strong case that it might serve as a substrate for a recombinant that lead to nCoV.

Kristian Andersen 12:30
Nope. Let's hope more sequences come out - would be so awesome to see an nCoV-like RBD and furin site. Would be critical evidence **against** cell culture hypothesis (which I'm still leaning towards).

Robert Garry 12:38
Definite lean for me too. Would buy Andrew a beer and Eddie a subscription to Geneious, if Ron Fouchier shares previously alluded to cell culture data showing cell culture passage produces a furin site in a CoV.

Kristian Andersen 12:49
Mike Farzan said that they see furin sites in culture too, but I can't find any papers on it! I'll ping him tomorrow and ask (R01 day today...) (edited)

Robert Garry 12:50
Great - ask for data....

Robert Garry 13:03
I hope Fazan or Fouchier have this data. It would render the already dead bioengineered scenario totally and completely dead.

👍 1

It would also make a strong case for the cell culture/accidental escape model.

Kristian Andersen 15:57
This is pretty nifty.

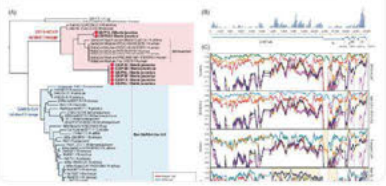
http://cov-glue.cvr.gla.ac.uk/#/replacement

Some of these mutations are interesting - human adaptive mutations...

Kristian Andersen 17:14
Eddie's recent tree

PDF ▾

File from iOS
PDF



February 5th, 2020

Andrew Rambaut 18:01
For your amusement: https://jameslyonsweiler.com/2020/02/02/moderately-strong-confirmation-of-a-laboratory-origin-of-2019-ncov/

jameslyonsweiler.com
Moderately Strong Confirmation of a Laboratory Origin of 2019-nCoV
James Lyons-Weiler, PhD 2-2-2020 Dr. Marc Wathelet commented that he was puzzled about my report of a spike protein gene homologous to part of the pShuttle-SN vector, given that spike glycoproteins...
Feb 2nd, 2020 (278 kB) ▾

1

See if you can work out what he has done here.

Latest messages

Eddie Holmes 19:05
Kristian, I confused here. In the figure that I sent you - which is from the paper that Tommy Lam is writing - the pango and nCoV seem to share a lot of the key sites. But this is not what your alignment shows. Correct? Does this include the pango sequence I sent you the other day? I don't think we are comparing the same things here. No cell culture involved.

Eddie Holmes 19:21
I have Geneious but I'm too old to deal with things that go out frame.

Kristian Andersen 19:53
Let me look into this a little closer tomorrow. The online pango sequence has a lot of missing bases, hence it wasn't included in the previous alignment. But as I'm eyeballing it at the moment, I can see it lining up better. I'll take a look tomorrow.

Eddie Holmes 20:11
Thanks. I'll get word more info from Tommy shortly - try and work out which sequence ID relates to which virus in the tree. It seems that P1L and P2S were sequenced by different groups (the one on the SRA is P1L and that from Tommy is P2S). I think they are both have very similar RBDs to humans.

February 6th, 2020

Kristian Andersen 01:00
> See if you can work out what he has done here.
I can't figure it out... tell me

2 replies Last reply 3 years ago

Eddie Holmes 02:01
Tommy says that the key seqs are P376, P377 and P378, from the SRA, and 'OurPangolin v2'. He merged them for some analyses as they are very similar.

Pango madness. (1). The more divergent cluster in the tree are from Guangxi. These do not have 2019-nCoV like RBDs. The cluster closer to 2019-nCoV are from Guangdong (seq IDs above). They are very similar to 2019-nCoV in RBD, sharing most of the key residues. Closer than RaTG13. Indeed, computational docking analyses (Rosetta) shows that the pangolin RDB have similar high binding affinity as 2019-nCoV RDB to human ACE2 (2). The two Guangdong viruses were sequenced by different groups at different times. No human cell culture evolve. (3). The similarity between the RBD of the Guangdong pangolins and 2019-nCoV is only at nonsynonymous sites. No movement in a tree of synonymous sites. So, convergence? How is all this explained? Remarkable that we have two clusters of pango viruses that are closely related to 2019-nCoV but that differ so profoundly in the RBD.

Andrew Rambaut 09:54
@Kristian do you have a genome alignment of everything in Geneious with annotations?

I mean all the bat SARS-r and the pangolins?

I think I am going to go to the WHO meeting in Geneva next week (I was invited by the modelling group I am on). But it might be good to see what crops up about all this.

Kristian Andersen 10:04
On my agenda today so I'll have that in a few hours

Andrew Rambaut 10:08
Thanks. I feel I need to do a deep dive into it all but my current data sets are a mess.

Kristian Andersen 10:16
Agreed

Just remember - the pangos are only S and some very incomplete (which concerns me a bit - the ones that are complete don't look like nCoV in the RBD, the ones that are incomplete do. I'm worried about data quality here, but I'll look into it)

Andrew Rambaut 10:24
Perhaps @Eddie Holmes can persuade them to sequence full genomes with some urgency?
💯 1

Kristian Andersen 13:08
I can't for the life of me get a good alignment with those additional pengos included... They seem very low quality. I'll continue... For now, here are spike protein alignments containing the bat, pengo, and some select human viruses. Changed the annotations to be more logical too.

2 files

- alignment_spike_nt.fasta.gz
  Gzip
- alignment_spike_nt.geneious
  Zip

Eddie Holmes 15:16
There are whole genomes. I just sent you S to make it easier, which clearly failed. I'll see if I can get all the sequence data.

Translate this

selected_RBD-whole.fas

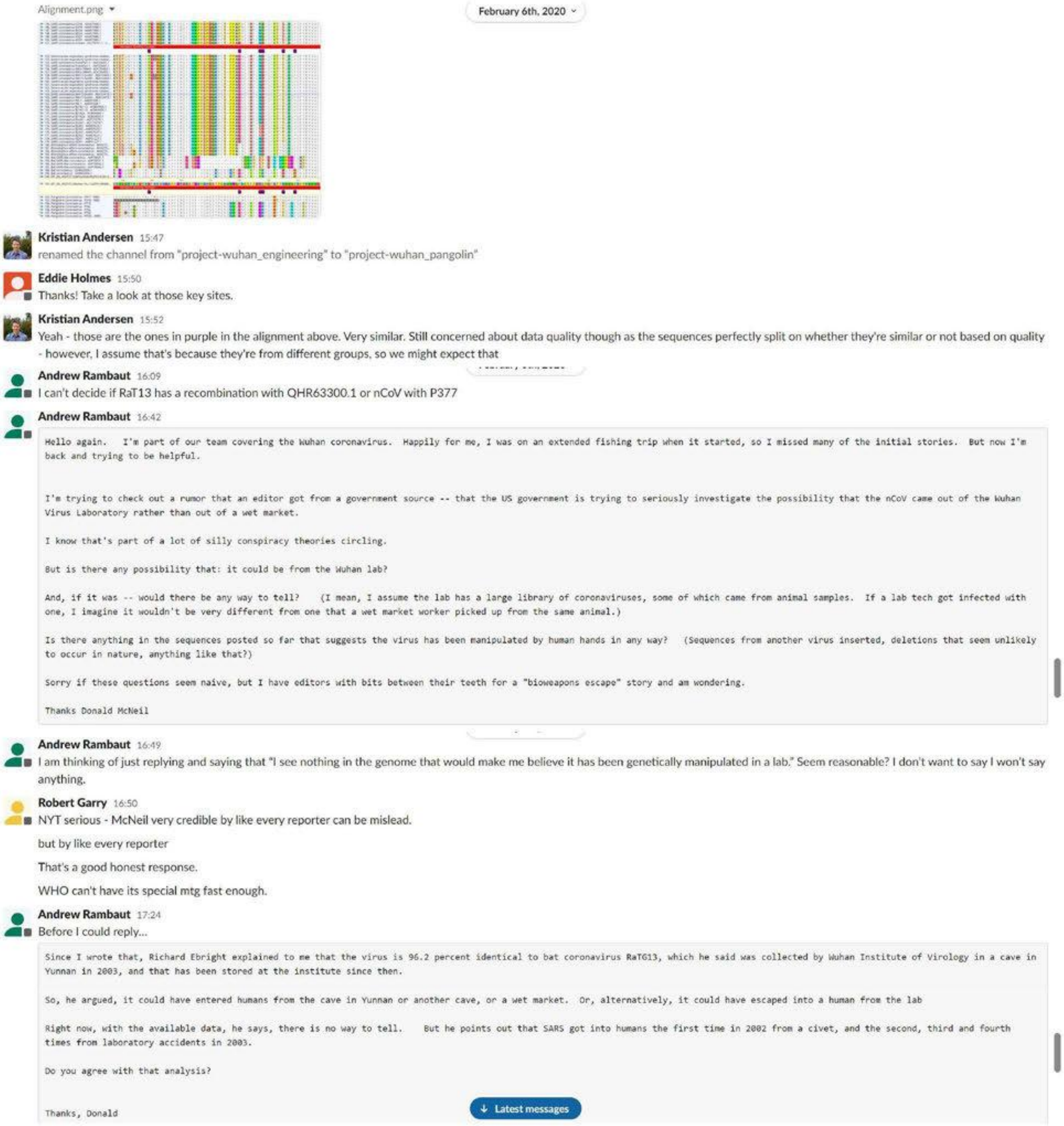
```
>2019-nCoV_EPI402124|BetaCoV/Wuhan/WIV04/2019(2)
AATATTACAAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATTCCGCATCATTTTCCACTTTTAAGTGTTATGGAGTGTCTCCTACTAAATTA
AATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGGAATTCTAACAATCTTGATTCTAAG
GTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGTAATGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAAT
GGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCAACTGTT
>EPI402131|BetaCoV/bat/Yunnan/RaTG13/2013|2013-07-24(2)
AATATTACAAACTTATGTCCTTTTGGTGAAGTTTTTAACGCCACCACATTCGCATCAGTTTATGCTTGGAACAGAAAGAGAATTAGCAACTGTGTTGCTGATTACTCTGTCCTATATAATTCCACTTCATTTTCTACCTTTAAATGTTATGGAGTGTCTCCTACTAAATTA
AATGATCTCTGCTTTACTAATGTTTATGCAGACTCATTTGTGATTACAGGTGATGAAGTCAGACAAATTGCGCCAGGACAAACTGGAAAGATTGCTGACTACAATTATAAACTACCAGATGATTTTACTGGTTGTGTTATAGCTTGGAATTCTAAGCATATT...
```

Eddie Holmes 15:35
Our Pangolin = Guangdong. GXP = Guangxi

Kristian Andersen 15:44
Here we go - I cleaned it up. Seems like we might have ourselves a pangolin recombinant...

2 files

- alignment_spike_aa.geneious
  Zip
- alignment_spike_aa.fasta.gz
  Gzip



Kristian Andersen 15:47
renamed the channel from "project-wuhan_engineering" to "project-wuhan_pangolin"

Eddie Holmes 15:50
Thanks! Take a look at those key sites.

Kristian Andersen 15:52
Yeah - those are the ones in purple in the alignment above. Very similar. Still concerned about data quality though as the sequences perfectly split on whether they're similar or not based on quality - however, I assume that's because they're from different groups, so we might expect that

Andrew Rambaut 16:09
I can't decide if RaT13 has a recombination with QHR63300.1 or nCoV with P377

Andrew Rambaut 16:42

> Hello again. I'm part of our team covering the Wuhan coronavirus. Happily for me, I was on an extended fishing trip when it started, so I missed many of the initial stories. But now I'm back and trying to be helpful.
>
> I'm trying to check out a rumor that an editor got from a government source -- that the US government is trying to seriously investigate the possibility that the nCoV came out of the Wuhan Virus Laboratory rather than out of a wet market.
>
> I know that's part of a lot of silly conspiracy theories circling.
>
> But is there any possibility that: it could be from the Wuhan lab?
>
> And, if it was -- would there be any way to tell? (I mean, I assume the lab has a large library of coronaviruses, some of which came from animal samples. If a lab tech got infected with one, I imagine it wouldn't be very different from one that a wet market worker picked up from the same animal.)
>
> Is there anything in the sequences posted so far that suggests the virus has been manipulated by human hands in any way? (Sequences from another virus inserted, deletions that seem unlikely to occur in nature, anything like that?)
>
> Sorry if these questions seem naive, but I have editors with bits between their teeth for a "bioweapons escape" story and am wondering.
>
> Thanks Donald McNeil

Andrew Rambaut 16:49
I am thinking of just replying and saying that "I see nothing in the genome that would make me believe it has been genetically manipulated in a lab." Seem reasonable? I don't want to say I won't say anything.

Robert Garry 16:50
NYT serious - McNeil very credible by like every reporter can be mislead.

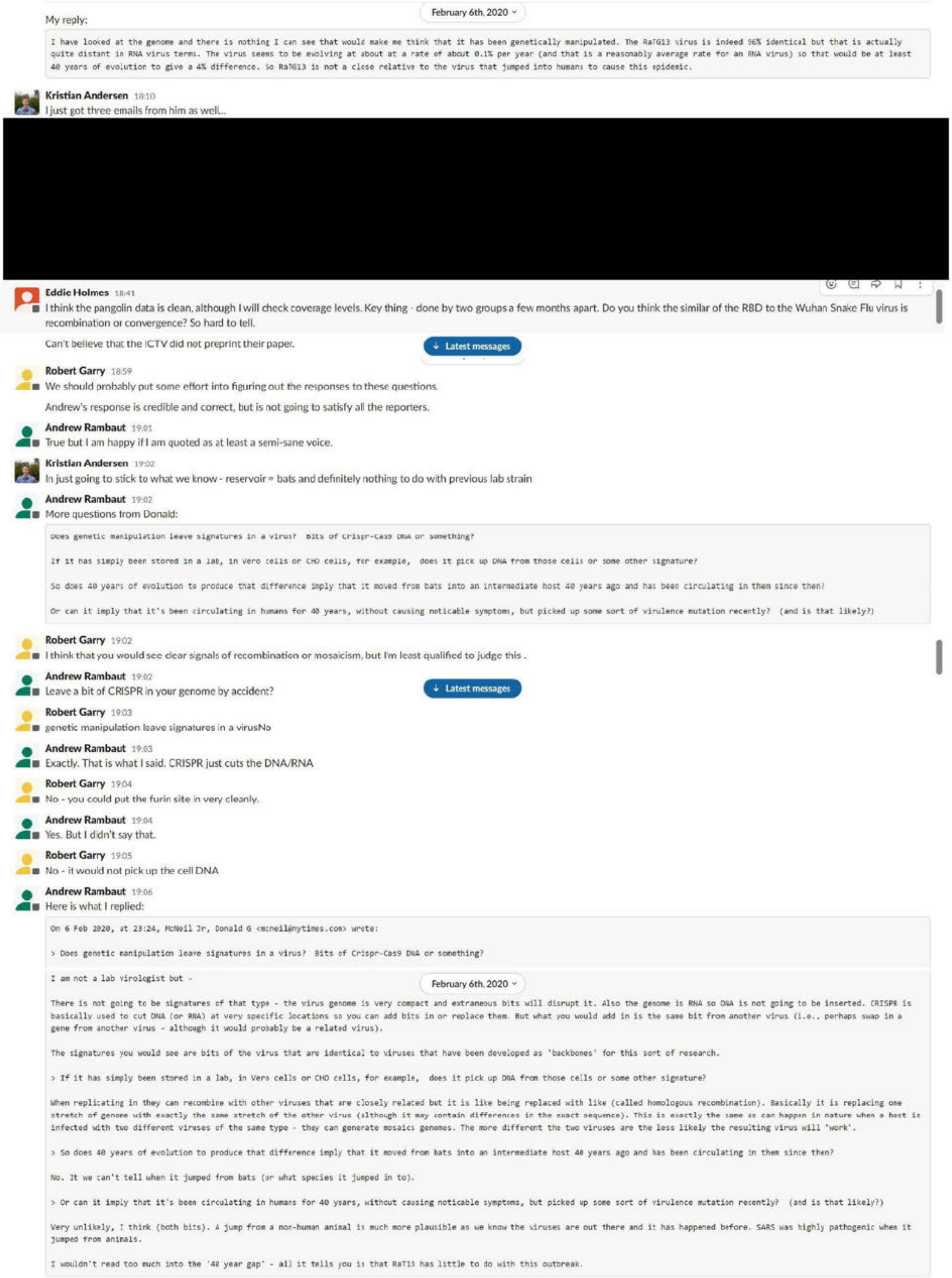
but by like every reporter

That's a good honest response.

WHO can't have its special mtg fast enough.

Andrew Rambaut 17:24
Before I could reply...

> Since I wrote that, Richard Ebright explained to me that the virus is 96.2 percent identical to bat coronavirus RaTG13, which he said was collected by Wuhan Institute of Virology in a cave in Yunnan in 2003, and that has been stored at the institute since then.
>
> So, he argued, it could have entered humans from the cave in Yunnan or another cave, or a wet market. Or, alternatively, it could have escaped into a human from the lab
>
> Right now, with the available data, he says, there is no way to tell. But he points out that SARS got into humans the first time in 2002 from a civet, and the second, third and fourth times from laboratory accidents in 2003.
>
> Do you agree with that analysis?
>
> Thanks, Donald



February 6th, 2020

My reply:

```
I have looked at the genome and there is nothing I can see that would make me think that it has been genetically manipulated. The RaTG13 virus is indeed 96% identical but that is actually quite distant in RNA virus terms. The virus seems to be evolving at about at a rate of about 0.1% per year (and that is a reasonably average rate for an RNA virus) so that would be at least 40 years of evolution to give a 4% difference. So RaTG13 is not a close relative to the virus that jumped into humans to cause this epidemic.
```

Kristian Andersen 18:10
I just got three emails from him as well...

[redacted]

Eddie Holmes 18:41
I think the pangolin data is clean, although I will check coverage levels. Key thing - done by two groups a few months apart. Do you think the similar of the RBD to the Wuhan Snake Flu virus is recombination or convergence? So hard to tell.

Can't believe that the ICTV did not preprint their paper.

Robert Garry 18:59
We should probably put some effort into figuring out the responses to these questions.

Andrew's response is credible and correct, but is not going to satisfy all the reporters.

Andrew Rambaut 19:01
True but I am happy if I am quoted as at least a semi-sane voice.

Kristian Andersen 19:02
In just going to stick to what we know - reservoir = bats and definitely nothing to do with previous lab strain

Andrew Rambaut 19:02
More questions from Donald:

```
Does genetic manipulation leave signatures in a virus?  Bits of Crispr-Cas9 DNA or something?

If it has simply been stored in a lab, in Vero cells or CHO cells, for example,  does it pick up DNA from those cells or some other signature?

So does 40 years of evolution to produce that difference imply that it moved from bats into an intermediate host 40 years ago and has been circulating in them since then?

Or can it imply that it's been circulating in humans for 40 years, without causing noticable symptoms, but picked up some sort of virulence mutation recently?  (and is that likely?)
```

Robert Garry 19:02
I think that you would see clear signals of recombination or mosaicism, but I'm least qualified to judge this .

Andrew Rambaut 19:02
Leave a bit of CRISPR in your genome by accident?

Robert Garry 19:03
genetic manipulation leave signatures in a virusNo

Andrew Rambaut 19:03
Exactly. That is what I said. CRISPR just cuts the DNA/RNA

Robert Garry 19:04
No - you could put the furin site in very cleanly.

Andrew Rambaut 19:04
Yes. But I didn't say that.

Robert Garry 19:05
No - it would not pick up the cell DNA

Andrew Rambaut 19:06
Here is what I replied:

```
On 6 Feb 2020, at 23:24, McNeil Jr, Donald G <mcneil@nytimes.com> wrote:

> Does genetic manipulation leave signatures in a virus?  Bits of Crispr-Cas9 DNA or something?

I am not a lab virologist but -

There is not going to be signatures of that type - the virus genome is very compact and extraneous bits will disrupt it. Also the genome is RNA so DNA is not going to be inserted. CRISPR is basically used to cut DNA (or RNA) at very specific locations so you can add bits in or replace them. But what you would add in is the same bit from another virus (i.e., perhaps swap in a gene from another virus - although it would probably be a related virus).

The signatures you would see are bits of the virus that are identical to viruses that have been developed as 'backbones' for this sort of research.

> If it has simply been stored in a lab, in Vero cells or CHO cells, for example,  does it pick up DNA from those cells or some other signature?

When replicating in they can recombine with other viruses that are closely related but it is like being replaced with like (called homologous recombination). Basically it is replacing one stretch of genome with exactly the same stretch of the other virus (although it may contain differences in the exact sequence). This is exactly the same as can happen in nature when a host is infected with two different viruses of the same type - they can generate mosaics genomes. The more different the two viruses are the less likely the resulting virus will 'work'.

> So does 40 years of evolution to produce that difference imply that it moved from bats into an intermediate host 40 years ago and has been circulating in them since then?

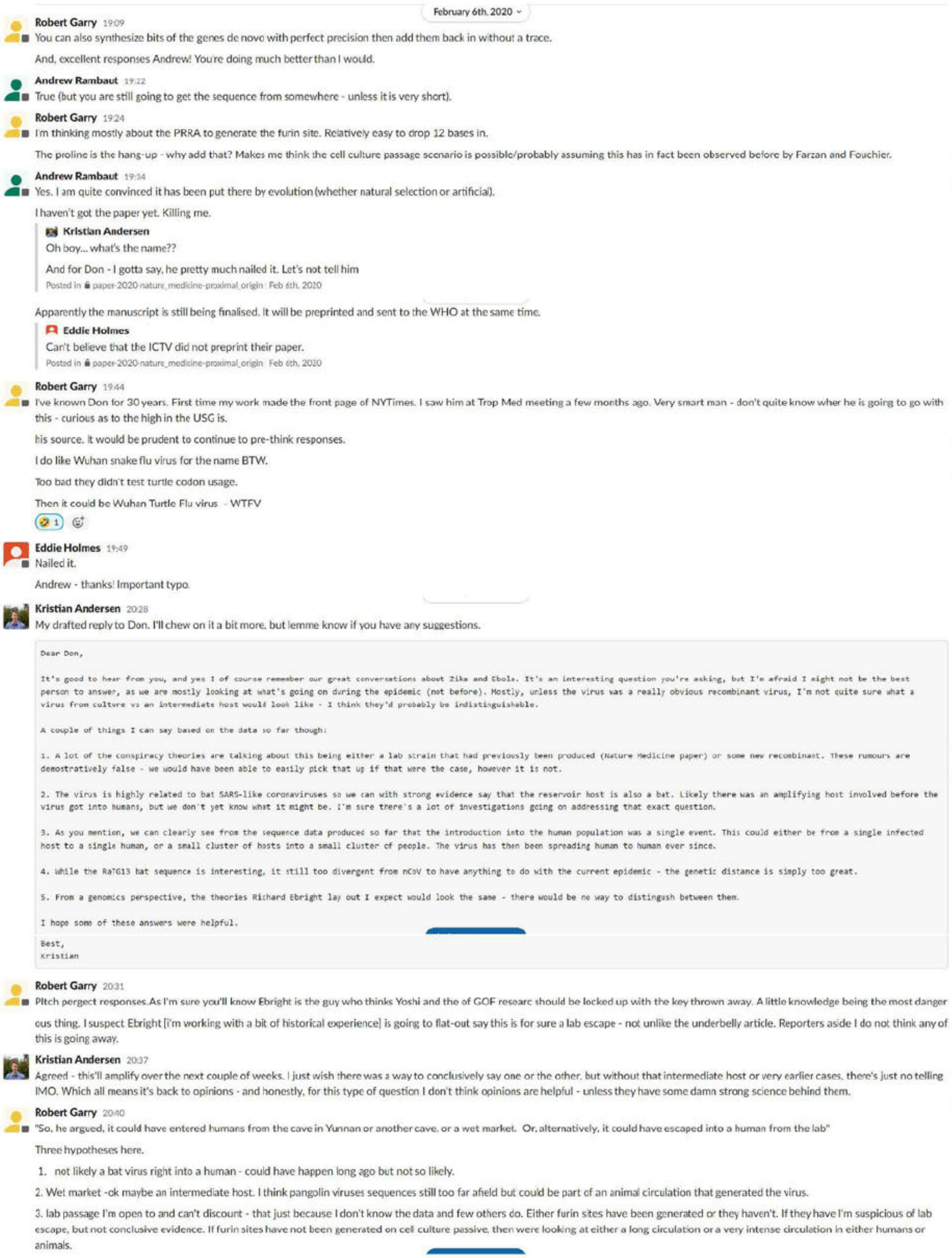
No. It we can't tell when it jumped from bats (or what species it jumped in to).

> Or can it imply that it's been circulating in humans for 40 years, without causing noticable symptoms, but picked up some sort of virulence mutation recently?  (and is that likely?)

Very unlikely, I think (both bits). A jump from a non-human animal is much more plausible as we know the viruses are out there and it has happened before. SARS was highly pathogenic when it jumped from animals.

I wouldn't read too much into the '40 year gap' - all it tells you is that RaT13 has little to do with this outbreak.
```

February 6th, 2020



February 6th, 2020

Robert Garry 19:09
You can also synthesize bits of the genes de novo with perfect precision then add them back in without a trace.

And, excellent responses Andrew! You're doing much better than I would.

Andrew Rambaut 19:22
True (but you are still going to get the sequence from somewhere - unless it is very short).

Robert Garry 19:24
I'm thinking mostly about the PRRA to generate the furin site. Relatively easy to drop 12 bases in.

The proline is the hang-up - why add that? Makes me think the cell culture passage scenario is possible/probably assuming this has in fact been observed before by Farzan and Fouchier.

Andrew Rambaut 19:34
Yes. I am quite convinced it has been put there by evolution (whether natural selection or artificial).

I haven't got the paper yet. Killing me.

> **Kristian Andersen**
> Oh boy... what's the name??
>
> And for Don - I gotta say, he pretty much nailed it. Let's not tell him
>
> Posted in 🔒 paper-2020-nature_medicine-proximal_origin | Feb 6th, 2020

Apparently the manuscript is still being finalised. It will be preprinted and sent to the WHO at the same time.

> **Eddie Holmes**
> Can't believe that the ICTV did not preprint their paper.
>
> Posted in 🔒 paper-2020-nature_medicine-proximal_origin | Feb 6th, 2020

Robert Garry 19:44
I've known Don for 30 years. First time my work made the front page of NYTimes. I saw him at Trop Med meeting a few months ago. Very smart man - don't quite know wher he is going to go with this - curious as to the high in the USG is.

his source. It would be prudent to continue to pre-think responses.

I do like Wuhan snake flu virus for the name BTW.

Too bad they didn't test turtle codon usage.

Then it could be Wuhan Turtle Flu virus - WTFV

🤣 1

Eddie Holmes 19:49
Nailed it.

Andrew - thanks! Important typo.

Kristian Andersen 20:28
My drafted reply to Don. I'll chew on it a bit more, but lemme know if you have any suggestions.

```
Dear Don,

It's good to hear from you, and yes I of course remember our great conversations about Zika and Ebola. It's an interesting question you're asking, but I'm afraid I might not be the best
person to answer, as we are mostly looking at what's going on during the epidemic (not before). Mostly, unless the virus was a really obvious recombinant virus, I'm not quite sure what a
virus from culture vs an intermediate host would look like - I think they'd probably be indistinguishable.

A couple of things I can say based on the data so far though:

1. A lot of the conspiracy theories are talking about this being either a lab strain that had previously been produced (Nature Medicine paper) or some new recombinant. These rumours are
demostratively false - we would have been able to easily pick that up if that were the case, however it is not.

2. The virus is highly related to bat SARS-like coronaviruses so we can with strong evidence say that the reservoir host is also a bat. Likely there was an amplifying host involved before the
virus got into humans, but we don't yet know what it might be. I'm sure there's a lot of investigations going on addressing that exact question.

3. As you mention, we can clearly see from the sequence data produced so far that the introduction into the human population was a single event. This could either be from a single infected
host to a single human, or a small cluster of hosts into a small cluster of people. The virus has then been spreading human to human ever since.

4. While the RaTG13 bat sequence is interesting, it still too divergent from nCoV to have anything to do with the current epidemic - the genetic distance is simply too great.

5. From a genomics perspective, the theories Richard Ebright lay out I expect would look the same - there would be no way to distingush between them.

I hope some of these answers were helpful.

Best,
Kristian
```

Robert Garry 20:31
Pitch pergect responses.As I'm sure you'll know Ebright is the guy who thinks Yoshi and the of GOF researc should be locked up with the key thrown away. A little knowledge being the most dangerous thing. I suspect Ebright [i'm working with a bit of historical experience] is going to flat-out say this is for sure a lab escape - not unlike the underbelly article. Reporters aside I do not think any of this is going away.

Kristian Andersen 20:37
Agreed - this'll amplify over the next couple of weeks. I just wish there was a way to conclusively say one or the other, but without that intermediate host or very earlier cases, there's just no telling IMO. Which all means it's back to opinions - and honestly, for this type of question I don't think opinions are helpful - unless they have some damn strong science behind them.

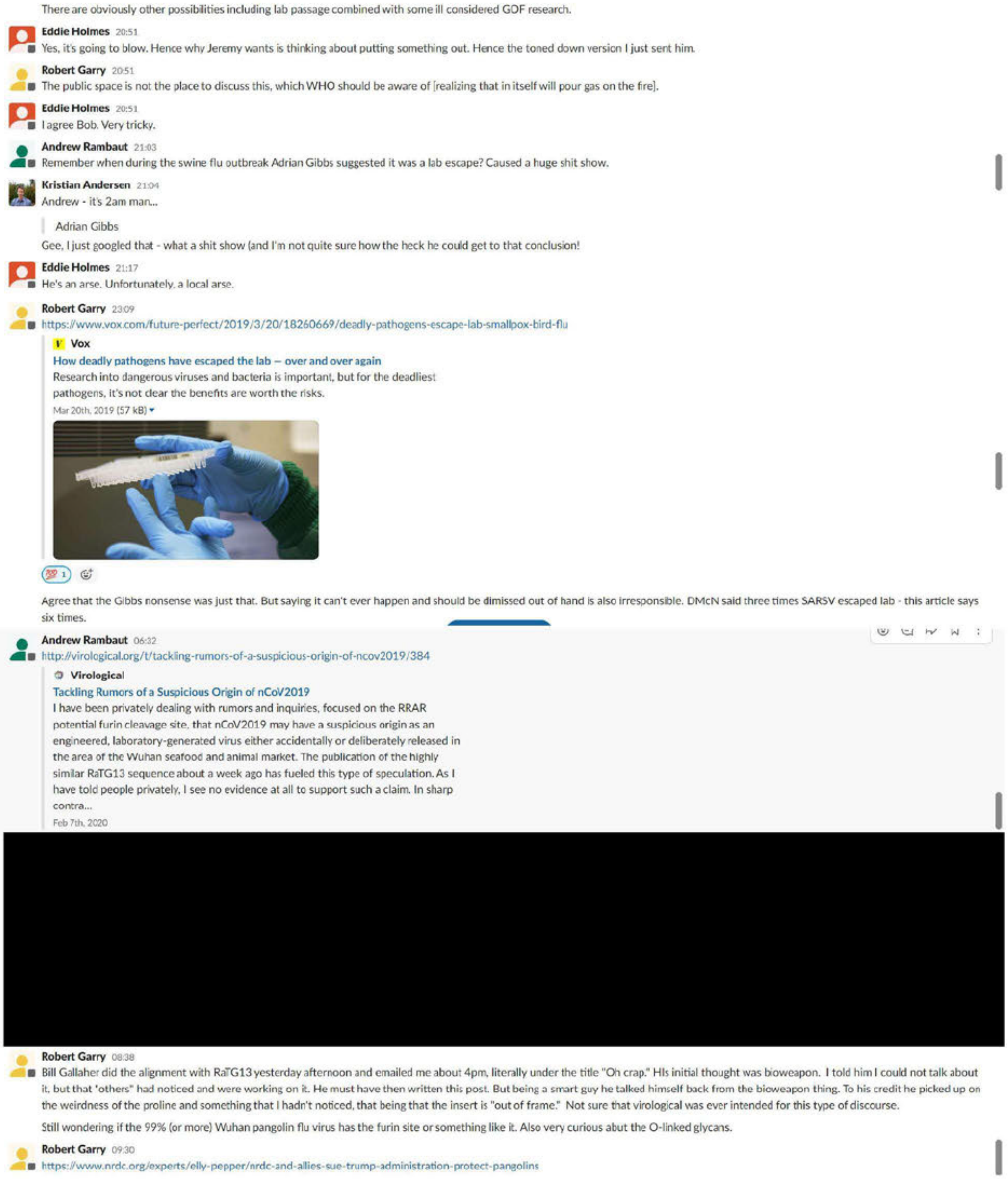
Robert Garry 20:40
"So, he argued, it could have entered humans from the cave in Yunnan or another cave, or a wet market. Or, alternatively, it could have escaped into a human from the lab"

Three hypotheses here.

1. not likely a bat virus right into a human - could have happen long ago but not so likely.

2. Wet market -ok maybe an intermediate host. I think pangolin viruses sequences still too far afield but could be part of an animal circulation that generated the virus.

3. lab passage I'm open to and can't discount - that just because I don't know the data and few others do. Either furin sites have been generated or they haven't. If they have I'm suspicious of lab escape, but not conclusive evidence. If furin sites have not been generated on cell culture passive, then were looking at either a long circulation or a very intense circulation in either humans or animals.



There are obviously other possibilities including lab passage combined with some ill considered GOF research.

Eddie Holmes 20:51
Yes, it's going to blow. Hence why Jeremy wants is thinking about putting something out. Hence the toned down version I just sent him.

Robert Garry 20:51
The public space is not the place to discuss this, which WHO should be aware of [realizing that in itself will pour gas on the fire].

Eddie Holmes 20:51
I agree Bob. Very tricky.

Andrew Rambaut 21:03
Remember when during the swine flu outbreak Adrian Gibbs suggested it was a lab escape? Caused a huge shit show.

Kristian Andersen 21:04
Andrew - it's 2am man...

> Adrian Gibbs

Gee, I just googled that - what a shit show (and I'm not quite sure how the heck he could get to that conclusion!

Eddie Holmes 21:17
He's an arse. Unfortunately, a local arse.

Robert Garry 23:09
https://www.vox.com/future-perfect/2019/3/20/18260669/deadly-pathogens-escape-lab-smallpox-bird-flu

> **Vox**
> How deadly pathogens have escaped the lab — over and over again
> Research into dangerous viruses and bacteria is important, but for the deadliest pathogens, it's not clear the benefits are worth the risks.
> Mar 20th, 2019 (57 kB)

💯 1

Agree that the Gibbs nonsense was just that. But saying it can't ever happen and should be dimissed out of hand is also irresponsible. DMcN said three times SARSV escaped lab - this article says six times.

Andrew Rambaut 06:32
http://virological.org/t/tackling-rumors-of-a-suspicious-origin-of-ncov2019/384

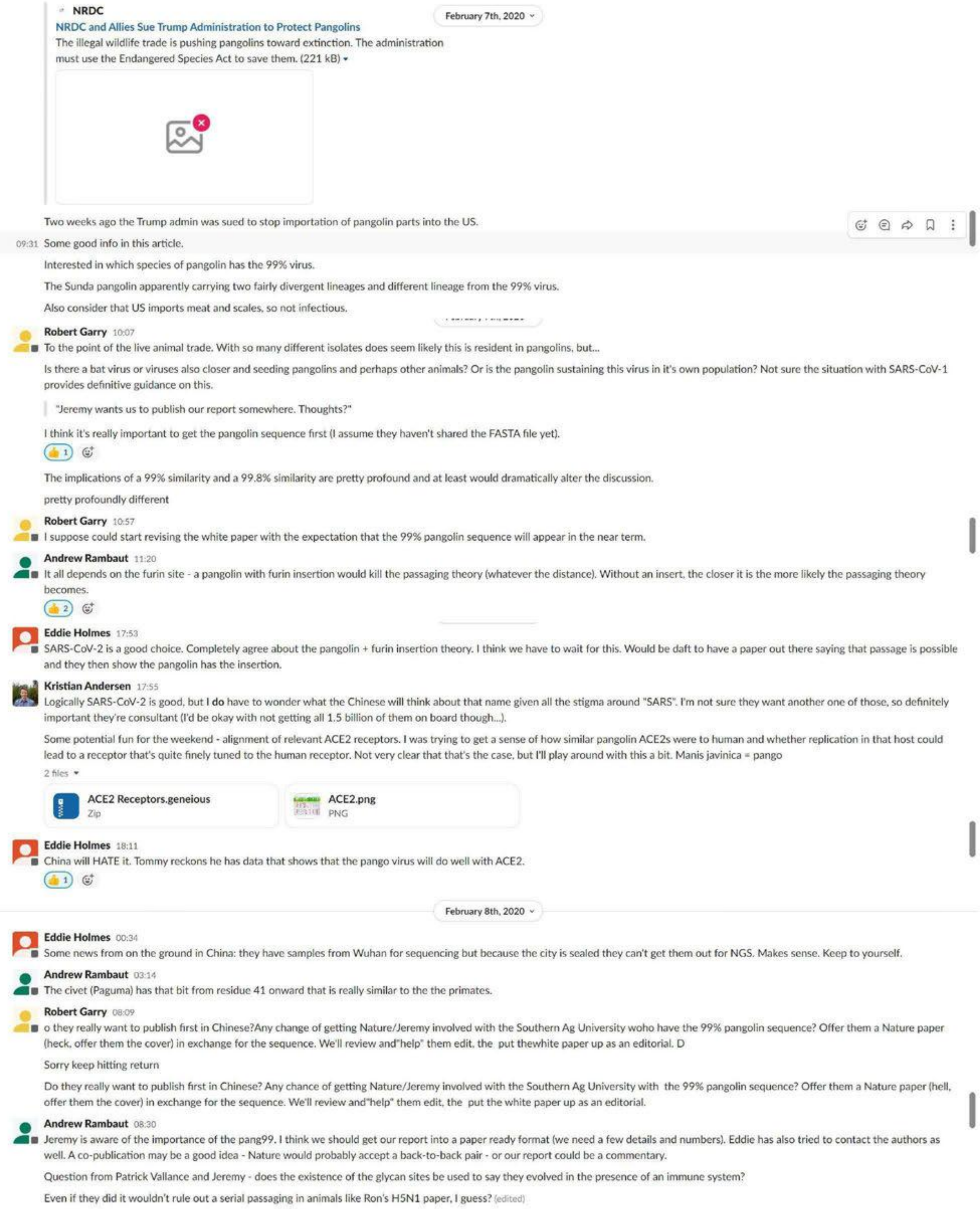
> **Virological**
> Tackling Rumors of a Suspicious Origin of nCoV2019
> I have been privately dealing with rumors and inquiries, focused on the RRAR potential furin cleavage site, that nCoV2019 may have a suspicious origin as an engineered, laboratory-generated virus either accidentally or deliberately released in the area of the Wuhan seafood and animal market. The publication of the highly similar RaTG13 sequence about a week ago has fueled this type of speculation. As I have told people privately, I see no evidence at all to support such a claim. In sharp contra...
> Feb 7th, 2020

[redacted]

Robert Garry 08:38
Bill Gallaher did the alignment with RaTG13 yesterday afternoon and emailed me about 4pm, literally under the title "Oh crap." HIs initial thought was bioweapon. I told him I could not talk about it, but that "others" had noticed and were working on it. He must have then written this post. But being a smart guy he talked himself back from the bioweapon thing. To his credit he picked up on the weirdness of the proline and something that I hadn't noticed, that being that the insert is "out of frame." Not sure that virological was ever intended for this type of discourse.

Still wondering if the 99% (or more) Wuhan pangolin flu virus has the furin site or something like it. Also very curious abut the O-linked glycans.

Robert Garry 09:30
https://www.nrdc.org/experts/elly-pepper/nrdc-and-allies-sue-trump-administration-protect-pangolins



NRDC

NRDC and Allies Sue Trump Administration to Protect Pangolins

The illegal wildlife trade is pushing pangolins toward extinction. The administration must use the Endangered Species Act to save them. (221 kB)

Two weeks ago the Trump admin was sued to stop importation of pangolin parts into the US.

09:31 Some good info in this article.

Interested in which species of pangolin has the 99% virus.

The Sunda pangolin apparently carrying two fairly divergent lineages and different lineage from the 99% virus.

Also consider that US imports meat and scales, so not infectious.

Robert Garry 10:07

To the point of the live animal trade. With so many different isolates does seem likely this is resident in pangolins, but...

Is there a bat virus or viruses also closer and seeding pangolins and perhaps other animals? Or is the pangolin sustaining this virus in it's own population? Not sure the situation with SARS-CoV-1 provides definitive guidance on this.

> "Jeremy wants us to publish our report somewhere. Thoughts?"

I think it's really important to get the pangolin sequence first (I assume they haven't shared the FASTA file yet).

👍 1

The implications of a 99% similarity and a 99.8% similarity are pretty profound and at least would dramatically alter the discussion.

pretty profoundly different

Robert Garry 10:57

I suppose could start revising the white paper with the expectation that the 99% pangolin sequence will appear in the near term.

Andrew Rambaut 11:20

It all depends on the furin site - a pangolin with furin insertion would kill the passaging theory (whatever the distance). Without an insert, the closer it is the more likely the passaging theory becomes.

👍 2

Eddie Holmes 17:53

SARS-CoV-2 is a good choice. Completely agree about the pangolin + furin insertion theory. I think we have to wait for this. Would be daft to have a paper out there saying that passage is possible and they then show the pangolin has the insertion.

Kristian Andersen 17:55

Logically SARS-CoV-2 is good, but I **do** have to wonder what the Chinese will think about that name given all the stigma around "SARS". I'm not sure they want another one of those, so definitely important they're consultant (I'd be okay with not getting all 1.5 billion of them on board though...).

Some potential fun for the weekend - alignment of relevant ACE2 receptors. I was trying to get a sense of how similar pangolin ACE2s were to human and whether replication in that host could lead to a receptor that's quite finely tuned to the human receptor. Not very clear that that's the case, but I'll play around with this a bit. Manis javinica = pango

2 files

ACE2 Receptors.geneious
Zip

ACE2.png
PNG

Eddie Holmes 18:11

China will HATE it. Tommy reckons he has data that shows that the pango virus will do well with ACE2.

👍 1

Eddie Holmes 00:34

Some news from on the ground in China: they have samples from Wuhan for sequencing but because the city is sealed they can't get them out for NGS. Makes sense. Keep to yourself.

Andrew Rambaut 03:14

The civet (Paguma) has that bit from residue 41 onward that is really similar to the the primates.

Robert Garry 08:09

o they really want to publish first in Chinese?Any change of getting Nature/Jeremy involved with the Southern Ag University woho have the 99% pangolin sequence? Offer them a Nature paper (heck, offer them the cover) in exchange for the sequence. We'll review and"help" them edit, the put thewhite paper up as an editorial. D

Sorry keep hitting return

Do they really want to publish first in Chinese? Any chance of getting Nature/Jeremy involved with the Southern Ag University with the 99% pangolin sequence? Offer them a Nature paper (hell, offer them the cover) in exchange for the sequence. We'll review and"help" them edit, the put the white paper up as an editorial.

Andrew Rambaut 08:30

Jeremy is aware of the importance of the pang99. I think we should get our report into a paper ready format (we need a few details and numbers). Eddie has also tried to contact the authors as well. A co-publication may be a good idea - Nature would probably accept a back-to-back pair - or our report could be a commentary.

Question from Patrick Vallance and Jeremy - does the existence of the glycan sites be used to say they evolved in the presence of an immune system?

Even if they did it wouldn't rule out a serial passaging in animals like Ron's H5N1 paper, I guess? (edited)

February 8th, 2020

Robert Garry 08:43

I'd say the existance of the glycans is pretty strong evidence of evolution in the presence of an immune system. I don't think it is random chance since the glycans appear in other betacoronaviruses that "evolve" a furin site, eg MHV and HKU1. MHV and HKU1 also simultaneously evolve a variable and sometimes large patch of O-linked glycans at the top of the prefusion (virion) form of the spike. Seems pretty clear this is immune based selection all around to me.

Yes serial passage in animals would do the same thing. There are a couple passage of H5N1 in chicken papers - the furin site appears in steps.

Hopefully the pangolin 99% CoV shows up with a furin site - if not as Andrew said passage becomes more likely.

If this is going high profile we need to add a few things.

A diagram outlining the three scenarios with cartoons of bats and pangolins. Don't make the cell culture passage scientist look asian (but maybe resemble an Ego guy). Could even have a bioweapon scenario with a big X.

Maybe some sort of diagram of the overall spike model - Kristian made a pdb, and so did I so can do this pointingg out the furin site and o glycan if this sounds like a possibility.

Andrew Rambaut 08:51

I have created a copy of the report to turn into something publishable: https://docs.google.com/document/d/14Hl21tdEyXQSXBBDC2KwHxSrKffyMdKWdMZGXxbd2z8/e

08:52 We need a carton picture of Peter Daszak to use in all the figures.

I don't think we should go anywhere near bioweapons - excluding lab constructs is sufficient.

It might be a good idea to nail the Lyons-Weiler stuff without mentioning it explictly - i.e., say there is no evidence of insertions or recombination from other known viruses (including SARS). The entire nCoV genome is descended from a putative common ancestor with RaTG13.

February 8th, 2020

Robert Garry 08:57

Stating the obvious: When the pangolin 99% sequence comes we're (and nobody better) are going to have to evaluate whether this jumped straight into people. We know the number of mutations from the SARS-CoV-1 market animals to people. Is this in the same range or does the pabgolin virus have too many mutations (incuding or not the furin or mucin) to be the immediate progenitor? Will need to include perhaps in a diagram.

Robert Garry 09:03

close enough?

image.png

1125 × 1390

Andrew Rambaut 09:04

That will do. Not implying anything about nefarious goings on.

Agreed. I was thinking of doing a quick analysis to estimate the date of the common ancestor with RaTG13 based on a reasonable range of rates. We could then reverse that and give the expected number of substitutions for a recent common ancestor - although I am not sure we know how recently a nCoV-pang99 MRCA would need to be. 1% divergence would imply about 5 years back in time (minimum - given current nCoV rate estimates). But we wouldn't expect to get the real progenitor unless it was basically in Wuhan market.

Robert Garry 09:10

Perfect

Robert Garry 09:17

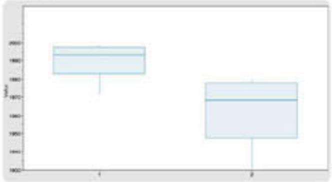
I could see the other pangolin sequences factoring in as well. If they are closer in the RBD - and as Kristian is teaching us they're pretty damn close, and pang99 is closer elsewhere except in the binding domain then you could have a recombinant. Should be "straightforward" or not to rule this out once pang99 comes.

Yeah - big differnce in implications between 99.0 and 99.8%. If I had to guess I'd say is closer to the former or else we'd be hearing how pang99 was nearly 100% similar.

Andrew Rambaut 09:32

Estimates of the date of common ancestor of nCoV and BaTG13 assuming a rate of 1e-3 (left) and 0.5e-3 (right)

image.png



95% credible intervals:
rate 1e-3: 1982.9271, 1997.564
rate 0.5e-3: 1947.6461, 1978.0808

So basically not more recently than 1997

Andrew Rambaut 09:43

@Robert Garry - I forwarded your reply about the glycans to Jeremy. He asks if it is OK to forward that to the whole group? (edited)

Robert Garry 09:55

sure!

Robert Garry 12:42

anyone want to take a stab at Tony Fauci

s question?

↓ Latest messages

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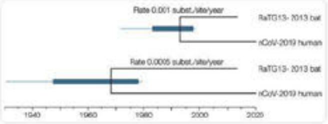
Andrew Rambaut 12:55

I guess the simple answer is no - there is no difference between a natural infection and a passaged infection. You could argue the transmission bottleneck might be larger?

TMRCA_figure.png



Robert Garry 13:03

Well - I already sent an answer - not incompatible with what you're saying - in the lab you can overcome the bottleneck.

Great looking figure!

Robert Garry 13:21

https://www.bbc.com/news/world-51429400

BBC - actual reporting - at least they usually try - we have very little of that left in the US.

Robert Garry 14:47

Comments - as predicted - by Ron Fouchier up on the email.

Eddie Holmes 15:32

Crap comments....basically just saying it can't be true.

↓ Latest messages

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Andrew Rambaut 15:43

Yes. Conflating the absence of evidence (passaging) with actual evidence against (engineering).

Argument about the other viruses is facile

Robert Garry 15:47

Agreed

Kristian Andersen 15:53

Super frustrating comments. To Ron's "As far as I am aware, no laboratory has worked on passaging the pangolin-origin virus, the bat-CoV RaTG13, or another closely related virus or had access to it prior to the outbreak" - not only has this been done, it's specifically being done in Wuhan. In BSL-2. That in itself means that we can't just dismiss a lab theory off hand by saying "not possible". That would be very foolhardy.

Kristian Andersen 16:04

The furin link keeps bugging me too - I can't find *any* good references on it in the published literature for CoVs. When I asked Mike, he linked to this paper, which doesn't really describe it either: https://jvi.asm.org/content/79/22/14451?ijkey=709aa5da9513e80f42db103ec19b539ed1cc350b&keytype2=tf_ipsecsha

February 8th, 2020

> **Journal of Virology**
>
> Murine Coronavirus with an Extended Host Range Uses Heparan Sulfate as an Entry Receptor
>
> Only a relatively few mutations in its spike protein allow the murine coronavirus to switch from a murine-restricted tropism to an extended host range by being passaged in vitro. One such virus that we studied had acquired two putative heparan sulfate-binding sites while preserving another site in the furin-cleavage motif. The adaptation of the virus through the use of heparan sulfate as an attachment/entry receptor was demonstrated by increased heparin binding as well as by inhibition of infection through treatment of cells and the virus with heparinase and heparin, respectively.
>
> Nov 15th, 2005

Robert Garry 16:06

Kristian you were on the NASEM call I think - who was it that volunteered that furin sites appear if you passage CoV in culture?

Andrew Rambaut 16:19

@Kristian With respect to this -

```
As to publishing this document in a journal, I am currently not in favor of doing so. I believe that publishing something that is open-ended could backfire at this stage. I think it's important that we try to gather additional evidence - including waiting on the pangolin virus sequences and further scrutinize the furin cleavage site and O-linked glycans - before publishing. That way we can (hopefully) come out with some strong conclusive statements that are based on the best data we have access to. I don't think we are there yet.
```

What do you think we should do?

What do you think we should do?

February 8th, 2020

Kristian Andersen 16:21

We should all just stay on Slack, that's what we should do - and not use email 😉. Check my other email... I definitely think we should move towards publication and create a separate document focused on that, but I think it's too early at the moment.

Btw - very strong comments from A+E here - it's unbelievable how conflicted Ron is.

Robert Garry 16:30

We now have (and we will get more) the pangolin data (Eddie has) we think we can tie this up even tighter with the next iteration and make a conclusive statement which will then be the go to scientific statement to refer to.

Eddie and I have just come off a call with the National Academy of Medicine in the US - who the White House has asked to produce a report on this....

Moving fast - don't think we should necessarily wait on the NAM to get something out there if pango99 seq is available.

Kristian Andersen 16:40

NASEM is useless - they'll have exactly zero... Too political an organization.

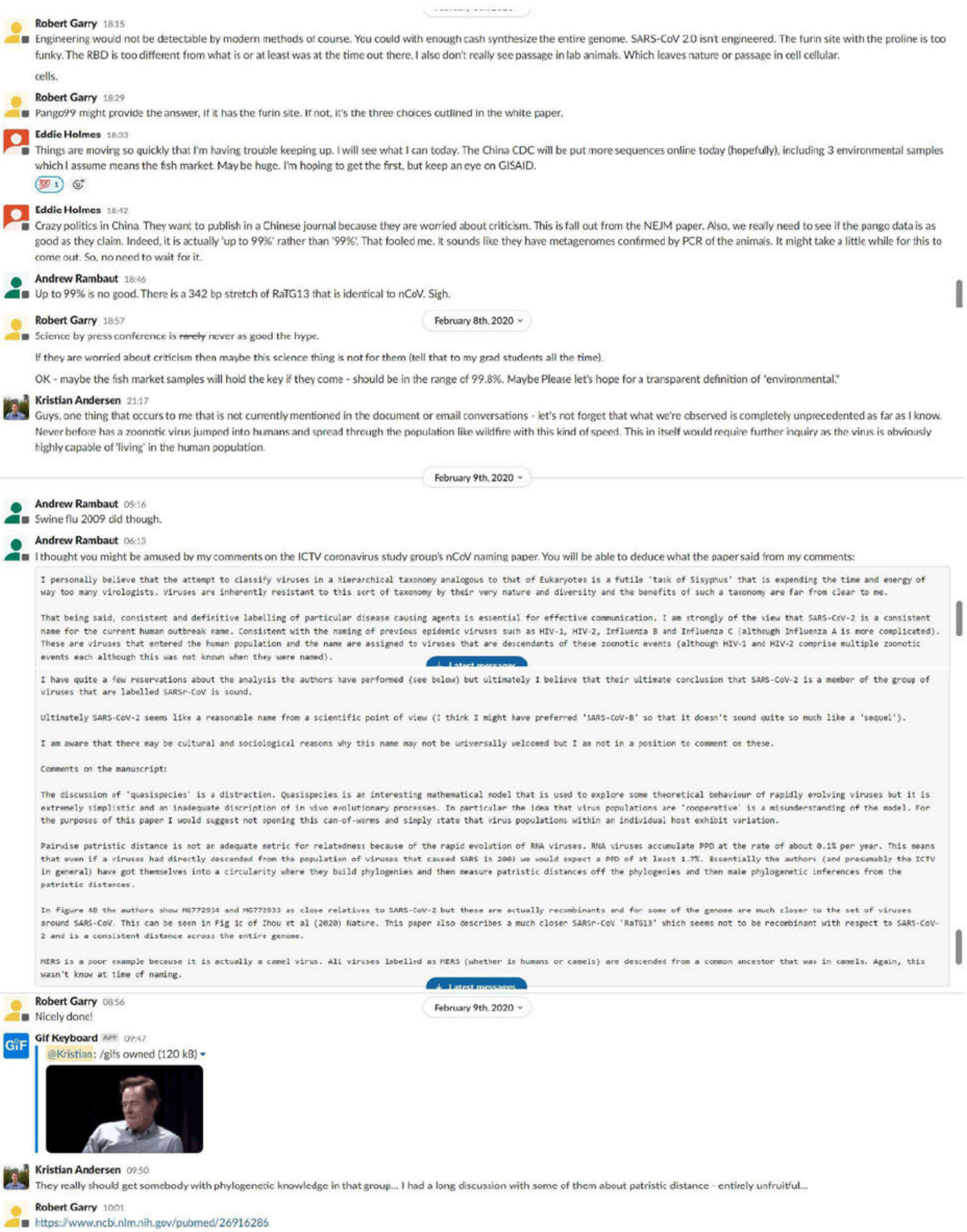
Kristian Andersen 17:52

So he agrees? "I do not understand Andrews argument " The sequence data clearly and unambiguously rules out any form of lab construct or engineering of the virus. ". Molecular biologists like myself can generate perfect copies of viruses without leaving a trace, eg the BamHI site. The arguments for and against passaging and engineering are the same if you ask me."

🤷

Robert Garry 18:10

Nature and passaging in cells or animals will generate unpredictable changes, thou we might make some rather generalized guesses as to what may pop up.



Robert Garry 18:15

Engineering would not be detectable by modern methods of course. You could with enough cash synthesize the entire genome. SARS-CoV 2.0 isn't engineered. The furin site with the proline is too funky. The RBD is too different from what is or at least was at the time out there. I also don't really see passage in lab animals. Which leaves nature or passage in cell cellular.

cells.

Robert Garry 18:29

Pango99 might provide the answer, if it has the furin site. If not, it's the three choices outlined in the white paper.

Eddie Holmes 18:33

Things are moving so quickly that I'm having trouble keeping up. I will see what I can today. The China CDC will be put more sequences online today (hopefully), including 3 environmental samples which I assume means the fish market. May be huge. I'm hoping to get the first, but keep an eye on GISAID.

💯 1

Eddie Holmes 18:42

Crazy politics in China. They want to publish in a Chinese journal because they are worried about criticism. This is fall out from the NEJM paper. Also, we really need to see if the pango data is as good as they claim. Indeed, it is actually 'up to 99%' rather than '99%'. That fooled me. It sounds like they have metagenomes confirmed by PCR of the animals. It might take a little while for this to come out. So, no need to wait for it.

Andrew Rambaut 18:46

Up to 99% is no good. There is a 342 bp stretch of RaTG13 that is identical to nCoV. Sigh.

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Robert Garry 18:57

Science by press conference is ~~rarely~~ never as good the hype.

If they are worried about criticism then maybe this science thing is not for them (tell that to my grad students all the time).

OK - maybe the fish market samples will hold the key if they come - should be in the range of 99.8%. Maybe Please let's hope for a transparent definition of "environmental."

Kristian Andersen 21:17

Guys, one thing that occurs to me that is not currently mentioned in the document or email conversations - let's not forget that what we're observed is completely unprecedented as far as I know. Never before has a zoonotic virus jumped into humans and spread through the population like wildfire with this kind of speed. This in itself would require further inquiry as the virus is obviously highly capable of 'living' in the human population.

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Andrew Rambaut 05:16

Swine flu 2009 did though.

Andrew Rambaut 06:13

I thought you might be amused by my comments on the ICTV coronavirus study group's nCoV naming paper. You will be able to deduce what the paper said from my comments:

> I personally believe that the attempt to classify viruses in a hierarchical taxonomy analogous to that of Eukaryotes is a futile 'task of Sisyphus' that is expending the time and energy of way too many virologists. Viruses are inherently resistant to this sort of taxonomy by their very nature and diversity and the benefits of such a taxonomy are far from clear to me.
>
> That being said, consistent and definitive labelling of particular disease causing agents is essential for effective communication. I am strongly of the view that SARS-CoV-2 is a consistent name for the current human outbreak name. Consistent with the naming of previous epidemic viruses such as HIV-1, HIV-2, Influenza B and Influenza C (although Influenza A is more complicated). These are viruses that entered the human population and the name are assigned to viruses that are descendants of these zoonotic events (although HIV-1 and HIV-2 comprise multiple zoonotic events each although this was not known when they were named).
>
> I have quite a few reservations about the analysis the authors have performed (see below) but ultimately I believe that their ultimate conclusion that SARS-CoV-2 is a member of the group of viruses that are labelled SARSr-CoV is sound.
>
> Ultimately SARS-CoV-2 seems like a reasonable name from a scientific point of view (I think I might have preferred 'SARS-CoV-B' so that it doesn't sound quite so much like a 'sequel').
>
> I am aware that there may be cultural and sociological reasons why this name may not be universally welcomed but I am not in a position to comment on these.
>
> Comments on the manuscript:
>
> The discussion of 'quasispecies' is a distraction. Quasispecies is an interesting mathematical model that is used to explore some theoretical behaviour of rapidly evolving viruses but it is extremely simplistic and an inadequate discription of in vivo evolutionary processes. In particular the idea that virus populations are 'cooperative' is a misunderstanding of the model. For the purposes of this paper I would suggest not opening this can-of-worms and simply state that virus populations within an individual host exhibit variation.
>
> Pairwise patristic distance is not an adequate metric for relatedness because of the rapid evolution of RNA viruses. RNA viruses accumulate PPD at the rate of about 0.1% per year. This means that even if a viruses had directly descended from the population of viruses that caused SARS in 2003 we would expect a PPD of at least 1.7%. Essentially the authors (and presumably the ICTV in general) have got themselves into a circularity where they build phylogenies and then measure patristic distances off the phylogenies and then make phylogenetic inferences from the patristic distances.
>
> In figure 4B the authors show MG772934 and MG772933 as close relatives to SARS-CoV-2 but these are actually recombinants and for some of the genome are much closer to the set of viruses around SARS-CoV. This can be seen in Fig 1c of Zhou et al (2020) Nature. This paper also describes a much closer SARSr-CoV 'RaTG13' which seems not to be recombinant with respect to SARS-CoV-2 and is a consistent distance across the entire genome.
>
> MERS is a poor example because it is actually a camel virus. All viruses labelled as MERS (whether in humans or camels) are descended from a common ancestor that was in camels. Again, this wasn't know at time of naming.

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Robert Garry 08:56

Nicely done!

Gif Keyboard APP 09:47

@Kristian: /gifs owned (120 kB)

Kristian Andersen 09:50

They really should get somebody with phylogenetic knowledge in that group... I had a long discussion with some of them about patristic distance - entirely unfruitful...

Robert Garry 10:01

https://www.ncbi.nlm.nih.gov/pubmed/26916286

Molecular epidemiology and evolutionary histories of human coronavirus OC43 and HKU1 among patients with upper respiratory tract infections in Kual... - PubMed - NCBI

Virol J. 2016 Feb 25;13:33. doi: 10.1186/s12985-016-0488-4. Research Support, Non-U.S. Gov't (13 kB)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4476415/

Genetic drift of human coronavirus OC43 spike gene during adaptive evolution

Coronaviruses (CoVs) continuously threaten human health. However, to date, the evolutionary mechanisms that govern CoV strain persistence in human populations have not been fully understood. In this study, we characterized the evolution of the major antigen-spike ...

https://www.ncbi.nlm.nih.gov/pubmed/21849456

Molecular epidemiology of human coronavirus OC43 reveals evolution of different genotypes over time and recent emergence of a novel genotype due to... - PubMed - NCBI

J Virol. 2011 Nov;85(21):11325-37. doi: 10.1128/JVI.05512-11. Epub 2011 Aug 17. Research Support, Non-U.S. Gov't (13 kB)

Robert Garry 10:14

Becoming more convinced that SARS-CoV-2 furin site and O-linked glycans has precedence in other beta-coronaviruses, MHV, HKU1 and OC43. Variable S1/S2 cleavage sites and variable O-linked glycans. Also pertinent is the adaptive evolution of the RBD in these viruses. Also recombination. The variable S1/S2 cleavage sites and O-linked glycans seen in other subgroup A virus, but at least not yet in the b subgroup containing SARS-CoVs and related bat viruses.

Robert Garry 15:14

A few new comments on the email chains. Six minutes apart.

https://abcnews.go.com/Politics/white-house-asks-scientists-investigate-origins-coronavirus/story?id=68807304ABC News' Chief Medical Correspondent Dr. Jennifer Ashton asked the director of the National Institute of Allergy and Infectious Disease about concerns that stem from misinformation online that the novel coronavirus could have been engineered or deliberately released. "There's always that concern," Dr. Anthony Fauci said. "And one of the things that people are doing right now is very carefully looking at sequences to see if there's even any possibility much less likelihood that that's going on. And you could ultimately determine that. So people are looking at it, but right now, the focus is on what are we going to do about what we have."

White House asks scientists to investigate origins of coronavirus

The White House asked scientists and medical experts to research the origins of the novel coronavirus, in part to counter misinformation about the outbreak. (89 kB)

I think Fauci gave the correct answer regarding engineering or deliberate release. You need to look. It follows and makes sense that you also look at accidental release as a possibility (something BTW that happened with SARS-CoV-1 SEVERAL times.

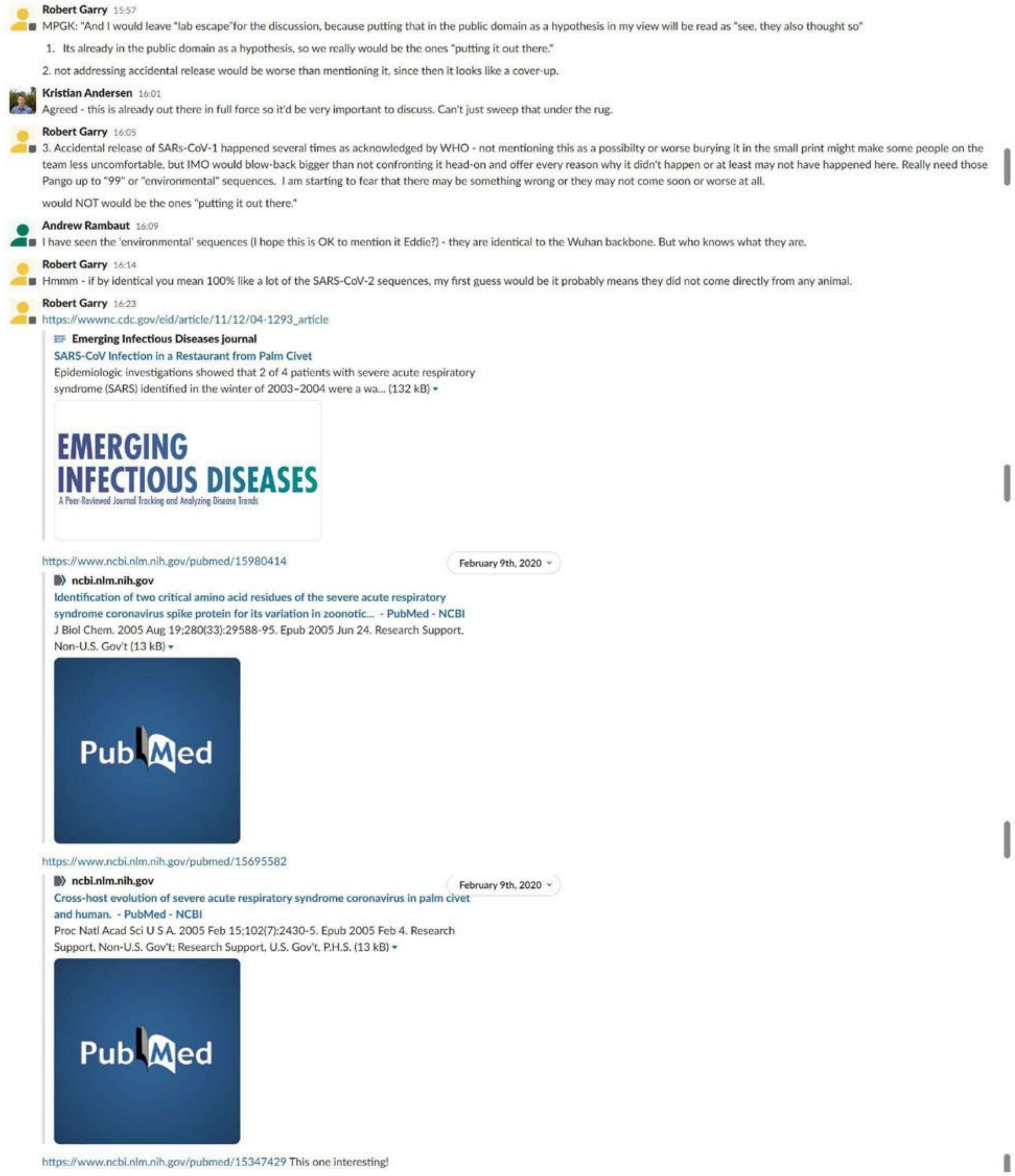
Call me conspiratorial (OK that horse left the barn), but I think there may be some hallway talk going on at Erasmus.

Kristian Andersen 15:39

I didn't realize both Ron and Marion are at Erasmus... Interesting. She makes some good points though that I agree on.

Good comments from Tony in that article - ever the politician.

[redacted]



Robert Garry 15:57

MPGK: "And I would leave "lab escape"for the discussion, because putting that in the public domain as a hypothesis in my view will be read as "see, they also thought so"

1. Its already in the public domain as a hypothesis, so we really would be the ones "putting it out there."

2. not addressing accidental release would be worse than mentioning it, since then it looks like a cover-up.

Kristian Andersen 16:01

Agreed - this is already out there in full force so it'd be very important to discuss. Can't just sweep that under the rug.

Robert Garry 16:05

3. Accidental release of SARs-CoV-1 happened several times as acknowledged by WHO - not mentioning this as a possibilty or worse burying it in the small print might make some people on the team less uncomfortable, but IMO would blow-back bigger than not confronting it head-on and offer every reason why it didn't happen or at least may not have happened here. Really need those Pango up to "99" or "environmental" sequences. I am starting to fear that there may be something wrong or they may not come soon or worse at all.

would NOT would be the ones "putting it out there."

Andrew Rambaut 16:09

I have seen the 'environmental' sequences (I hope this is OK to mention it Eddie?) - they are identical to the Wuhan backbone. But who knows what they are.

Robert Garry 16:14

Hmmm - if by identical you mean 100% like a lot of the SARS-CoV-2 sequences, my first guess would be it probably means they did not come directly from any animal.

Robert Garry 16:23

https://wwwnc.cdc.gov/eid/article/11/12/04-1293_article

Emerging Infectious Diseases journal

SARS-CoV Infection in a Restaurant from Palm Civet

Epidemiologic investigations showed that 2 of 4 patients with severe acute respiratory syndrome (SARS) identified in the winter of 2003–2004 were a wa... (132 kB) ▾

https://www.ncbi.nlm.nih.gov/pubmed/15980414

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ncbi.nlm.nih.gov

Identification of two critical amino acid residues of the severe acute respiratory syndrome coronavirus spike protein for its variation in zoonotic... - PubMed - NCBI

J Biol Chem. 2005 Aug 19;280(33):29588-95. Epub 2005 Jun 24. Research Support, Non-U.S. Gov't (13 kB) ▾

https://www.ncbi.nlm.nih.gov/pubmed/15695582

February 9th, 2020 ⌄

ncbi.nlm.nih.gov

Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. - PubMed - NCBI

Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4. Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S. (13 kB) ▾

https://www.ncbi.nlm.nih.gov/pubmed/15347429 This one interesting!

ncbi.nlm.nih.gov

Mutational dynamics of the SARS coronavirus in cell culture and human populations isolated in 2003. - PubMed - NCBI

BMC Infect Dis. 2004 Sep 6;4:32. Research Support, Non-U.S. Gov't (13 kB) ▾

Robert Garry 16:34

https://science.sciencemag.org/content/sci/early/2003/09/04/science.1087139.full.pdf Identical seems unexpected if from an animal source. Yes indeed would be good to know how the environment was sampled.

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Andrew Rambaut 17:58

Something that Richard Neher noticed - a mutation in ORF8 where the cluster sticking out with many of the recent cases matches RaTG13 (amino acid S) where as the so-called Wuhan outbreak sequences have a L:

image.png ▾

There is also a synonymous SNP in ORF1ab that shows the same pattern:

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image.png ▾

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This suggests a different rooting of the tree:

image.png ▾

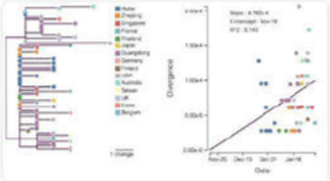
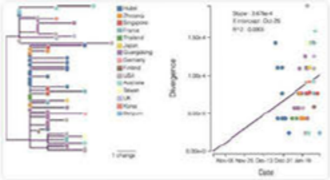


image.png ▾



Robert Garry 18:18
Very interesting and important. More evidence that the market was not the point source from which the outbreak sprang?

Andrew Rambaut 18:23
Need to see what the pangolin looks like!

Robert Garry 18:30
Oh yeah - the suspense is killing me....I suppose that's what beer is for.

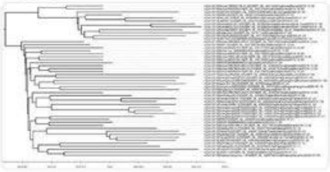
Eddie Holmes 18:37
Apologies, but I'm not going to be able to take part in these discussions much for a while because this storm has caused havoc. I've had no power for 24 hours and it might be another 24. It's a real mess. Need to do a clean up. A few things though: (i) what are we doing about this paper thing? I just can't get to it at the moment; (ii) the environmental seqs are spectacularly uninformative. Pretty shocking if this is the best they have; (iii) how do you interpret the alternative rooting? I can't work out the localities in the top clade.

96,000 houses without power. Alas, I live in the worst affected area. I only came into work to charge my devices.

Robert Garry 18:41
Nothing to apologize about - sorry for the mess, the distraction and the headaches.

Andrew Rambaut 18:43
This is the BEAST tree:

image.png ▾



Enforcing this root in BEAST doesn't really change things much. Rate 8.7e-4 (2.4e-4, 1.4e-3), TMRCA 2019-11-29 (2019-10-20, 2019-12-20). Exponential growth rate actually goes up - equivalent of a doubling time of 6.5 days.

Only one Wuhan sequence in the top clade but quite a few of the exports in that clade came from Wuhan.

You might think the bottom clade are from the market (human mediated spread?), top from prior circulating viruses.

Robert Garry 18:46
Waiting on pango up to 99. I was hoping the environmental samples would help, but the results made me uncomfortable. Afraid Pango99 might not be any more informative either. I think Kristian was going to take a stab at paper. The guidance from the email team not all that helpful either so far.

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Robert Garry 18:46
Waiting on pango up to 99. I was hoping the environmental samples would help, but the results made me uncomfortable. Afraid Pango99 might not be any more informative either. I think Kristian was going to take a stab at paper. The guidance from the email team not all that helpful either so far.

Eddie Holmes 19:00
Andrew, can I pass this info back to China CDC? Hopefully might loosen them to send more data.

Andrew Rambaut 19:55
Of course!

Nick Loman and I were looking at the genomes that went up yesterday (9 of them?). Some of them have weird errors in them (rows of 4 SNPS and things). We don't really know what is causing these errors.

Eddie Holmes 20:07
Thanks.

[redacted]

Kristian Andersen 22:12
@Andrew Rambaut did you take a look at the environmental samples? They look Wuham to me, but not particularly basal to the rest... Tells us nothing. I'm a little suspicious of these...

Kristian Andersen 22:31
Rooting of this tree in general is weird. Keeping the origin in Wuhan and taking RaTG13 into consideration it looks to me as if WH04 (406801) is the most logical root, but the RTT on that tree is hopeless. Multiple closely space intros? 🤷‍♂️ (edited)

↓ Latest messages

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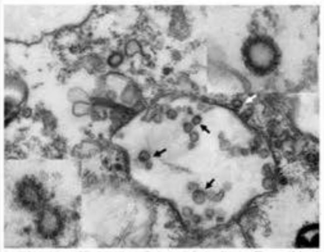
Robert Garry 09:17

I have some questions about this EM.

image.png



A typical coronavirus particle structure in pangolins is seen via electron microscopy. (Photo provided to chinadaily.com.cn)

http://www.chinadaily.com.cn/a/202002/07/WS5e3d1daca310128217275d93.html

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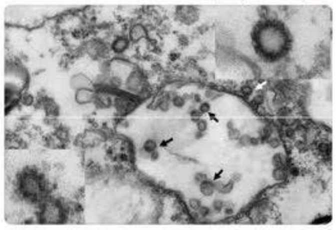
chinadaily.com.cn

Pangolin could be coronavirus intermediate host: Study - Chinadaily.com.cn

The pangolin might be a potential intermediate host of the novel coronavirus, as genome sequences of the disease strain separated from the animals were 99 percent identical to those found in infected people, a study has discovered. (102 kB)



From another article:

February 10th, 2020

GUANGZHOU, Feb. 7 (Xinhua) -- The genome sequence of the novel coronavirus strain separated from pangolins was 99 percent identical to that from infected people, indicating pangolins may be an intermediate host of the virus, a study has found.
The study was led by the South China Agricultural University. According to Liu Yahong, president of the university, the research team analyzed more than 1,000 metagenome samples of wild animals and found pangolins as the most likely intermediate host.
Molecular biological detection revealed that the positive rate of Betacoronavirus in pangolins was 70 percent. Researchers further isolated the virus and observed its structure with an electron microscope. They found that the genome sequence of the coronavirus strain was 99 percent identical to those in infected people.

Assuming this an accurate account the researchers did metagenomic studies of 1000 wild animal samples. Then they assembled genomes,and analyzed them.

Here's what keep me up last night:

THEN the "Researchers further isolated the virus and observed its structure with an electron microscope."

So - they grew it in cell culture. Those picture looks to me like growth in cultured cells - probably Vero. You can't get EM pictures out of animal tissues like this. Furthermore the virus is growing pretty damn well in those cells.

Robert Garry 09:41

This doesn't happen overnight. This likely means that the metagenomic study etc happen a while back. My BIGGEST question how far back. The first I heard of pangolin sequences on Virological about 10 days ago. My second BIG question - if they grew it in culture as they said how much did the virus change on passage? They surely did not grow the virus in pangolin cells. Gentlemen please walk me back on where my mind is wondering....

Andrew Rambaut 10:03

99% is not close enough.

Kristian Andersen 10:08

Those Guangdong sequences do look mighty basal though 😉

I think the likelihood of them quickly throwing these into culture to 'snap' some EM pictures is pretty high. Doesn't mean much though - getting EM and sequences within a couple of weeks is pretty reasonable if you know exactly what to do (these folks had a paper on pango sequences last year, so I assume they do).

Robert Garry 10:21

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6893680/figure/viruses-11-00979-f005/

PubMed Central (PMC)

Viral Metagenomics Revealed Sendai Virus and Coronavirus Infection of Malayan Pangolins (Manis javanica)

Pangolins are endangered animals in urgent need of protection. Identifying and cataloguing the viruses carried by pangolins is a logical approach to evaluate the range of potential pathogens and help with conservation. This study provides insight into ...

THis one?

Seems like different group in Guangdong than South China Ag but maybe they came together.

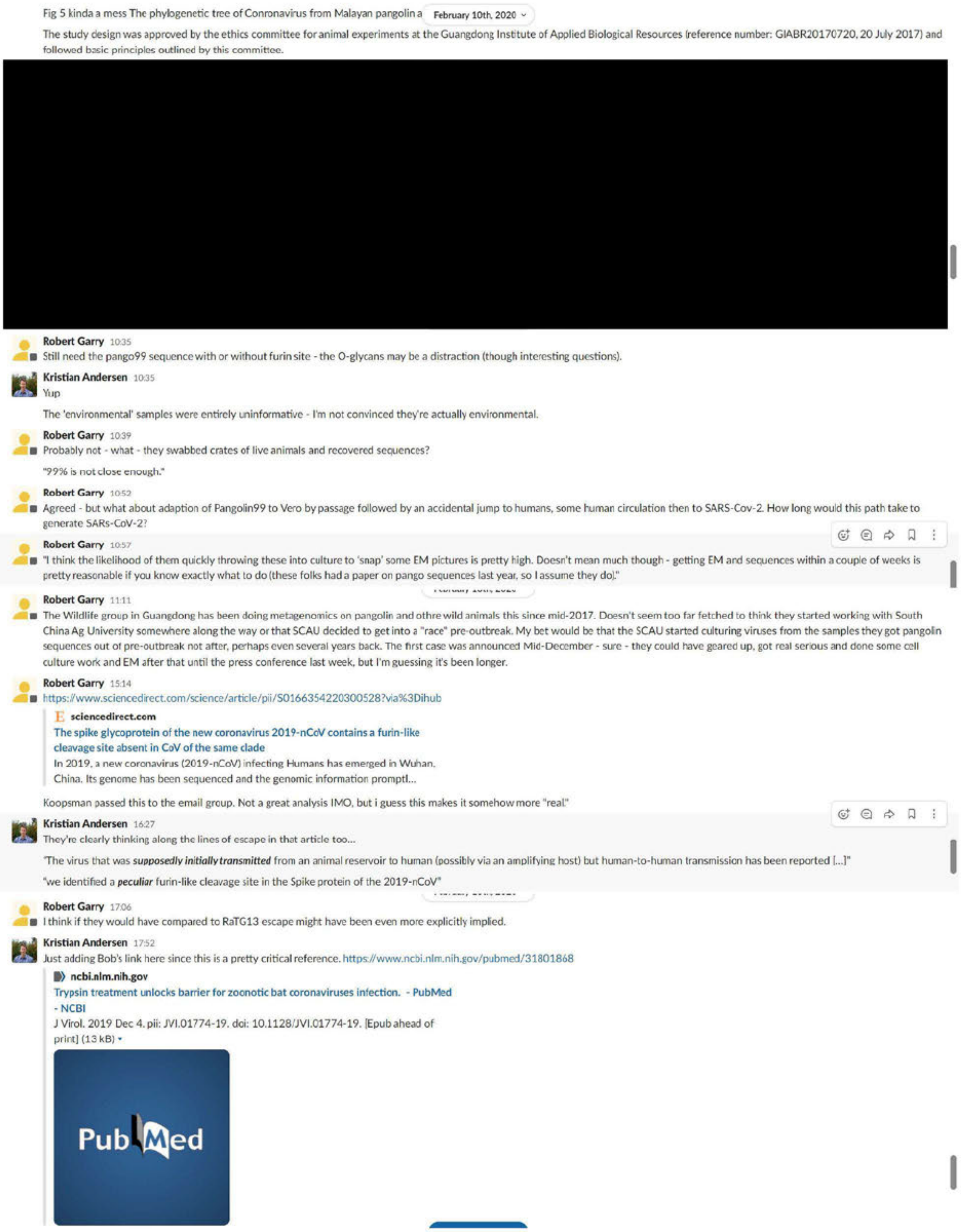


Fig 5 kinda a mess The phylogenetic tree of Conronavirus from Malayan pangolin a

The study design was approved by the ethics committee for animal experiments at the Guangdong Institute of Applied Biological Resources (reference number: GIABR20170720, 20 July 2017) and followed basic principles outlined by this committee.

[redacted]

Robert Garry 10:35
Still need the pango99 sequence with or without furin site - the O-glycans may be a distraction (though interesting questions).

Kristian Andersen 10:35
Yup

The 'environmental' samples were entirely uninformative - I'm not convinced they're actually environmental.

Robert Garry 10:39
Probably not - what - they swabbed crates of live animals and recovered sequences?

"99% is not close enough."

Robert Garry 10:52
Agreed - but what about adaption of Pangolin99 to Vero by passage followed by an accidental jump to humans, some human circulation then to SARS-Cov-2. How long would this path take to generate SARs-CoV-2?

Robert Garry 10:57
"I think the likelihood of them quickly throwing these into culture to 'snap' some EM pictures is pretty high. Doesn't mean much though - getting EM and sequences within a couple of weeks is pretty reasonable if you know exactly what to do (these folks had a paper on pango sequences last year, so I assume they do)."

Robert Garry 11:11
The Wildlife group in Guangdong has been doing metagenomics on pangolin and othre wild animals this since mid-2017. Doesn't seem too far fetched to think they started working with South China Ag University somewhere along the way or that SCAU decided to get into a "race" pre-outbreak. My bet would be that the SCAU started culturing viruses from the samples they got pangolin sequences out of pre-outbreak not after, perhaps even several years back. The first case was announced Mid-December - sure - they could have geared up, got real serious and done some cell culture work and EM after that until the press conference last week, but I'm guessing it's been longer.

Robert Garry 15:14
https://www.sciencedirect.com/science/article/pii/S0166354220300528?via%3Dihub

sciencedirect.com
The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade
In 2019, a new coronavirus (2019-nCoV) infecting Humans has emerged in Wuhan, China. Its genome has been sequenced and the genomic information promptl...

Koopsman passed this to the email group. Not a great analysis IMO, but i guess this makes it somehow more "real."

Kristian Andersen 16:27
They're clearly thinking along the lines of escape in that article too...

"The virus that was ***supposedly initially transmitted*** from an animal reservoir to human (possibly via an amplifying host) but human-to-human transmission has been reported [...]"

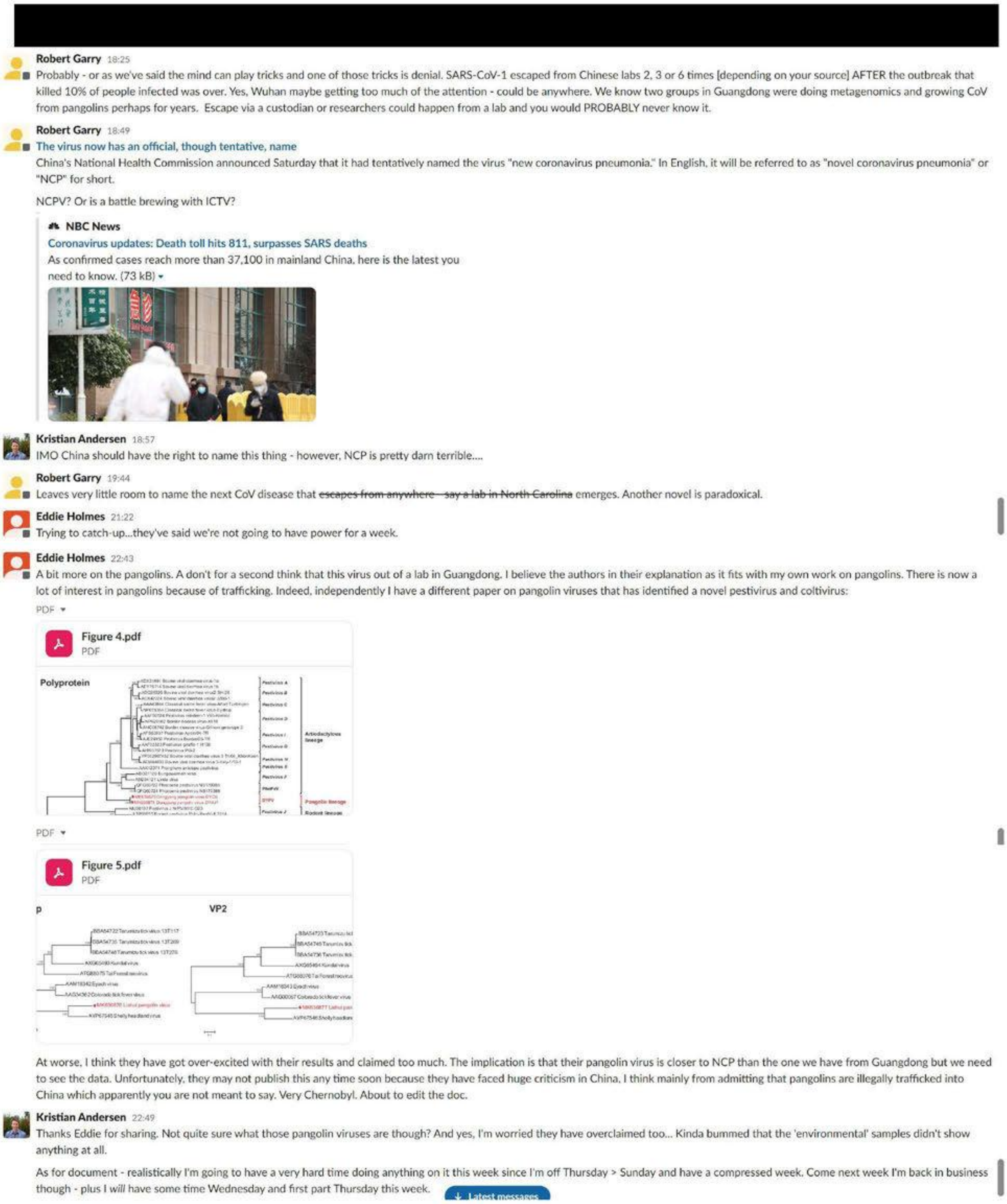
"we identified a ***peculiar*** furin-like cleavage site in the Spike protein of the 2019-nCoV"

Robert Garry 17:06
I think if they would have compared to RaTG13 escape might have been even more explicitly implied.

Kristian Andersen 17:52
Just adding Bob's link here since this is a pretty critical reference. https://www.ncbi.nlm.nih.gov/pubmed/31801868

ncbi.nlm.nih.gov
Trypsin treatment unlocks barrier for zoonotic bat coronaviruses infection. - PubMed - NCBI
J Virol. 2019 Dec 4. pii: JVI.01774-19. doi: 10.1128/JVI.01774-19. [Epub ahead of print] (13 kB)

[redacted]



Robert Garry 18:25
Probably - or as we've said the mind can play tricks and one of those tricks is denial. SARS-CoV-1 escaped from Chinese labs 2, 3 or 6 times [depending on your source] AFTER the outbreak that killed 10% of people infected was over. Yes, Wuhan maybe getting too much of the attention - could be anywhere. We know two groups in Guangdong were doing metagenomics and growing CoV from pangolins perhaps for years. Escape via a custodian or researchers could happen from a lab and you would PROBABLY never know it.

Robert Garry 18:49
The virus now has an official, though tentative, name
China's National Health Commission announced Saturday that it had tentatively named the virus "new coronavirus pneumonia." In English, it will be referred to as "novel coronavirus pneumonia" or "NCP" for short.

NCPV? Or is a battle brewing with ICTV?

> **NBC News**
> Coronavirus updates: Death toll hits 811, surpasses SARS deaths
> As confirmed cases reach more than 37,100 in mainland China, here is the latest you need to know. (73 kB) ▾

Kristian Andersen 18:57
IMO China should have the right to name this thing - however, NCP is pretty darn terrible....

Robert Garry 19:44
Leaves very little room to name the next CoV disease that ~~escapes from anywhere - say a lab in North Carolina~~ emerges. Another novel is paradoxical.

Eddie Holmes 21:22
Trying to catch-up...they've said we're not going to have power for a week.

Eddie Holmes 22:43
A bit more on the pangolins. A don't for a second think that this virus out of a lab in Guangdong. I believe the authors in their explanation as it fits with my own work on pangolins. There is now a lot of interest in pangolins because of trafficking. Indeed, independently I have a different paper on pangolin viruses that has identified a novel pestivirus and coltivirus:

PDF ▾

Figure 4.pdf
PDF

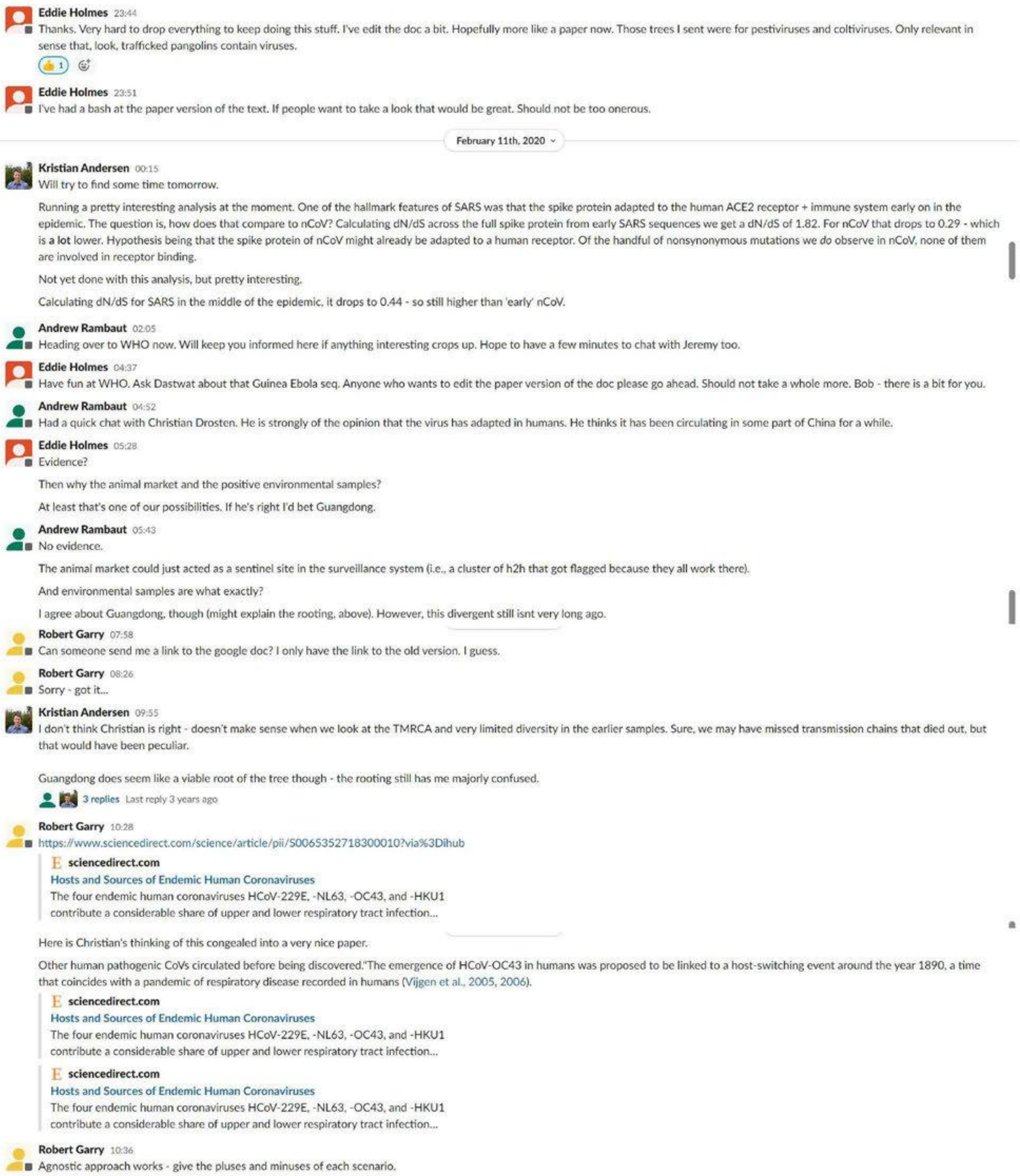
PDF ▾

Figure 5.pdf
PDF

At worse, I think they have got over-excited with their results and claimed too much. The implication is that their pangolin virus is closer to NCP than the one we have from Guangdong but we need to see the data. Unfortunately, they may not publish this any time soon because they have faced huge criticism in China, I think mainly from admitting that pangolins are illegally trafficked into China which apparently you are not meant to say. Very Chernobyl. About to edit the doc.

Kristian Andersen 22:49
Thanks Eddie for sharing. Not quite sure what those pangolin viruses are though? And yes, I'm worried they have overclaimed too... Kinda bummed that the 'environmental' samples didn't show anything at all.

As for document - realistically I'm going to have a very hard time doing anything on it this week since I'm off Thursday > Sunday and have a compressed week. Come next week I'm back in business though - plus I *will* have some time Wednesday and first part Thursday this week.



Eddie Holmes 23:44
Thanks. Very hard to drop everything to keep doing this stuff. I've edit the doc a bit. Hopefully more like a paper now. Those trees I sent were for pestiviruses and coltiviruses. Only relevant in sense that, look, trafficked pangolins contain viruses.

👍 1

Eddie Holmes 23:51
I've had a bash at the paper version of the text. If people want to take a look that would be great. Should not be too onerous.

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Kristian Andersen 00:15
Will try to find some time tomorrow.

Running a pretty interesting analysis at the moment. One of the hallmark features of SARS was that the spike protein adapted to the human ACE2 receptor + immune system early on in the epidemic. The question is, how does that compare to nCoV? Calculating dN/dS across the full spike protein from early SARS sequences we get a dN/dS of 1.82. For nCoV that drops to 0.29 - which is **a lot** lower. Hypothesis being that the spike protein of nCoV might already be adapted to a human receptor. Of the handful of nonsynonymous mutations we *do* observe in nCoV, none of them are involved in receptor binding.

Not yet done with this analysis, but pretty interesting.

Calculating dN/dS for SARS in the middle of the epidemic, it drops to 0.44 - so still higher than 'early' nCoV.

Andrew Rambaut 02:05
Heading over to WHO now. Will keep you informed here if anything interesting crops up. Hope to have a few minutes to chat with Jeremy too.

Eddie Holmes 04:37
Have fun at WHO. Ask Dastwat about that Guinea Ebola seq. Anyone who wants to edit the paper version of the doc please go ahead. Should not take a whole more. Bob - there is a bit for you.

Andrew Rambaut 04:52
Had a quick chat with Christian Drosten. He is strongly of the opinion that the virus has adapted in humans. He thinks it has been circulating in some part of China for a while.

Eddie Holmes 05:28
Evidence?

Then why the animal market and the positive environmental samples?

At least that's one of our possibilities. If he's right I'd bet Guangdong.

Andrew Rambaut 05:43
No evidence.

The animal market could just acted as a sentinel site in the surveillance system (i.e., a cluster of h2h that got flagged because they all work there).

And environmental samples are what exactly?

I agree about Guangdong, though (might explain the rooting, above). However, this divergent still isnt very long ago.

Robert Garry 07:58
Can someone send me a link to the google doc? I only have the link to the old version. I guess.

Robert Garry 08:26
Sorry - got it...

Kristian Andersen 09:55
I don't think Christian is right - doesn't make sense when we look at the TMRCA and very limited diversity in the earlier samples. Sure, we may have missed transmission chains that died out, but that would have been peculiar.

Guangdong does seem like a viable root of the tree though - the rooting still has me majorly confused.

3 replies Last reply 3 years ago

Robert Garry 10:28
https://www.sciencedirect.com/science/article/pii/S0065352718300010?via%3Dihub

> **sciencedirect.com**
> Hosts and Sources of Endemic Human Coronaviruses
> The four endemic human coronaviruses HCoV-229E, -NL63, -OC43, and -HKU1 contribute a considerable share of upper and lower respiratory tract infection...

Here is Christian's thinking of this congealed into a very nice paper.

Other human pathogenic CoVs circulated before being discovered."The emergence of HCoV-OC43 in humans was proposed to be linked to a host-switching event around the year 1890, a time that coincides with a pandemic of respiratory disease recorded in humans (Vijgen et al., 2005, 2006).

> **sciencedirect.com**
> Hosts and Sources of Endemic Human Coronaviruses
> The four endemic human coronaviruses HCoV-229E, -NL63, -OC43, and -HKU1 contribute a considerable share of upper and lower respiratory tract infection...

> **sciencedirect.com**
> Hosts and Sources of Endemic Human Coronaviruses
> The four endemic human coronaviruses HCoV-229E, -NL63, -OC43, and -HKU1 contribute a considerable share of upper and lower respiratory tract infection...

Robert Garry 10:36
Agnostic approach works - give the pluses and minuses of each scenario.

[redacted]

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Robert Garry 10:50

"Calculating dN/dS across the full spike protein from early SARS sequences we get a dN/dS of 1.82. For nCoV that drops to 0.29 - which is **a lot** lower."

Can you calculate dN/dS for the pangolin spike sequences? They are pretty divergent.

Great everybody comes up with different names. I'm starting to like WTFV more and more...

Kristian Andersen 12:00

> Can you calculate dN/dS for the pangolin spike sequences? They are pretty divergent

Yeah, that could be done, but the sequences are a little sketchy so I'm not quite sure what we'll find.

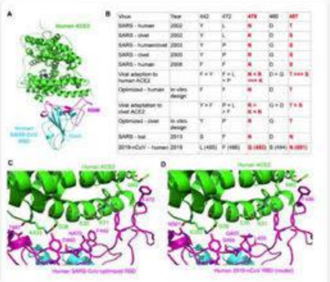
[redacted]

Robert Garry 12:38

AS for a new figure is there some way to for lack of a better word dumb down a figure like this from Baric?

My fear is that most readers eyes will glaze over at the sequence alignment and maybe worse a crystal structure.

image.png



[redacted]

Andrew Rambaut 14:26

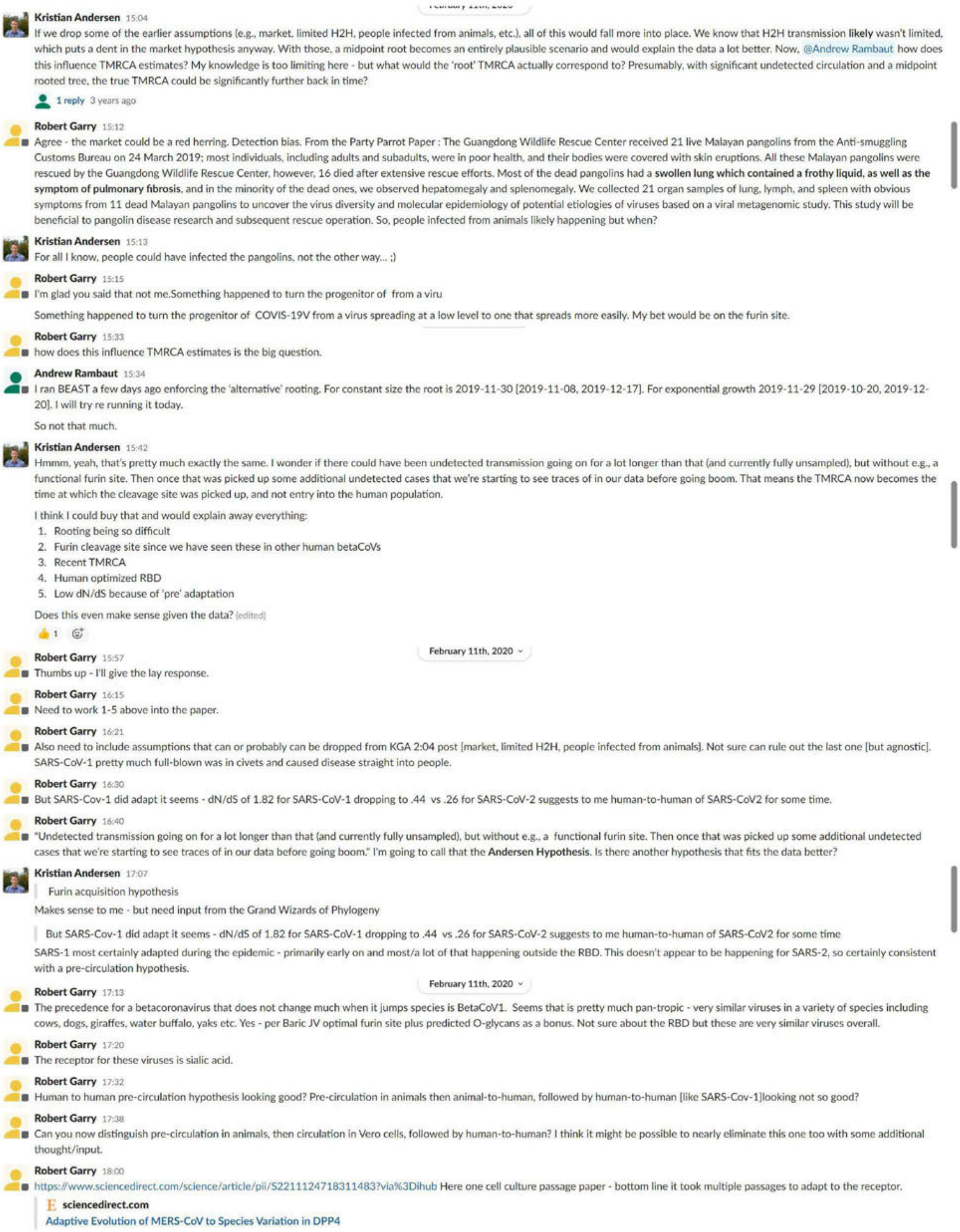
Going to chat with Jeremy tomorrow morning. I am beginning to be more convinced about the mid-point root. I think that means a long pre-detection period in Wuhan (possibly outside). Basically once you lose the market as the origin, all bets are off.

Kristian Andersen 14:34

Yeah, I think that's an interesting possibility too Andrew - and the root is definitely challenging. Thing is, given what we're seeing on the cruise ships, in the hospitals and communities, clearly this thing spreads extremely easily between humans - so as you say, it's highly plausible that while the market was were it was detected (and potentially amplified) it's not because of an animal reservoir there, it's because of extended human-to-human transmission. If you look at the environmental samples they also look like patient samples - which would be consistent in such a scenario.

Andrew Rambaut 14:46

That is my thought. I suspect the surveillance system picked it up because it was a market - this is essentially an avian influenza surveillance system. But it may have just been spread within the market.



Kristian Andersen 15:04
If we drop some of the earlier assumptions (e.g., market, limited H2H, people infected from animals, etc.), all of this would fall more into place. We know that H2H transmission **likely** wasn't limited, which puts a dent in the market hypothesis anyway. With those, a midpoint root becomes an entirely plausible scenario and would explain the data a lot better. Now, @Andrew Rambaut how does this influence TMRCA estimates? My knowledge is too limiting here - but what would the 'root' TMRCA actually correspond to? Presumably, with significant undetected circulation and a midpoint rooted tree, the true TMRCA could be significantly further back in time?

1 reply 3 years ago

Robert Garry 15:12
Agree - the market could be a red herring. Detection bias. From the Party Parrot Paper : The Guangdong Wildlife Rescue Center received 21 live Malayan pangolins from the Anti-smuggling Customs Bureau on 24 March 2019; most individuals, including adults and subadults, were in poor health, and their bodies were covered with skin eruptions. All these Malayan pangolins were rescued by the Guangdong Wildlife Rescue Center, however, 16 died after extensive rescue efforts. Most of the dead pangolins had a **swollen lung which contained a frothy liquid, as well as the symptom of pulmonary fibrosis**, and in the minority of the dead ones, we observed hepatomegaly and splenomegaly. We collected 21 organ samples of lung, lymph, and spleen with obvious symptoms from 11 dead Malayan pangolins to uncover the virus diversity and molecular epidemiology of potential etiologies of viruses based on a viral metagenomic study. This study will be beneficial to pangolin disease research and subsequent rescue operation. So, people infected from animals likely happening but when?

Kristian Andersen 15:13
For all I know, people could have infected the pangolins, not the other way... ;)

Robert Garry 15:15
I'm glad you said that not me.Something happened to turn the progenitor of from a viru

Something happened to turn the progenitor of COVIS-19V from a virus spreading at a low level to one that spreads more easily. My bet would be on the furin site.

Robert Garry 15:33
how does this influence TMRCA estimates is the big question.

Andrew Rambaut 15:34
I ran BEAST a few days ago enforcing the 'alternative' rooting. For constant size the root is 2019-11-30 [2019-11-08, 2019-12-17]. For exponential growth 2019-11-29 [2019-10-20, 2019-12-20]. I will try re running it today.

So not that much.

Kristian Andersen 15:42
Hmmm, yeah, that's pretty much exactly the same. I wonder if there could have been undetected transmission going on for a lot longer than that (and currently fully unsampled), but without e.g., a functional furin site. Then once that was picked up some additional undetected cases that we're starting to see traces of in our data before going boom. That means the TMRCA now becomes the time at which the cleavage site was picked up, and not entry into the human population.

I think I could buy that and would explain away everything:

1. Rooting being so difficult
2. Furin cleavage site since we have seen these in other human betaCoVs
3. Recent TMRCA
4. Human optimized RBD
5. Low dN/dS because of 'pre' adaptation

Does this even make sense given the data? (edited)

👍 1

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Robert Garry 15:57
Thumbs up - I'll give the lay response.

Robert Garry 16:15
Need to work 1-5 above into the paper.

Robert Garry 16:21
Also need to include assumptions that can or probably can be dropped from KGA 2:04 post [market, limited H2H, people infected from animals]. Not sure can rule out the last one [but agnostic]. SARS-CoV-1 pretty much full-blown was in civets and caused disease straight into people.

Robert Garry 16:30
But SARS-Cov-1 did adapt it seems - dN/dS of 1.82 for SARS-CoV-1 dropping to .44 vs .26 for SARS-CoV-2 suggests to me human-to-human of SARS-CoV2 for some time.

Robert Garry 16:40
"Undetected transmission going on for a lot longer than that (and currently fully unsampled), but without e.g., a functional furin site. Then once that was picked up some additional undetected cases that we're starting to see traces of in our data before going boom." I'm going to call that the **Andersen Hypothesis**. Is there another hypothesis that fits the data better?

Kristian Andersen 17:07
> Furin acquisition hypothesis

Makes sense to me - but need input from the Grand Wizards of Phylogeny

> But SARS-Cov-1 did adapt it seems - dN/dS of 1.82 for SARS-CoV-1 dropping to .44 vs .26 for SARS-CoV-2 suggests to me human-to-human of SARS-CoV2 for some time

SARS-1 most certainly adapted during the epidemic - primarily early on and most/a lot of that happening outside the RBD. This doesn't appear to be happening for SARS-2, so certainly consistent with a pre-circulation hypothesis.

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Robert Garry 17:13
The precedence for a betacoronavirus that does not change much when it jumps species is BetaCoV1. Seems that is pretty much pan-tropic - very similar viruses in a variety of species including cows, dogs, giraffes, water buffalo, yaks etc. Yes - per Baric JV optimal furin site plus predicted O-glycans as a bonus. Not sure about the RBD but these are very similar viruses overall.

Robert Garry 17:20
The receptor for these viruses is sialic acid.

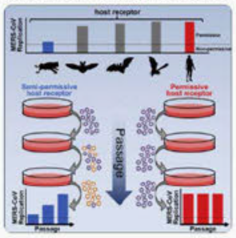
Robert Garry 17:32
Human to human pre-circulation hypothesis looking good? Pre-circulation in animals then animal-to-human, followed by human-to-human [like SARS-Cov-1]looking not so good?

Robert Garry 17:38
Can you now distinguish pre-circulation in animals, then circulation in Vero cells, followed by human-to-human? I think it might be possible to nearly eliminate this one too with some additional thought/input.

Robert Garry 18:00
https://www.sciencedirect.com/science/article/pii/S2211124718311483?via%3Dihub Here one cell culture passage paper - bottom line it took multiple passages to adapt to the receptor.

> E **sciencedirect.com**
> Adaptive Evolution of MERS-CoV to Species Variation in DPP4

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) likely originated in bats and passed to humans through dromedary camels; however, the genetic ... (85 kB) ▾



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC249560/

× **PubMed Central (PMC)**

Generation of seal influenza virus variants pathogenic for chickens, because of hemagglutinin cleavage site changes.

Influenza virus A/seal/Mass/1/80 (H7N7) was adapted to grow in MDCK cells and chicken embryo cells (CEC) in the absence of exogenous protease. The biological properties of the virus variants obtained coincided with intracellular activation of the hemagglutinin ...

Kristian Andersen 18:04

I don't think any of these can be eliminated or confirmed at this stage, but a couple of things:

1. All data seems to be consistent with the pre-circulation hypothesis posed above
2. O-linked glycans and low dN/dS not so consistent with passage in cell culture - furin cleavage site and optimal human ACE2 RBD very consistent
3. Low dN/dS and optimal human ACE2 RBD not so consistent with passage in animal model - furin cleavage site very consistent
4. Low dN/dS, furin cleavage site, and optimal human ACE2 RBD not so consistent with direct spillover - epi data consistent

1 reply 3 years ago

Robert Garry 18:04

Likewise many many passages in chick embryo cells to generate a polybasic cleavage in flu v. You can do it by *cell culture passage* but you really need to be trying to do it.

Robert Garry 18:11

Agree! Grand Wizards of Phylogeny need to poke holes, if there are any. Need to firm up precedence of undetected circulation in humans prior to emergence of HKU1, OC43, NL63, 229E - Drosten review has some of this.

Can you make a figure of the dN/dS data? Does this hold throughout the genome or just spike?

Andrew Rambaut 18:18

That MERS paper - why do people think MERS is adapted to humans? It has never transmitted for more than about a month in humans. No adaptations that arise in humans would get back into the camels. It is a camel virus. It is adapted to camels and just happens to replicate in humans.

I am not convinced about dN/dS either - where do you get a dN/dS for SARS of 1.82? Across the whole genome?

Sounds artifactual to me.

Robert Garry 18:20

Agree - bad premise, but they tried passaging MERS CoV in cell culture and it was pretty hard to get the virus to adapt - that was my point.

Andrew Rambaut 18:21

Fair enough. I just have heard here people talking about MERS as a human virus.

Robert Garry 18:22

MERS-CoV another one that should be looked at for dN/dS.

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Kristian Andersen 18:45

Yeah, don't get the MERS stuff - doesn't make sense.

For SARS/nCoV I'm specifically looking at the spike protein (for now) - comparing SARS early in the outbreak to in the middle of it. For SARS this has been done by others as well https://www.ncbi.nlm.nih.gov/pubmed/14752165

ncbi.nlm.nih.gov

Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. - PubMed - NCBI

Science. 2004 Mar 12;303(5664):1666-9. Epub 2004 Jan 29. (13 kB) ▾

Eddie Holmes 20:06

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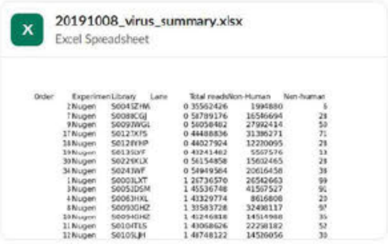
Sorry, need to catch up. Had to teach a class! One a year. Yes, MERS is a camel virus. Pisses me off that people say it is a bat virus. Anyway, I have trouble with the human pre-adaptation idea: (i) I don't see why the market is analogous to AIV screening unless Andrew knows something I don't. I think the best surveillance takes place in the hospitals; (ii) the main reason why I've been to Wuhan a few times is to take part in this big lung wash study (BAL) study we have going on. We have meta-transcriptomic data of ~600 people reporting to Wuhan Central Hospital with respiratory disease. We have their full meta-transcriptomes but it is taking an age to analyse because the data set is so big. I'm going to attach the raw virus data here (keep to yourself). I think these are from 2018 but I have to check. There are CoVs but nothing new. I need to double-check with my Mang but he is about best in world about this. The cells in yellow are confirmed, the others per lane reflect index-hopping. Obviously, not conclusive, but a representative sample that the virus was not there then. I suppose we need to get this published ASAP?

Excel Spreadsheet ▾



20191008_virus_summary.xlsx

Excel Spreadsheet

Order	Experimen	Library	Lane	Total reads	Non-Human	Non-human
2	Nugen	S0041ZHM	0	35562426	1994880	5
7	Nugen	S0088CGJ	0	58789176	16546694	28
9	Nugen	S0093WGL	0	56058482	27992414	50
17	Nugen	S0127XFS	0	44488836	31386271	71
18	Nugen	S0128YHP	0	44027924	12220095	28
19	Nugen	S0135GYF	0	43241482	5567576	13
30	Nugen	S0229KLX	0	56154858	15602465	28
34	Nugen	S0243WF	0	54949584	20616458	38
1	Nugen	S0003LXT	1	26736570	26542663	99
3	Nugen	S0052DSM	1	45536748	41567527	91
4	Nugen	S0063HXL	1	43329774	8616808	20
8	Nugen	S0093GHZ	1	33583728	32498117	97
10	Nugen	S0094GHZ	1	41246818	14514988	35
11	Nugen	S0104TLS	1	43068626	22258182	52
12	Nugen	S0105LJH	1	48748122	14520056	30

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Andrew Rambaut 01:07

About emails - no problem with Ian being on it. His question here...

```
Selection during passage

1.    Are we suggesting that the furin cleavage site evolved from de novo mutations or through recombination?
```

Do we think the furin insertion could have occurred one AA at a time? Seems unlikely as you have to insert a whole codon at a time. And if I remember for AIV sometimes the actual insertion is from elsewhere in the virus genome (not sure about this - it has been a while since I looked at this).

With respect to the pre-December circulation - I don't think we can say that it was more than a month or two and in that time the numbers would be very small. If 2 months with a double time of 6 days we have about 1000 people. But that pre-supposes the exponential growth rate we see now which presumably is the result of the furin sit.

I still can't see it circulating long enough with stuttering chains of transmission for it to evolve the furin site (and whatever else) and then take off. This stuff can't happen easily or it would have happened in SARS

Kristian Andersen 02:04

All I know is my head hurts...

Furin site probably could be step by step - increasing its ability to be cleaved little by little. Codons come and go sometimes in RNA viruses, so I wouldn't be that surprised (e.g., we have seen it in Lassa and Ebola, but not Zika and West Nile).

I still think the pre-circulation theory might have some legs, but I agree not perfect.

Did you explore routing more? I tried masking some sites in earlier samples that are suspect and also ran beast without time information under a couple of different models. Creates some beautifully midpoint rooted trees.

Eddie Holmes 03:24

I've added Ian to the Google docs. I'll edit a draft now and hopefully he can add some wise words.

[redacted]

Andrew Rambaut 03:31

Had a chat with Jeremy this morning. Really not much more to say.

Eddie Holmes 03:32

You mean for the doc?

Andrew Rambaut 03:33

Just that he still thinks it is important to get a matter-of-fact paper out there.

Eddie Holmes 03:36

Yes, let's just finish it.

Much as I think it is dumb, we need to use COVID-19. The ICTV are a bunch of twats.

Plus Jeremy is WHO linked

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Andrew Rambaut 03:47

Problem is that COVID-19 is the disease. We could start to call it COVID-19-CoV if we want to troll ICTV

I am doing an up-to-date BEAST analysis which we could use to discuss timing of TMRCA. Will then use the rate we get there to estimate divergence to RaTG13. Will be a minimum date but we could make that clear.

Can we use GISAID data? Would need the ackn. table but there is also the bit about attempting collaboration (for all submitters?).

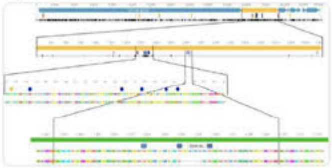
Eddie Holmes 03:55

Shit, you're right, so confusing. I think adding GISAID data is a good idea. Table can go online.

Andrew Rambaut 04:49

Needs quite a lot of work but what about a figure like this?

image.png ▾



Andrew Rambaut 05:14
The amino acid alignment insets could include a few more bats and SARS and you could let me know (@Kristian) which you want and which residues to show. I am happy to un-Geneious it.

Perhaps a sliding window similarity plot along the top to show how unrecombinant it is?

Eddie Holmes 05:22
👍

Eddie Holmes 05:56
Bloody obvious when you think about it: https://www.express.co.uk/news/world/1240664/coronavirus-news-latest-china-origin-meteorite-scientists-health-warning-death-toll-latest

Express.co.uk
Coronavirus came from METEORITE which hit China last year - bombshell scientist claim
THE deadly coronavirus which has killed more than 1,000 people globally came from a meteorite which hit China last year, scientists have sensationally claimed.
Feb 11th, 2020 (58 kB)

Andrew Rambaut 06:10
snake-space-flu

Robert Garry 07:53
At least gives an alternative tMCRA - not quite ready to add another scenario.

Robert Garry 08:03
from alexander and brown ref

All the current evidence indicates that HPAI viruses arise by mutation after LPAI viruses of the H5 or H7 subtype have been introduced into poultry. Several mechanisms may be responsible for this mutation. For most HPAI viruses, there appears to have been spontaneous duplication of purine triplets, which results in the insertion of basic amino acids at the HA0 cleavage site, and this seems to occur due to a transcription error by the polymerase complex (76). However, as pointed out by Perdue et al. (76), this is clearly not the only mechanism by which HPAI viruses arise, as some appear to result from nucleotide substitution rather than insertion, while others have insertions without repeating nucleotides. The Chile 2002 (107) and the Canada 2004 (75) H7N3 HPAI viruses have emerged as the result of an entirely different mechanism and show distinct and unusual cleavage site amino acid sequences. They appear to have arisen as a result of recombination with other genes (the nucleoprotein gene and matrix gene, respectively), resulting in an insertion at the cleavage site of 11 amino acids for the Chile virus and seven amino acids for the Canadian virus.

I think Kristian is on to something with the dN/dS but more analysis needed.

Cell. 2015 Jun 18;161(7):1516-26. doi: 10.1016/j.cell.2015.06.007.

February 12th, 2020

ncbi.nlm.nih.gov
Ebola Virus Epidemiology, Transmission, and Evolution during Seven Months in Sierra Leone. - PubMed - NCBI
Cell. 2015 Jun 18;161(7):1516-26. doi: 10.1016/j.cell.2015.06.007. Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S. (13 kB)

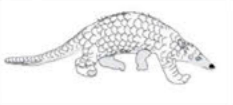
PubMed

I like Andrew's new figure too.

Robert Garry 09:38
Speaking of figures - of which we need several, some perhaps the more technical like the detailed alignments can be supplemental.

I started 45 minutes and did not finish a pango cartoon - a "scenario" diagram MIGHT be useful or it might be totally unhelpful - particularly since the main targets for this piece are not all virologists/evolutionary biologists.

image.png



Andrew Rambaut 09:44
Great. A quick sketch of Peter D to be our 'human' would be good.

(coincidental similarity, of course)

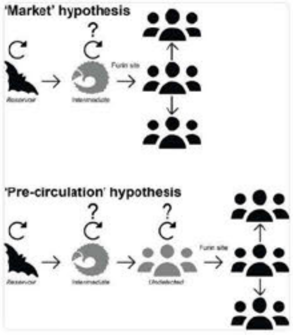
February 12th, 2020

Robert Garry 10:09
Do you think something like this is too much coincidence?

image.png

Kristian Andersen 13:34
I like Andrew's figure a lot - so yes, let's have something like that. I agree with Bob that having a schematic outlining the various scenarios would be critical as well - here's one I got started on for a talk I'm giving later today. Wouldn't be this one for the paper, but could serve as a starting point?

transmission.jpg



I think it's important we investigate the dN/dS difference more in-depth as it could provide critical clues that we currently don't have - if the spike protein evolves greatly after CoV jumps into humans but we don't observe that in nCoV, then that's very important information worth including. I have reached out to Andrew, so hopefully I can wrestle him away for a few minutes to discuss 😉.

Final point - now would probably be a good time to reach out to Clare to make sure that this is of interest to them and also get a sense of what specific things they might want addressed. Do y'all want me to reach out to her?

I'll get on the document too, but I'm pinned down at the moment - I'll have time possibly later today, but otherwise tomorrow AM. I'll then be gone until Sunday AM (with no internet - I'll be in the middle of the desert...). (edited)

Robert Garry 13:48
Yes - ping Clare - give her a little background about the email group.

Robert Garry 16:26
What about these?

image.png

👍 1

Robert Garry 16:48
I don't know about this one.

image.png

February 12th, 2020

Eddie Holmes 17:58
Kristian, if you could reach out to Clare that would be grand. She's had way too many emails from me. Jeremy said that he would speak to Magda. I don't think we should have a picture of the pangolin as an intermediate host. Might be them, but I bet these CoVs will be found in a whole range of animals. I don't think we want to come down to heavily on the side of pangolins for now. I would just putting a bloody great question mark there. Or use a generic rodent sort of thing.

Kristian Andersen 18:01
👍

Eddie Holmes 18:07
Why has the name of the virus in the paper been changed back to 2019-nCoV when that is now out-of-date? I changed them all to SARS-CoV-2 and now it has been changed back.

Kristian Andersen 18:10
I think Ian might be responsible... looking at the version history. We should stick to SARS-CoV-2 I think?

Emailed Clare - will let y'all know what she says. If it's a no, Science would likely be interested and Cell would take it for sure.

Robert Garry 18:20
Change change to generic rodent with question mark

image.png ▾

Or change any other aspect as well.

even "Peter"

February 12th, 2020

Eddie Holmes 18:33
Yes: (1) generic rodent with ?; (2) we need to use SARS-CoV-2. As soon as Ian as finished I'll do another global find-and-replace.

Robert Garry 18:45
Other edits corrections suggestions welcome.

image.png ▾

February 12th, 2020

Robert Garry 19:28
Could do something like these diagrams for SARS-CoV-1 and MERS-CoV for the supplemental file. Good contrast...

Robert Garry 20:15
Maybe change orange CoVs to "partial" adaptation to human receptor? Maybe change receptor to ACE-2?

Eddie Holmes 21:40
Done more on the text. Looks good.

Kristian Andersen 22:23
I'm wiped - but will take a good close look and provide edits first thing tomorrow.

Eddie Holmes 22:26
Get some rest!

February 13th, 2020

Andrew Rambaut 04:24
I'll be able to get on it today.

Eddie Holmes 04:25
That would be great.

Robert Garry 09:42
https://www.ncbi.nlm.nih.gov/pubmed/17402195

ncbi.nlm.nih.gov

February 13th, 2020

[Study on the dynamic prevalence of serum antibody against severe acute respiratory syndrome coronavirus in employees from wild animal market in Gu... - PubMed - NCBI

Zhonghua Liu Xing Bing Xue Za Zhi. 2006 Nov;27(11):950-2. English Abstract

(13 kB) ▾

Kristian Andersen 09:49

Clare got back to me with a "Yes please!". She suggested this was probably a "Perspective"

File from iOS ▾

February 13th, 2020

Clare Thomas

to Kristian

Dear Kristian,

Yes please! It sounds possibly like a Perspective. I would love to take a look and consider whether it might be suitable for Nature.

All the best,

Clare

...

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Andrew Rambaut 09:52

I was thinking that something along the lines of a perspective as we are basically synthesising information.

Kristian Andersen 10:02

Yup, agreed. I'll take a look as well shortly

Robert Garry 10:27

February 13th, 2020

That's good news.

Kristian Andersen 11:31

A couple of guidelines for the Perspective format - it's similar to a Review, but we have more flexibility in terms of content and length (can/should be short): https://www.nature.com/nature/for-authors/other-subs

Main thing - 200 word synopsis and we can include a fair number of figures, so we might consider having maybe three?

Nature

Other types of submissions | Nature

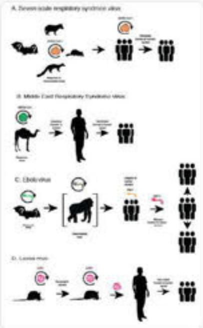
Other types of submissions

Robert Garry 12:29

February 13th, 2020

Was thinking of something like this for the supplement, especially if Kristian develops some convincing dN/dS data comparing SARS-CoV-1 and -2 maybe other viruses.

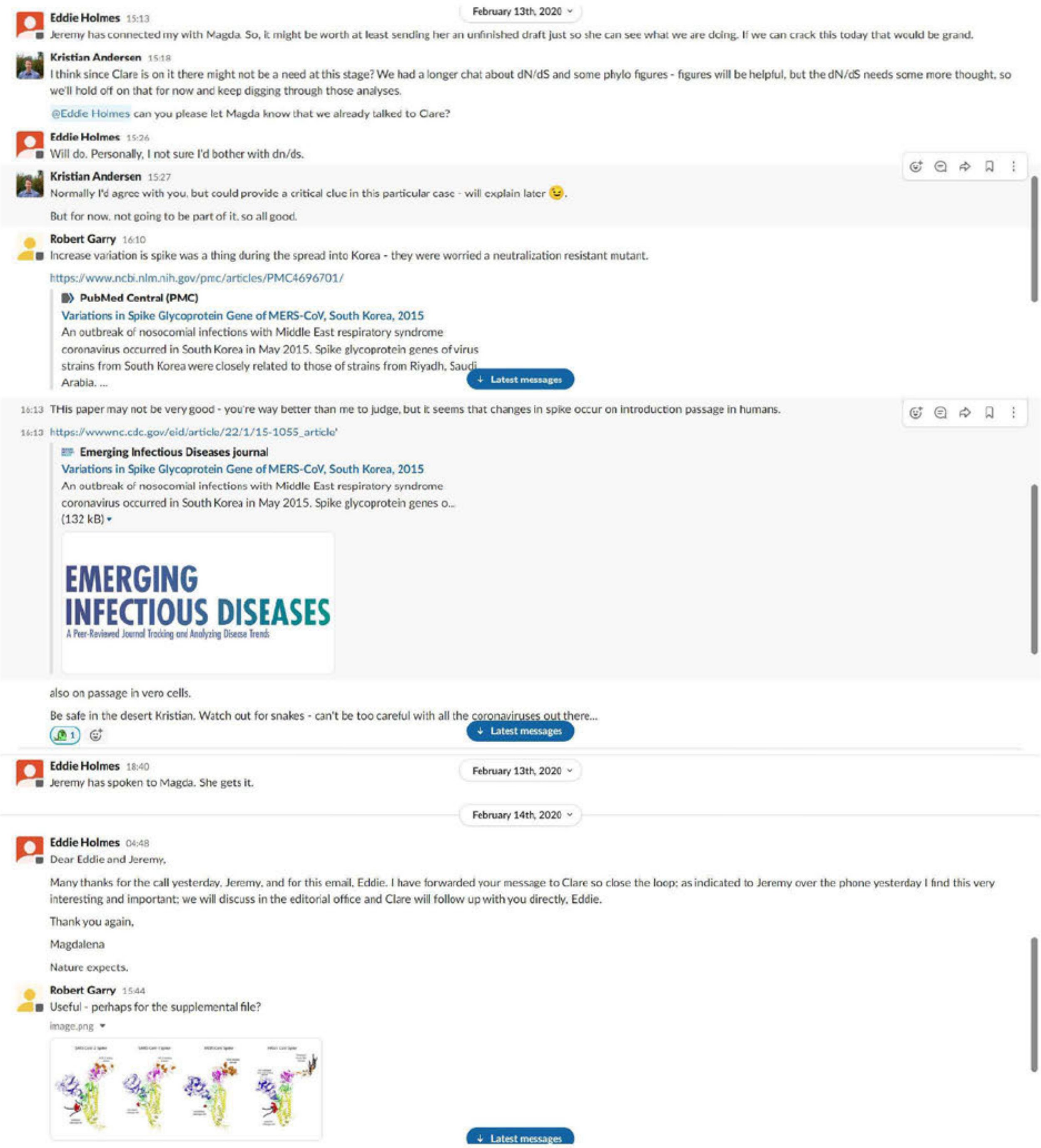
image.png ▾



Also I probably haven't captured the best flow for the various scenarios bu throwing this out for discussion and maybe learning something.

We might want to go with other "generic" "humans" at some point.

↓ Latest messages



February 13th, 2020

Eddie Holmes 15:13

Jeremy has connected my with Magda. So, it might be worth at least sending her an unfinished draft just so she can see what we are doing. If we can crack this today that would be grand.

Kristian Andersen 15:18

I think since Clare is on it there might not be a need at this stage? We had a longer chat about dN/dS and some phylo figures - figures will be helpful, but the dN/dS needs some more thought, so we'll hold off on that for now and keep digging through those analyses.

@Eddie Holmes can you please let Magda know that we already talked to Clare?

Eddie Holmes 15:26

Will do. Personally, I not sure I'd bother with dn/ds.

Kristian Andersen 15:27

Normally I'd agree with you, but could provide a critical clue in this particular case - will explain later 😉.

But for now, not going to be part of it, so all good.

Robert Garry 16:10

Increase variation is spike was a thing during the spread into Korea - they were worried a neutralization resistant mutant.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4696701/

> **PubMed Central (PMC)**
> Variations in Spike Glycoprotein Gene of MERS-CoV, South Korea, 2015
> An outbreak of nosocomial infections with Middle East respiratory syndrome coronavirus occurred in South Korea in May 2015. Spike glycoprotein genes of virus strains from South Korea were closely related to those of strains from Riyadh, Saudi Arabia. ...

16:13 THis paper may not be very good - you're way better than me to judge, but it seems that changes in spike occur on introduction passage in humans.

16:13 https://wwwnc.cdc.gov/eid/article/22/1/15-1055_article'

> **Emerging Infectious Diseases journal**
> Variations in Spike Glycoprotein Gene of MERS-CoV, South Korea, 2015
> An outbreak of nosocomial infections with Middle East respiratory syndrome coronavirus occurred in South Korea in May 2015. Spike glycoprotein genes o...
> (132 kB)

also on passage in vero cells.

Be safe in the desert Kristian. Watch out for snakes - can't be too careful with all the coronaviruses out there...

February 13th, 2020

Eddie Holmes 18:40

Jeremy has spoken to Magda. She gets it.

February 14th, 2020

Eddie Holmes 04:48

Dear Eddie and Jeremy,

Many thanks for the call yesterday, Jeremy, and for this email, Eddie. I have forwarded your message to Clare so close the loop; as indicated to Jeremy over the phone yesterday I find this very interesting and important; we will discuss in the editorial office and Clare will follow up with you directly, Eddie.

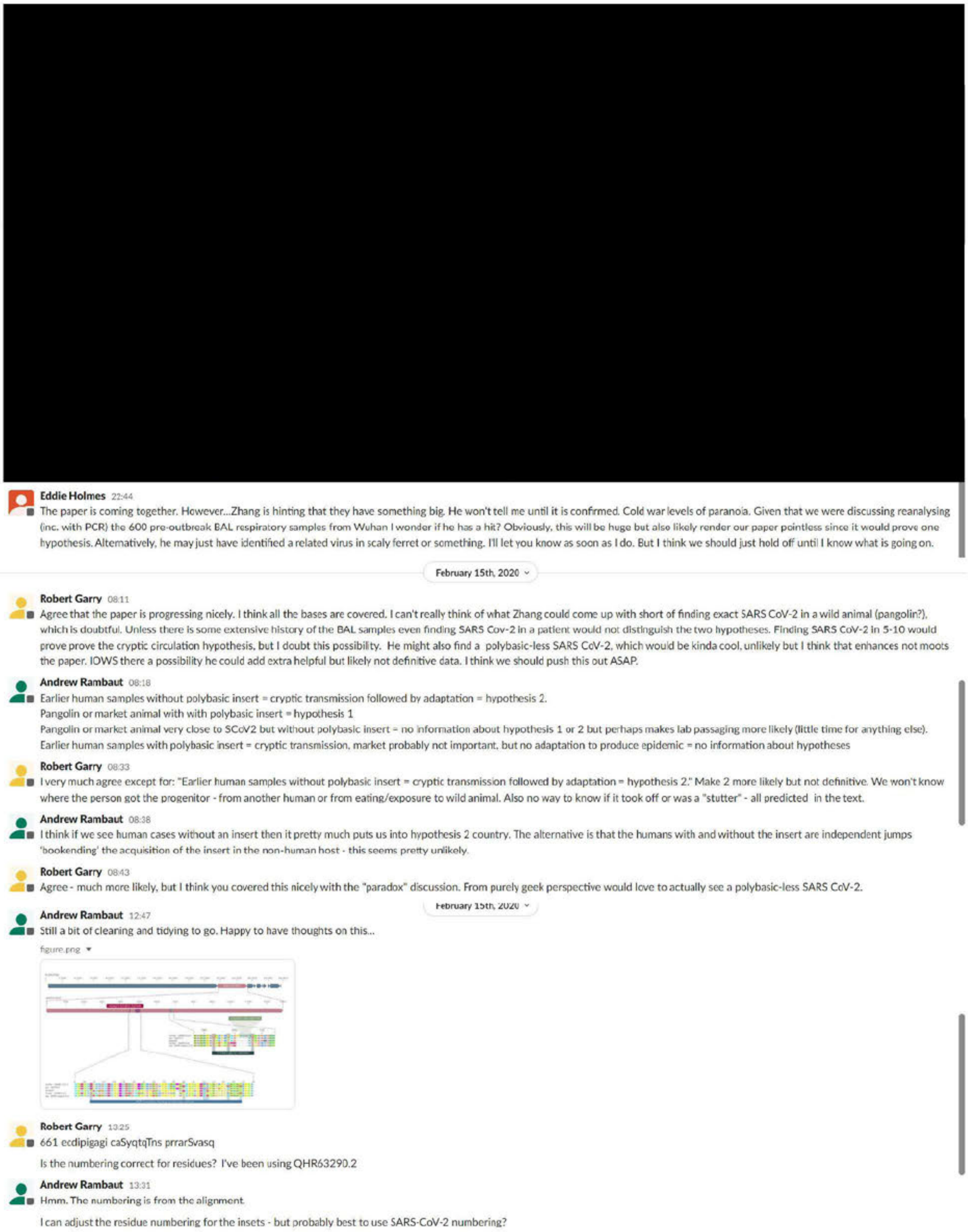
Thank you again,

Magdalena

Nature expects.

Robert Garry 15:44

Useful - perhaps for the supplemental file?



[redacted]

Eddie Holmes 22:44

The paper is coming together. However...Zhang is hinting that they have something big. He won't tell me until it is confirmed. Cold war levels of paranoia. Given that we were discussing reanalysing (inc. with PCR) the 600 pre-outbreak BAL respiratory samples from Wuhan I wonder if he has a hit? Obviously, this will be huge but also likely render our paper pointless since it would prove one hypothesis. Alternatively, he may just have identified a related virus in scaly ferret or something. I'll let you know as soon as I do. But I think we should just hold off until I know what is going on.

February 15th, 2020

Robert Garry 08:11

Agree that the paper is progressing nicely. I think all the bases are covered. I can't really think of what Zhang could come up with short of finding exact SARS CoV-2 in a wild animal (pangolin?), which is doubtful. Unless there is some extensive history of the BAL samples even finding SARS Cov-2 in a patient would not distinguish the two hypotheses. Finding SARS CoV-2 in 5-10 would prove prove the cryptic circulation hypothesis, but I doubt this possibilty. He might also find a polybasic-less SARS CoV-2, which would be kinda cool, unlikely but I think that enhances not moots the paper. IOWS there a possibility he could add extra helpful but likely not definitive data. I think we should push this out ASAP.

Andrew Rambaut 08:18

Earlier human samples without polybasic insert = cryptic transmission followed by adaptation = hypothesis 2.
Pangolin or market animal with with polybasic insert = hypothesis 1
Pangolin or market animal very close to SCoV2 but without polybasic insert = no information about hypothesis 1 or 2 but perhaps makes lab passaging more likely (little time for anything else).
Earlier human samples with polybasic insert = cryptic transmission, market probably not important, but no adaptation to produce epidemic = no information about hypotheses

Robert Garry 08:33

I very much agree except for: "Earlier human samples without polybasic insert = cryptic transmission followed by adaptation = hypothesis 2." Make 2 more likely but not definitive. We won't know where the person got the progenitor - from another human or from eating/exposure to wild animal. Also no way to know if it took off or was a "stutter" - all predicted in the text.

Andrew Rambaut 08:38

I think if we see human cases without an insert then it pretty much puts us into hypothesis 2 country. The alternative is that the humans with and without the insert are independent jumps 'bookending' the acquisition of the insert in the non-human host - this seems pretty unlikely.

Robert Garry 08:43

Agree - much more likely, but I think you covered this nicely with the "paradox" discussion. From purely geek perspective would love to actually see a polybasic-less SARS CoV-2.

February 15th, 2020

Andrew Rambaut 12:47

Still a bit of cleaning and tidying to go. Happy to have thoughts on this...

Robert Garry 13:25

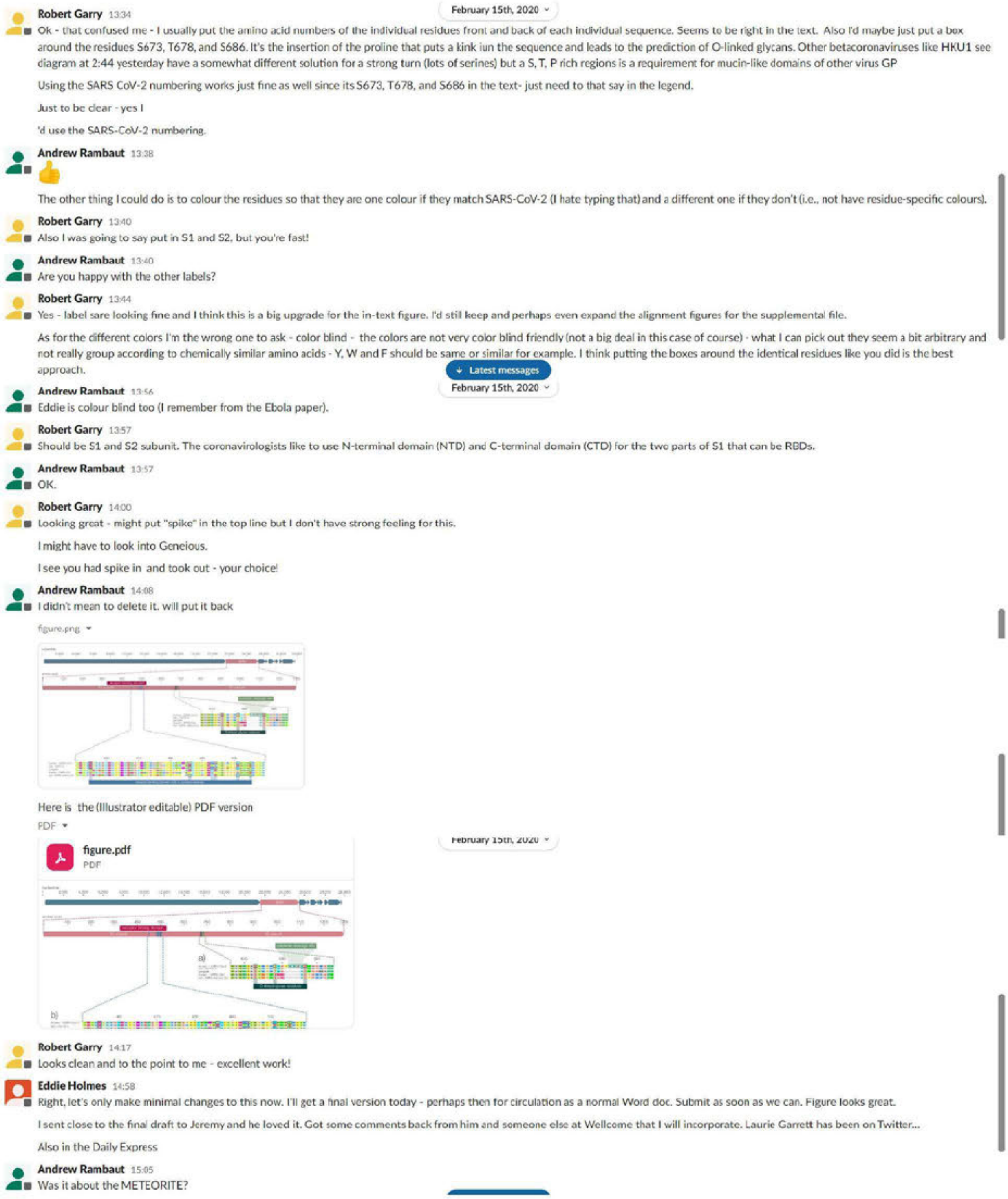
661 ecdipigagi caSyqtqTns prrarSvasq

Is the numbering correct for residues? I've been using QHR63290.2

Andrew Rambaut 13:31

Hmm. The numbering is from the alignment

I can adjust the residue numbering for the insets - but probably best to use SARS-CoV-2 numbering?



Robert Garry 13:34
Ok - that confused me - I usually put the amino acid numbers of the individual residues front and back of each individual sequence. Seems to be right in the text. Also I'd maybe just put a box around the residues S673, T678, and S686. It's the insertion of the proline that puts a kink iun the sequence and leads to the prediction of O-linked glycans. Other betacoronaviruses like HKU1 see diagram at 2:44 yesterday have a somewhat different solution for a strong turn (lots of serines) but a S, T, P rich regions is a requirement for mucin-like domains of other virus GP

Using the SARS CoV-2 numbering works just fine as well since its S673, T678, and S686 in the text- just need to that say in the legend.

Just to be clear - yes I

'd use the SARS-CoV-2 numbering.

Andrew Rambaut 13:38
👍

The other thing I could do is to colour the residues so that they are one colour if they match SARS-CoV-2 (I hate typing that) and a different one if they don't (i.e., not have residue-specific colours).

Robert Garry 13:40
Also I was going to say put in S1 and S2, but you're fast!

Andrew Rambaut 13:40
Are you happy with the other labels?

Robert Garry 13:44
Yes - label sare looking fine and I think this is a big upgrade for the in-text figure. I'd still keep and perhaps even expand the alignment figures for the supplemental file.

As for the different colors I'm the wrong one to ask - color blind - the colors are not very color blind friendly (not a big deal in this case of course) - what I can pick out they seem a bit arbitrary and not really group according to chemically similar amino acids - Y, W and F should be same or similar for example. I think putting the boxes around the identical residues like you did is the best approach.

Andrew Rambaut 13:56
Eddie is colour blind too (I remember from the Ebola paper).

Robert Garry 13:57
Should be S1 and S2 subunit. The coronavirologists like to use N-terminal domain (NTD) and C-terminal domain (CTD) for the two parts of S1 that can be RBDs.

Andrew Rambaut 13:57
OK.

Robert Garry 14:00
Looking great - might put "spike" in the top line but I don't have strong feeling for this.

I might have to look into Geneious.

I see you had spike in and took out - your choice!

Andrew Rambaut 14:08
I didn't mean to delete it. will put it back

Here is the (Illustrator editable) PDF version

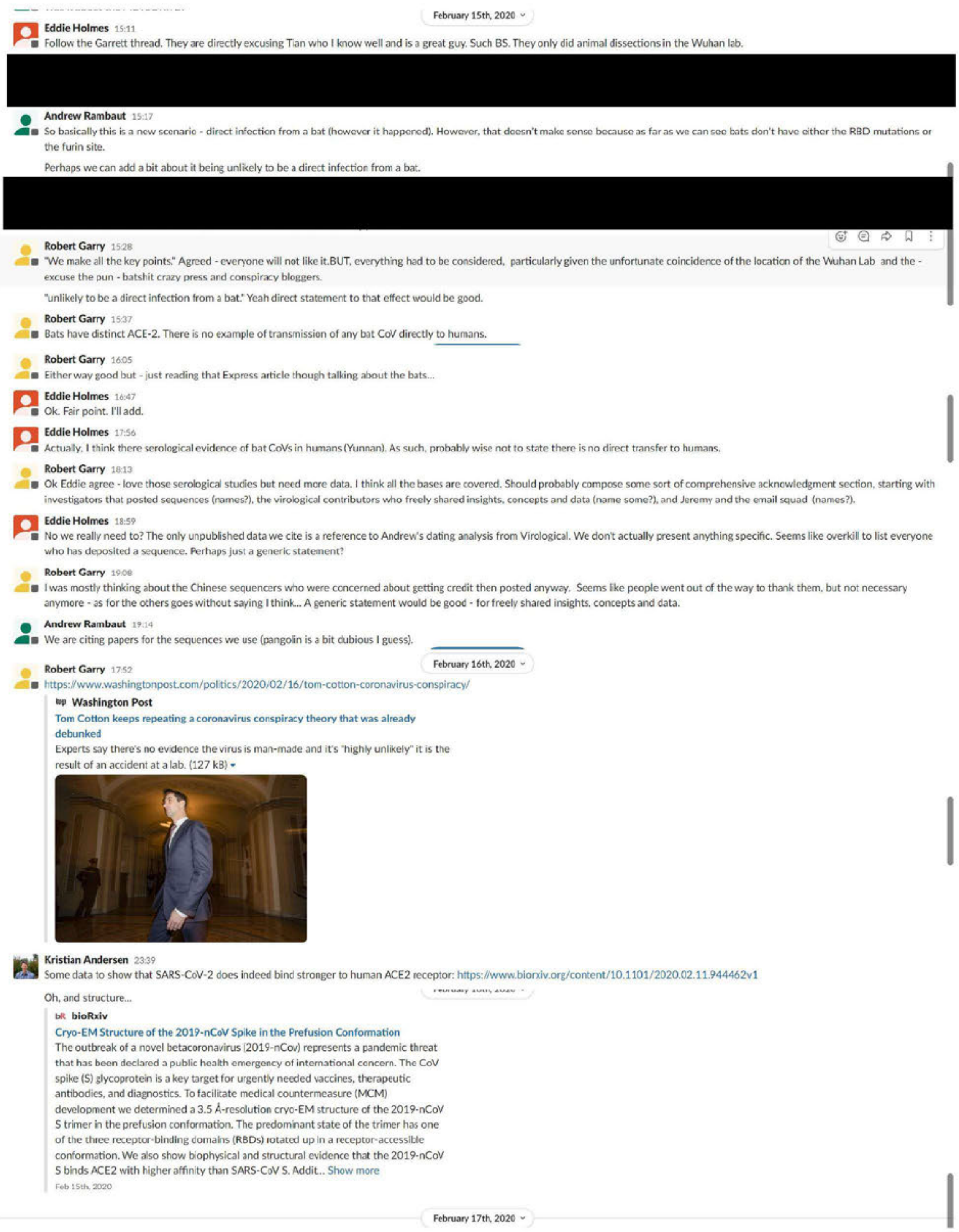
Robert Garry 14:17
Looks clean and to the point to me - excellent work!

Eddie Holmes 14:58
Right, let's only make minimal changes to this now. I'll get a final version today - perhaps then for circulation as a normal Word doc. Submit as soon as we can. Figure looks great.

I sent close to the final draft to Jeremy and he loved it. Got some comments back from him and someone else at Wellcome that I will incorporate. Laurie Garrett has been on Twitter...

Also in the Daily Express

Andrew Rambaut 15:05
Was it about the METEORITE?



February 15th, 2020

Eddie Holmes 15:11
Follow the Garrett thread. They are directly excusing Tian who I know well and is a great guy. Such BS. They only did animal dissections in the Wuhan lab.

[redacted]

Andrew Rambaut 15:17
So basically this is a new scenario - direct infection from a bat (however it happened). However, that doesn't make sense because as far as we can see bats don't have either the RBD mutations or the furin site.

Perhaps we can add a bit about it being unlikely to be a direct infection from a bat.

[redacted]

Robert Garry 15:28
"We make all the key points." Agreed - everyone will not like it.BUT, everything had to be considered, particularly given the unfortunate coincidence of the location of the Wuhan Lab and the - excuse the pun - batshit crazy press and conspiracy bloggers.

"unlikely to be a direct infection from a bat." Yeah direct statement to that effect would be good.

Robert Garry 15:37
Bats have distinct ACE-2. There is no example of transmission of any bat CoV directly to humans.

Robert Garry 16:05
Either way good but - just reading that Express article though talking about the bats...

Eddie Holmes 16:47
Ok. Fair point. I'll add.

Eddie Holmes 17:56
Actually, I think there serological evidence of bat CoVs in humans (Yunnan). As such, probably wise not to state there is no direct transfer to humans.

Robert Garry 18:13
Ok Eddie agree - love those serological studies but need more data. I think all the bases are covered. Should probably compose some sort of comprehensive acknowledgment section, starting with investigators that posted sequences (names?), the virological contributors who freely shared insights, concepts and data (name some?), and Jeremy and the email squad (names?).

Eddie Holmes 18:59
No we really need to? The only unpublished data we cite is a reference to Andrew's dating analysis from Virological. We don't actually present anything specific. Seems like overkill to list everyone who has deposited a sequence. Perhaps just a generic statement?

Robert Garry 19:08
I was mostly thinking about the Chinese sequencers who were concerned about getting credit then posted anyway. Seems like people went out of the way to thank them, but not necessary anymore - as for the others goes without saying I think... A generic statement would be good - for freely shared insights, concepts and data.

Andrew Rambaut 19:14
We are citing papers for the sequences we use (pangolin is a bit dubious I guess).

February 16th, 2020

Robert Garry 17:52
https://www.washingtonpost.com/politics/2020/02/16/tom-cotton-coronavirus-conspiracy/

Washington Post
Tom Cotton keeps repeating a coronavirus conspiracy theory that was already debunked
Experts say there's no evidence the virus is man-made and it's "highly unlikely" it is the result of an accident at a lab. (127 kB)

Kristian Andersen 23:39
Some data to show that SARS-CoV-2 does indeed bind stronger to human ACE2 receptor: https://www.biorxiv.org/content/10.1101/2020.02.11.944462v1

Oh, and structure...

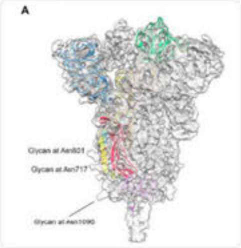
bioRxiv
Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation
The outbreak of a novel betacoronavirus (2019-nCov) represents a pandemic threat that has been declared a public health emergency of international concern. The CoV spike (S) glycoprotein is a key target for urgently needed vaccines, therapeutic antibodies, and diagnostics. To facilitate medical countermeasure (MCM) development we determined a 3.5 Å-resolution cryo-EM structure of the 2019-nCoV S trimer in the prefusion conformation. The predominant state of the trimer has one of the three receptor-binding domains (RBDs) rotated up in a receptor-accessible conformation. We also show biophysical and structural evidence that the 2019-nCoV S binds ACE2 with higher affinity than SARS-CoV S. Addit... Show more
Feb 15th, 2020

February 17th, 2020

February 17th, 2020

Robert Garry 08:46
This is from the sup file

image.png



Those are probably the o-linked glycans - they were just guessing what that density is.

Andrew Rambaut 08:48
Are those antibody accessible?

Robert Garry 08:49
That's the trimer so yes - right on the outside.

February 17th, 2020

Andrew Rambaut 08:49
Cool.

Robert Garry 08:52
It's "only" a 3.5 angstrom structure which is good for cryo. But leaves a lot to modeling and imagination. THere are >20 n-linked glycans

THe o-linked ones probably longer and less structured, but the fact that that density is there is as you said pretty cool.

Kristian Andersen 09:26
Cool. Any insights as to what that cleavage site might do?

Kristian Andersen 09:38
Just skimmed through the manuscript and will read more closely later today - probably best to wait with edits (if any) until we hear back from Clare. I DO notice my name is misspelled though 😉.
Andrew, corrected it on the Virological version.

Robert Garry 10:24
They haven't posted their coordinates yet. I'm guessing still refining the models which takes computer time. They did modify the PRRAR site to PGSAS, but this would leave the O-linkages. At the very least what they labeled as glycans at 717 and 801 likely aren't - they are too high up.
👍 1

Robert Garry 10:31
I think that is the English spelling of "Andersen." *Nature* you know.

The version on virological is pretty good - Jeremy is asking for it - makes a much stronger case against biuoengineering.

While you were dodging rattlers did you come to any insights re dN/dS????

February 17th, 2020

Andrew Rambaut 10:35
The version on the GoogleDoc is out of date. I am just going to fix the figure.

Kristian Andersen 10:40
I'm gonna spike Eddie's drink for pulling this out of Google and into Word 😉.

Finally woke up and properly read through the whole thing - it's very good and balanced IMO. I'm sure we'll have chance to provide updates...

Will work on dN/dS today - let's see where that takes us.

Robert Garry 10:40
There is a SARs that should be SARS. Sorry not to pick up on the 5 vs 6 thing.

Robert Garry 10:46
"Will work on dN/dS today - let's see where that takes us." I think that it could be VERY important,even decisive. But the current version will be pretty understandable by the policy people who I am most concerned about at the moment. The structure/binding kinetic paper came at just the right time. MUCH stronger argument against bioweapon, which is just what is needed now to counter the Fox News crowd and others. There are plenty of follow-up manuscripts where dN/dS, polybasic and O-linked sites across the CoV family, etc could go...

Kristian Andersen 10:53
Totally agree - main issue is that it'll pull us more in a research direction as opposed to perspective so it could get tricky. But I'll work on it and write up a Virological post probably tomorrow or Wednesday - we can then see where this takes us.

As for Fox News - Tom Cotton is trending with COVID-19 on the Twitters at the moment. I gotta say - the guy isn't totally wrong. (although, of course, the reason why they're doing this has nothing to do with the virus and everything to do with they China commentary, so obviously wrong).

February 17th, 2020

Andrew Rambaut 11:09
People are picking up on the fact that we don't rule out animal passaging.

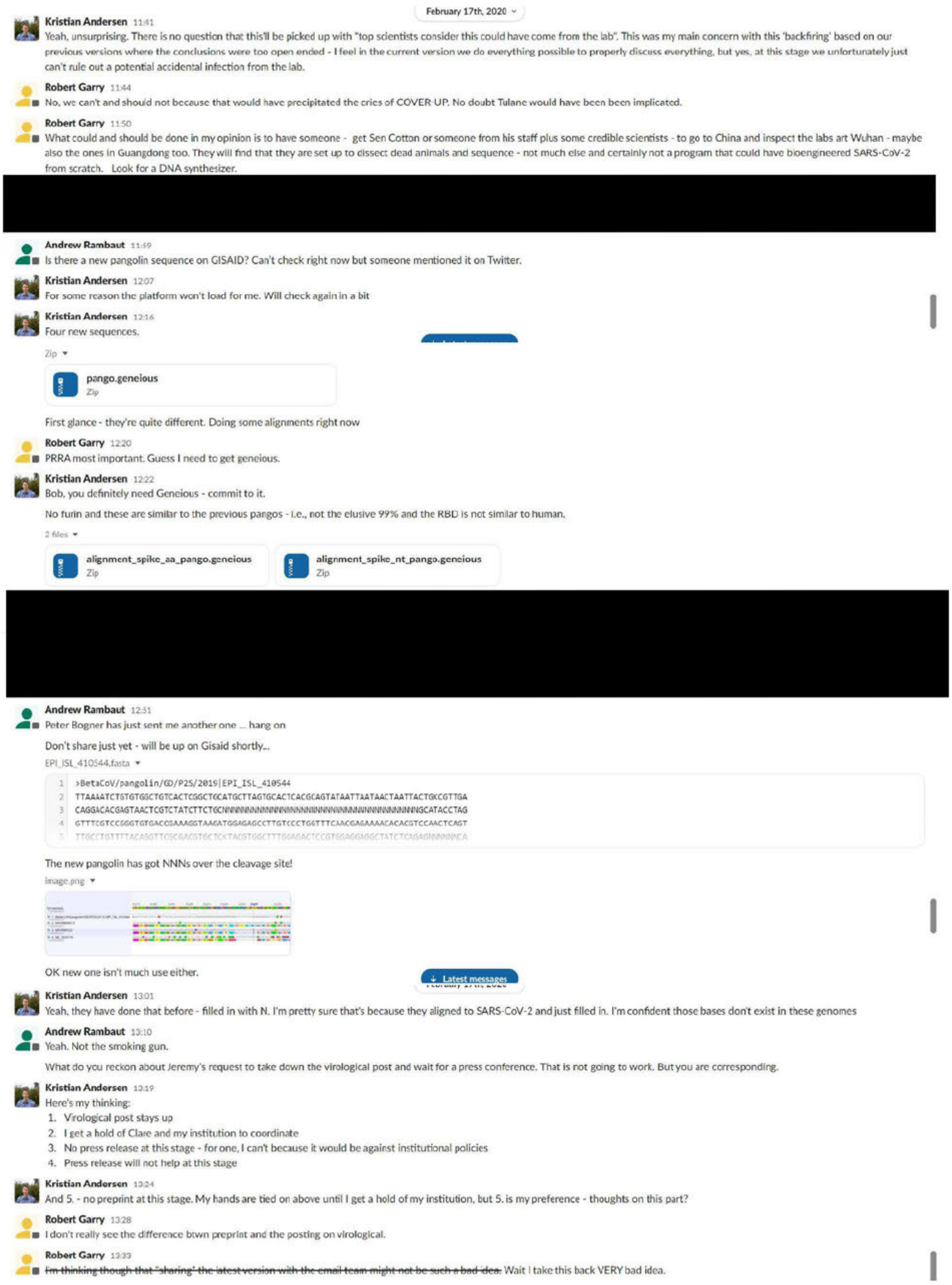
(which we don't because it is still plausible)

image.png

Jaybird

Also, I believe the theory that the virus was found in the wild and the isolated in the lab - not engineered in the lab - then an engineer was infected and released it to the population. Your article does not refute this scenario.

8:33 AM · 2/17/20 · Twitter for iPhone



Kristian Andersen 11:41

Yeah, unsurprising. There is no question that this'll be picked up with "top scientists consider this could have come from the lab". This was my main concern with this 'backfiring' based on our previous versions where the conclusions were too open ended - I feel in the current version we do everything possible to properly discuss everything, but yes, at this stage we unfortunately just can't rule out a potential accidental infection from the lab.

Robert Garry 11:44

No, we can't and should not because that would have precipitated the cries of COVER UP. No doubt Tulane would have been been implicated.

Robert Garry 11:50

What could and should be done in my opinion is to have someone - get Sen Cotton or someone from his staff plus some credible scientists - to go to China and inspect the labs art Wuhan - maybe also the ones in Guangdong too. They will find that they are set up to dissect dead animals and sequence - not much else and certainly not a program that could have bioengineered SARS-CoV-2 from scratch. Look for a DNA synthesizer.

[redacted]

Andrew Rambaut 11:59

Is there a new pangolin sequence on GISAID? Can't check right now but someone mentioned it on Twitter.

Kristian Andersen 12:07

For some reason the platform won't load for me. Will check again in a bit

Kristian Andersen 12:16

Four new sequences.

First glance - they're quite different. Doing some alignments right now

Robert Garry 12:20

PRRA most important. Guess I need to get geneious.

Kristian Andersen 12:22

Bob, you definitely need Geneious - commit to it.

No furin and these are similar to the previous pangos - i.e., not the elusive 99% and the RBD is not similar to human.

[redacted]

Andrew Rambaut 12:51

Peter Bogner has just sent me another one ... hang on

Don't share just yet - will be up on Gisaid shortly...

```
>BetaCoV/pangolin/GD/P2S/2019|EPI_ISL_410544
TTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGKCGTTGA
CAGGACACGAGTAACTCGTCTATCTTCTGCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGCATACCTAG
GTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAACACACGTCCAACTCAGT
TTGCCTGTTTTACAGGTTCGCGACGTGCTCKTACGTGGCTTTGGAGACTCCGTGGAGGAGGCTATCTCAGAGNNNNNNCA
```

The new pangolin has got NNNs over the cleavage site!

OK new one isn't much use either.

Kristian Andersen 13:01

Yeah, they have done that before - filled in with N. I'm pretty sure that's because they aligned to SARS-CoV-2 and just filled in. I'm confident those bases don't exist in these genomes

Andrew Rambaut 13:10

Yeah. Not the smoking gun.

What do you reckon about Jeremy's request to take down the virological post and wait for a press conference. That is not going to work. But you are corresponding.

Kristian Andersen 13:19

Here's my thinking:

1. Virological post stays up
2. I get a hold of Clare and my institution to coordinate
3. No press release at this stage - for one, I can't because it would be against institutional policies
4. Press release will not help at this stage

Kristian Andersen 13:24

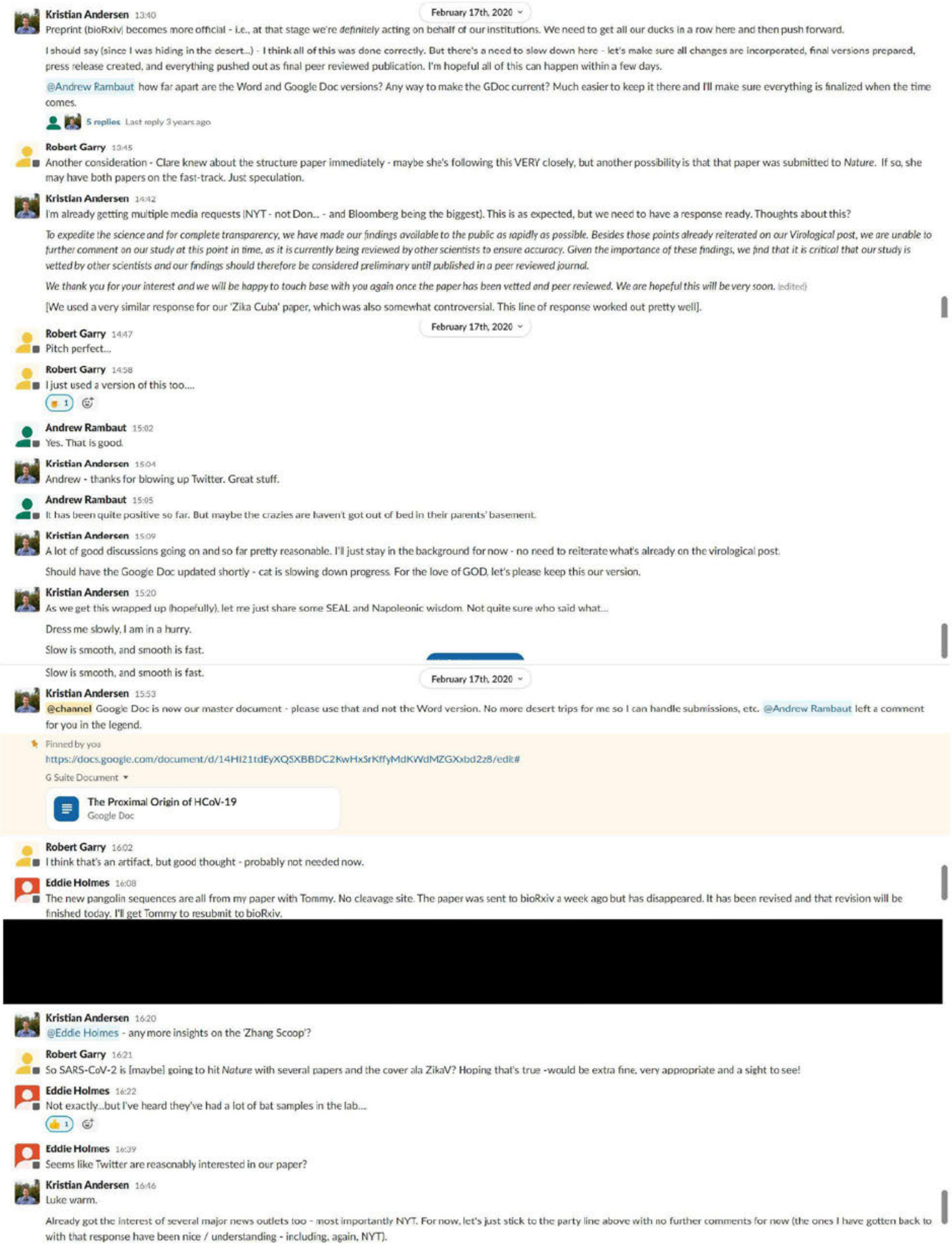
And 5. - no preprint at this stage. My hands are tied on above until I get a hold of my institution, but 5. is my preference - thoughts on this part?

Robert Garry 13:28

I don't really see the difference btwn preprint and the posting on virological.

Robert Garry 13:33

~~I'm thinking though that "sharing" the latest version with the email team might not be such a bad idea.~~ Wait I take this back VERY bad idea.



February 17th, 2020

Kristian Andersen 13:40
Preprint (bioRxiv) becomes more official - i.e., at that stage we're *definitely* acting on behalf of our institutions. We need to get all our ducks in a row here and then push forward.

I should say (since I was hiding in the desert...) - I think all of this was done correctly. But there's a need to slow down here - let's make sure all changes are incorporated, final versions prepared, press release created, and everything pushed out as final peer reviewed publication. I'm hopeful all of this can happen within a few days.

@Andrew Rambaut how far apart are the Word and Google Doc versions? Any way to make the GDoc current? Much easier to keep it there and I'll make sure everything is finalized when the time comes.

5 replies Last reply 3 years ago

Robert Garry 13:45
Another consideration - Clare knew about the structure paper immediately - maybe she's following this VERY closely, but another possibility is that that paper was submitted to *Nature*. If so, she may have both papers on the fast-track. Just speculation.

Kristian Andersen 14:42
I'm already getting multiple media requests (NYT - not Don... - and Bloomberg being the biggest). This is as expected, but we need to have a response ready. Thoughts about this?

To expedite the science and for complete transparency, we have made our findings available to the public as rapidly as possible. Besides those points already reiterated on our Virological post, we are unable to further comment on our study at this point in time, as it is currently being reviewed by other scientists to ensure accuracy. Given the importance of these findings, we find that it is critical that our study is vetted by other scientists and our findings should therefore be considered preliminary until published in a peer reviewed journal.

We thank you for your interest and we will be happy to touch base with you again once the paper has been vetted and peer reviewed. We are hopeful this will be very soon. (edited)

[We used a very similar response for our 'Zika Cuba' paper, which was also somewhat controversial. This line of response worked out pretty well].

February 17th, 2020

Robert Garry 14:47
Pitch perfect...

Robert Garry 14:58
I just used a version of this too....
1

Andrew Rambaut 15:02
Yes. That is good.

Kristian Andersen 15:04
Andrew - thanks for blowing up Twitter. Great stuff.

Andrew Rambaut 15:05
It has been quite positive so far. But maybe the crazies are haven't got out of bed in their parents' basement.

Kristian Andersen 15:09
A lot of good discussions going on and so far pretty reasonable. I'll just stay in the background for now - no need to reiterate what's already on the virological post.

Should have the Google Doc updated shortly - cat is slowing down progress. For the love of GOD, let's please keep this our version.

Kristian Andersen 15:20
As we get this wrapped up (hopefully), let me just share some SEAL and Napoleonic wisdom. Not quite sure who said what...

Dress me slowly, I am in a hurry.

Slow is smooth, and smooth is fast.

Slow is smooth, and smooth is fast.

February 17th, 2020

Kristian Andersen 15:53
@channel Google Doc is now our master document - please use that and not the Word version. No more desert trips for me so I can handle submissions, etc. @Andrew Rambaut left a comment for you in the legend.

Pinned by you
https://docs.google.com/document/d/14HI21tdEyXQSXBBDC2KwHxSrKffyMdKWdMZGXxbd2z8/edit#
G Suite Document ▾

The Proximal Origin of HCoV-19
Google Doc

Robert Garry 16:02
I think that's an artifact, but good thought - probably not needed now.

Eddie Holmes 16:08
The new pangolin sequences are all from my paper with Tommy. No cleavage site. The paper was sent to bioRxiv a week ago but has disappeared. It has been revised and that revision will be finished today. I'll get Tommy to resubmit to bioRxiv.

[redacted]

Kristian Andersen 16:20
@Eddie Holmes - any more insights on the 'Zhang Scoop'?

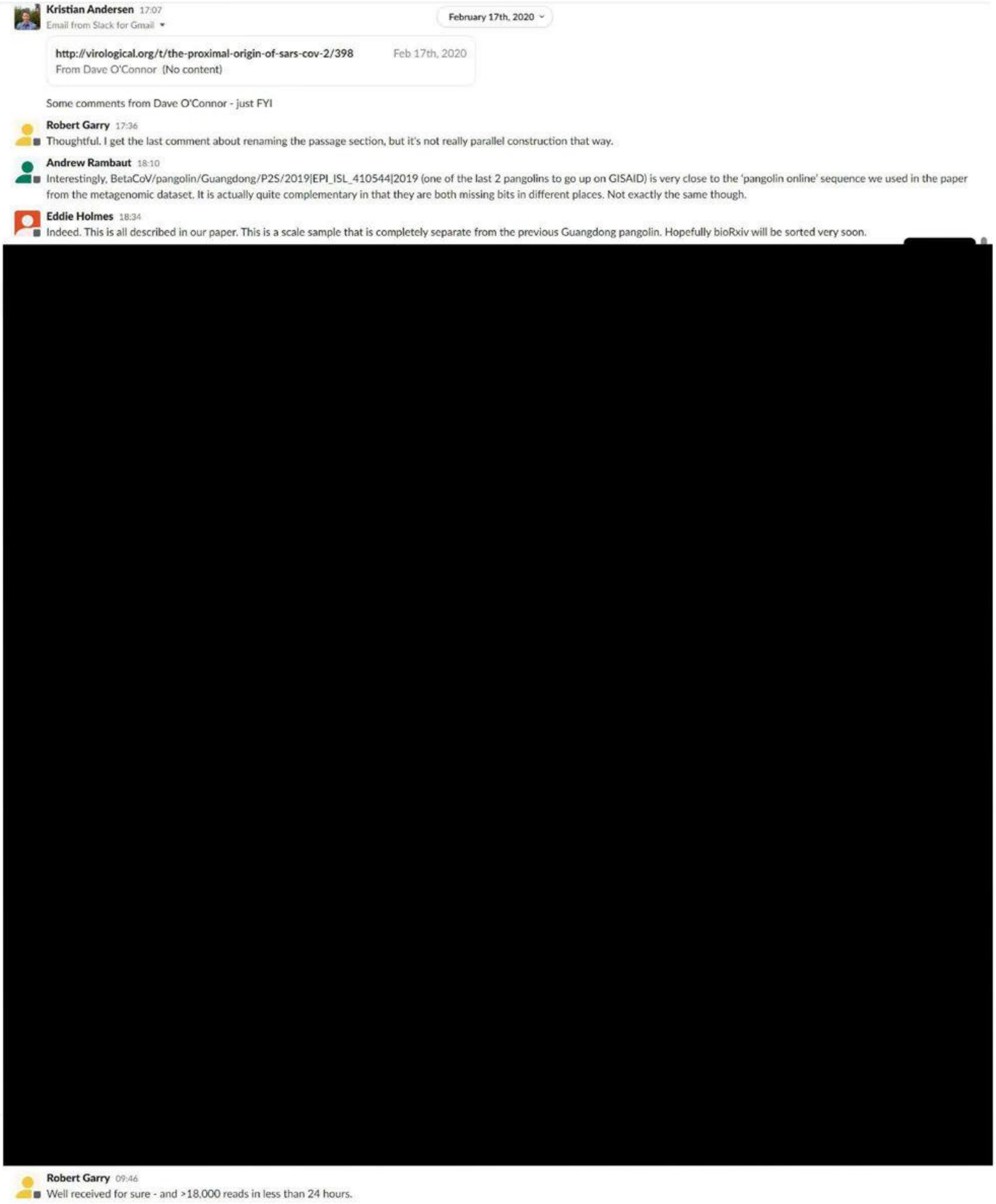
Robert Garry 16:21
So SARS-CoV-2 is [maybe] going to hit *Nature* with several papers and the cover ala ZikaV? Hoping that's true -would be extra fine, very appropriate and a sight to see!

Eddie Holmes 16:22
Not exactly...but I've heard they've had a lot of bat samples in the lab....
👍 1

Eddie Holmes 16:39
Seems like Twitter are reasonably interested in our paper?

Kristian Andersen 16:46
Luke warm.

Already got the interest of several major news outlets too - most importantly NYT. For now, let's just stick to the party line above with no further comments for now (the ones I have gotten back to with that response have been nice / understanding - including, again, NYT).



Kristian Andersen 17:07
Email from Slack for Gmail

http://virological.org/t/the-proximal-origin-of-sars-cov-2/398 Feb 17th, 2020
From Dave O'Connor (No content)

Some comments from Dave O'Connor - just FYI

Robert Garry 17:36
Thoughtful. I get the last comment about renaming the passage section, but it's not really parallel construction that way.

Andrew Rambaut 18:10
Interestingly, BetaCoV/pangolin/Guangdong/P2S/2019|EPI_ISL_410544|2019 (one of the last 2 pangolins to go up on GISAID) is very close to the 'pangolin online' sequence we used in the paper from the metagenomic dataset. It is actually quite complementary in that they are both missing bits in different places. Not exactly the same though.

Eddie Holmes 18:34
Indeed. This is all described in our paper. This is a scale sample that is completely separate from the previous Guangdong pangolin. Hopefully bioRxiv will be sorted very soon.

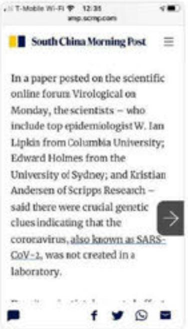
[redacted]

Robert Garry 09:46
Well received for sure - and >18,000 reads in less than 24 hours.

February 18th, 2020

Kristian Andersen 15:37
Sorry Andrew and Bob that you didn't quite make the cut to be a "Top Epidemiologist". Hilarious 🥳

File from iOS ▾



In a paper posted on the scientific online forum Virological on Monday, the scientists – who include top epidemiologist W. Ian Lipkin from Columbia University; Edward Holmes from the University of Sydney; and Kristian Andersen of Scripps Research – said there were crucial genetic clues indicating that the coronavirus, also known as SARS-CoV-2, was not created in a laboratory.

February 18th, 2020

Andrew Rambaut 15:54
I don't think you get that sobriquet, Kristian (or Eddie). You are just a 'scientist'.

Kristian Andersen 15:57
That's just like your opinion, man.

I think you might be right. 😭

Robert Garry 17:14
It's all fine - I'm just going to keep plugging along best I can...

And yeah Ian got the top billing and a title. Eddie and Kristian were sorta afterthoughts. Oh well...

Andrew Rambaut 17:45
We have our first citation in the Lancet: https://www.thelancet.com/pb-assets/Lancet/pdfs/S0140673620304189.pdf

Robert Garry 18:09
Must have been added in proof I guess.

Andrew Rambaut 18:11
I signed the petition too.

You know that 'top epidemiologist' is cockney rhyming slang for 'call my proctologist'?

🤣 1

February 18th, 2020

Andrew Rambaut 18:52
I think this is 'pango99'

EPI_ISL_410721.fasta ▾

```
>BetaCoV/pangolin/Guandong/1/2020|EPI_ISL_410721
CACGCAGTATAATTAATAGCTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGT
TTCGTCCGTGTTGCAGCCGATCATCAGCATACCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTC
CCTGGTTTCAACGAGAAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGG
AGACTCCGTGGAGGAGGCTATCTCAGAGGCACGTCAACATCTCAAGGATGGCACTTGTGGCTTAGTAGAGGTTGAAAAAG
```

Robert Garry 18:52
I signed it too, but I'm fearful I'm going to start getting requests to donate to GVP.

1 reply 3 years ago

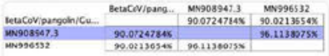
February 18th, 2020

Andrew Rambaut 18:53
Pango99 (if that is what it is) doesn't have the furin site.

image.png ▾

When they say 'up to 99%' they mean an average og 90%

image.png ▾



	BetaCoV/pang...	MN908947.3	MN996532
BetaCoV/pangolin/Gu...		90.0724784%	90.0213654%
MN908947.3	90.0724784%		96.1138075%
MN996532	90.0213654%	96.1138075%	

February 18th, 2020

Kristian Andersen 18:56
Hmmm

What's the RBD like?

Also, this was picked up in Guangdong in January of this year? The more pango sequences I see the less likely I find that they are intermediate - I think they're just one of many animals with SARS-like CoVs

Andrew Rambaut 19:00
Zip

SARS-CoV-2_BaTG13_Pangolin.geneious
Zip

I think they are picking it up at markets or staging areas?

Very like in MERS in camels - lots of really short recombinations.

Suggests lots of coinfections

But basically the same as the pangolin online in RBD

Kristian Andersen 19:04
Yeah, basically looks like a better sequenced version of the "pangolin online" sequence. Interesting with the RBD for sure.

Andrew Rambaut 19:05
Ignore - that was Ns

February 18th, 2020

Kristian Andersen 19:05
Yup

Looks highly similar to me

Andrew Rambaut 19:05
image.png

Kristian Andersen 19:06
Question is - did they recently realize that pangolins carry CoVs and then grew them in the lab to see if they could infect human cells? This is quite a high probability event.

Clearly none of these pangolin sequences were the source though

The RBD is very intriguing - if it's not lab, then definitely recombination (also high probability event)

Robert Garry 19:08
The NTD of S different than SARS-CoV-2, but yes the RBD thereafter very similar except the PRRA. And yes that looks like a CoV that could infect people. But recombinant with what?

Kristian Andersen 19:09
Recombinants can be anything really - could be bat and pango, just all pango, pango and intermediate, etc.

Could even be human and pango.

Andrew Rambaut 19:10
Yes. But both the pangolin and the SARS2 lineage will have diverged since the recombination.

It could have jumped either way as well.

Kristian Andersen 19:15
Definitely

Robert Garry 19:18
Do we need to add a line or two about recombination to the paper - at least put the word in as a potential?

Kristian Andersen 19:23
Yeah, we probably should. Let's wait until we hear back from Nature before doing any tweaks though - I talked to Clare this morning and I'm hoping end of this week.

Robert Garry 19:28
Depends on who they sent it to - the twittering has been closer to 99% [positive] than the pangolin sequence. A few diehards might object to even whiffing at the possibility of a lab escape, but I didn't get the sense from the public reactions that that was offensive to most. Clearly stating no bioengineering seems to be the take home, plus that it is well done and needed.

Kristian Andersen 19:37
I think there are two camps in the interpretation of the paper: (1) definitely didn't come from the lab, (2) they said they can't rule out it came from the lab so it definitely came from the lab.

🤷

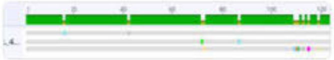
February 18th, 2020

Andrew Rambaut 20:08
New pangolin is at least a much better sequence. See the recombinations in spike nicely:

image.png

Just the RBD:

image.png



Kristian Andersen 20:12
Yup, pretty cool to see. Since that 'online' sequence was kinda stitched together, I'm also happy to see a higher quality sequence for this

Andrew Rambaut 20:14
Yes. I am also strongly moving towards the idea that these poor bastards are becoming infected in the live animal chain from some other animal (ferret-badgers).

Robert Garry 21:12
Maybe a couple of animals - hence the several lineages?

Are there really that many differences at the 5' end? Or is that sequencing error?

February 18th, 2020

Kristian Andersen 21:18
I think that's probably real

You have Geneious now Bob - check the alignment 😉

Robert Garry 21:22
Geneious is on my office desktop - but if I was there I'd be blasting the 5' end of Pango90 looking for a match.

Kristian Andersen 21:27
"No significant similarity found"... Hmmm

2 files ▾

RaTG13 vs nCoV and pango vs nCoV. Big dip in similarity between pango and nCoV in the 5' end of the spike. Interesting. Could be recombination breakpoint.

Robert Garry 21:38
Hmmm - that's unexpected. Did you run a protein blast?

Kristian Andersen 21:59
Here's a tblastx: https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Get&RID=4T8H83NH014

Robert Garry 22:08
So you ran the blastx on the 5' sequence and nothing? That's very strange?

Kristian Andersen 22:10
No, the tblastx has hits to various CoVs (via the link above) - including HKUs. The blastn didn't return anything.

Eddie Holmes 23:08
There are a few points to note: (i) there are 2 lineages of pango CoVs, smuggled into different provinces (Guangxi & Guangdong), that are BOTH close to SARS-CoV-2. If there were just caught in the chain, why the geographical separation? That seems non-random to me. Why both viruses like SARS-CoV-2?; (ii) how to explain similarity to SARS-CoV-2 in the RBD? In the RBD the pango CoVs are the closest relative to SARS-CoV-2. If it is recombination, what is recombining with what? Interestingly, if you do an RBD tree on synonymous sites only then the pango CoVs are more distant to RaTG13 again. So, I don't think you can exclude convergence. But what is driving that? Very clearly, there are more animals involved in this but it is very hard to work out what is moving to what.

1

3 replies Last reply 3 years ago

[illegible]

Eddie Holmes 23:11
The new pango virus is almost identical to ours. They totally over-hyped in that press release. Mind you. Universities always over-hype these things.

PDF ▾

fullg_wSCAU.pdf
PDF

February 19th, 2020

Andrew Rambaut 01:58
Morning.

Kristian Andersen 01:59
'night.

Andrew Rambaut 02:00
Look at the alignment I posted above.

image.png ▾

Kristian Andersen 02:01
Yeah... true - recombination.

Andrew Rambaut 02:01
You can see then 5' end. But also that RaTG13 has a patch of differences in the RBD. It looks like it had a recombination in?

Two things - need to look if that recombination in 5' spike extends into 3' ORF1ab. Second look if the RBD patch in RaTG13 is also visible in the nucs.

Kristian Andersen 02:04
This is what you guys saw in MERS?

Andrew Rambaut 02:08
This sort of thing - extensive recombination but often of quite short regions. Nowhere near as diverse as this.

It is a bit crazy that you can swap in so many amino acids and it still works.

Kristian Andersen 02:10
Probably vast majority of times it doesn't. I think the only reasonable explanation is that there is a **fuck ton** of CoVs circulating in a bunch of different animals in some parts of China (edited)

Do we know if anybody has ever done passive surveillance in any of these 'wet' markets? Would be interesting to know if one would find all sorts of CoVs circulating. You know, similar to what GVP has suggested doing... I don't know if any of these figures are accurate, but I think I saw 70% infectivity rates in some of the captured pangolins - that's very very high.

[which, if true, probably also means that they're reservoirs and not merely intermediates]

Eddie Holmes 03:29
I still don't quite totally see RBD recombination into the pangolin sequence. I see it the bit where is divergent, but where does it acquire the human sequence?

Eddie Holmes 03:36
I'm not doubting that there's recombination. Obvious. But I need see where it makes the human and pangolin sequence so close in the RBD?

Andrew Rambaut 04:02
I plan to do a more detailed analysis today. Will post here.

Eddie Holmes 04:05
Or are you saying that the RaTG13 RBD has recombined out? Couldn't that little cluster of mutations just be receptor adaptation?

February 19th, 2020

Andrew Rambaut 04:06
Need to look in the synonymous.

Eddie Holmes 04:06
PDF ▾

Figure3_2020-02-18-6am.pdf
PDF

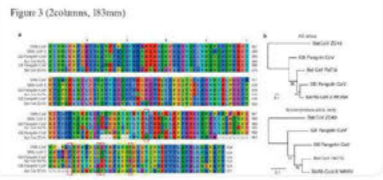


Figure 3 (2columns, 183mm)

Andrew Rambaut 04:06
Either way this happened a while back and there are overlayed mutations.

Eddie Holmes 04:07
Here are Tommy's trees for the RBD

Eddie Holmes 04:12
Here's a rough amino acid tree of the RBD. Pretty striking.

PDF ▾

selected_RBD_whole_wSCAU_aa_phyml.pdf
PDF

For the RBD I can't quite choose between recombination or convergence, or both?

In unrelated news I hear that our proximal origins paper has been very big news in China...

February 19th, 2020

Andrew Rambaut 04:49
In a good way?

Andrew Rambaut 04:54
It definitely looks like the nucleotides follow the amino acids:

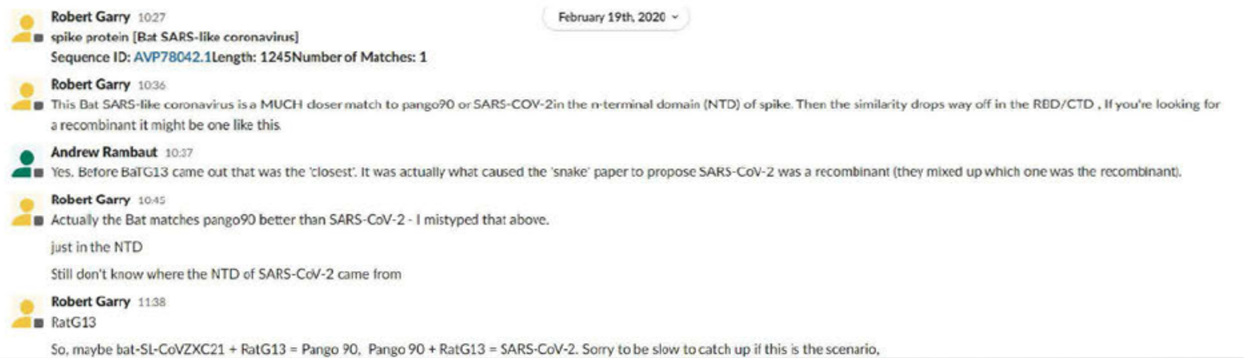
image.png ▾

I will add in all of Tommy's ones and a few outgroups and keep looking.

In all but 1 of the 6 key residues, the pangolin and the human virus use the same codon. The exception is a A/T transversion in the third position.

Robert Garry 05:05
The Guangdong Wildlife Rescue Center received 21 live Malayan pangolins from the Anti-smuggling Customs Bureau on 24 March 2019; most individuals, including adults and subadults, were in poor health, and their bodies were covered with skin eruptions. All these Malayan pangolins were rescued by the Guangdong Wildlife Rescue Center, however, 16 died after extensive rescue efforts. Most of the dead pangolins had a swollen lung which contained a frothy liquid, as well as the symptom of pulmonary fibrosis, and in the minority of the dead ones, we observed hepatomegaly and splenomegaly. We collected 21 organ samples of lung, lymph, and spleen with obvious symptoms from 11 dead Malayan pangolins to uncover the virus diversity and molecular epidemiology of potential etiologies of viruses based on a viral metagenomic study. This study will be beneficial to pangolin disease research and subsequent rescue operation.One or several members of the *Coronaviridae* families were identified in 2 out of the 11 *M. javanica* individuals (individual 07 and 08).

From the part parrot viruses paper. I don't think in current ref list but probably should be.



Robert Garry 10:27
spike protein [Bat SARS-like coronavirus]
Sequence ID: AVP78042.1Length: 1245Number of Matches: 1

Robert Garry 10:36
This Bat SARS-like coronavirus is a MUCH doser match to pango90 or SARS-COV-2in the n-terminal domain (NTD) of spike. Then the similarity drops way off in the RBD/CTD , If you're looking for a recombinant it might be one like this.

Andrew Rambaut 10:37
Yes. Before BaTG13 came out that was the 'closest'. It was actually what caused the 'snake' paper to propose SARS-CoV-2 was a recombinant (they mixed up which one was the recombinant).

Robert Garry 10:45
Actually the Bat matches pango90 better than SARS-CoV-2 - I mistyped that above.

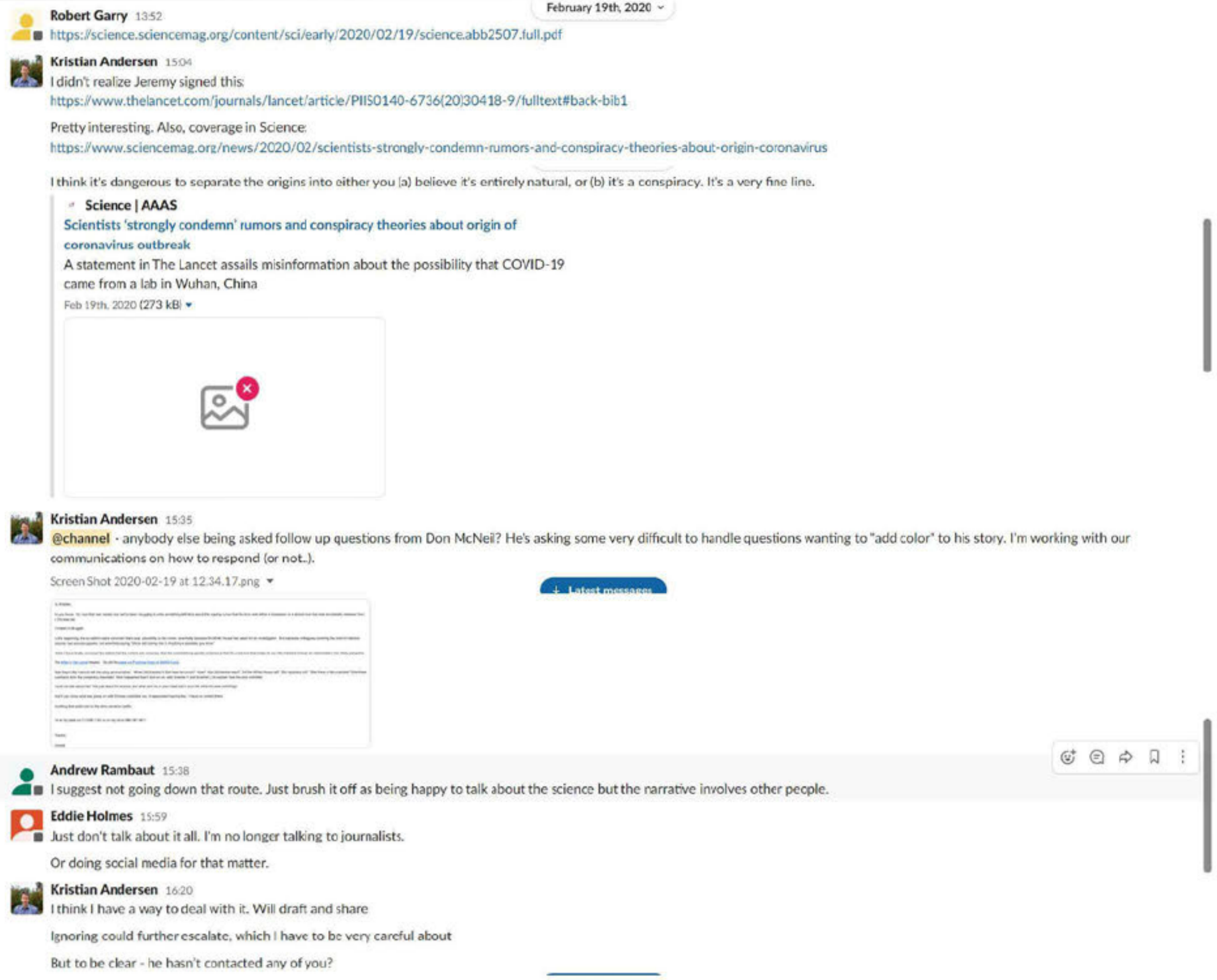
just in the NTD

Still don't know where the NTD of SARS-CoV-2 came from

Robert Garry 11:38
RatG13

So, maybe bat-SL-CoVZXC21 + RatG13 = Pango 90, Pango 90 + RatG13 = SARS-CoV-2. Sorry to be slow to catch up if this is the scenario,

[redacted]



Robert Garry 13:52
https://science.sciencemag.org/content/sci/early/2020/02/19/science.abb2507.full.pdf

Kristian Andersen 15:04
I didn't realize Jeremy signed this:
https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30418-9/fulltext#back-bib1

Pretty interesting. Also, coverage in Science:
https://www.sciencemag.org/news/2020/02/scientists-strongly-condemn-rumors-and-conspiracy-theories-about-origin-coronavirus

I think it's dangerous to separate the origins into either you (a) believe it's entirely natural, or (b) it's a conspiracy. It's a very fine line.

> **Science | AAAS**
> Scientists 'strongly condemn' rumors and conspiracy theories about origin of coronavirus outbreak
> A statement in The Lancet assails misinformation about the possibility that COVID-19 came from a lab in Wuhan, China
> Feb 19th, 2020 (273 kB)

Kristian Andersen 15:35
@channel - anybody else being asked follow up questions from Don McNeil? He's asking some very difficult to handle questions wanting to "add color" to his story. I'm working with our communications on how to respond (or not.).

Screen Shot 2020-02-19 at 12.34.17.png

Andrew Rambaut 15:38
I suggest not going down that route. Just brush it off as being happy to talk about the science but the narrative involves other people.

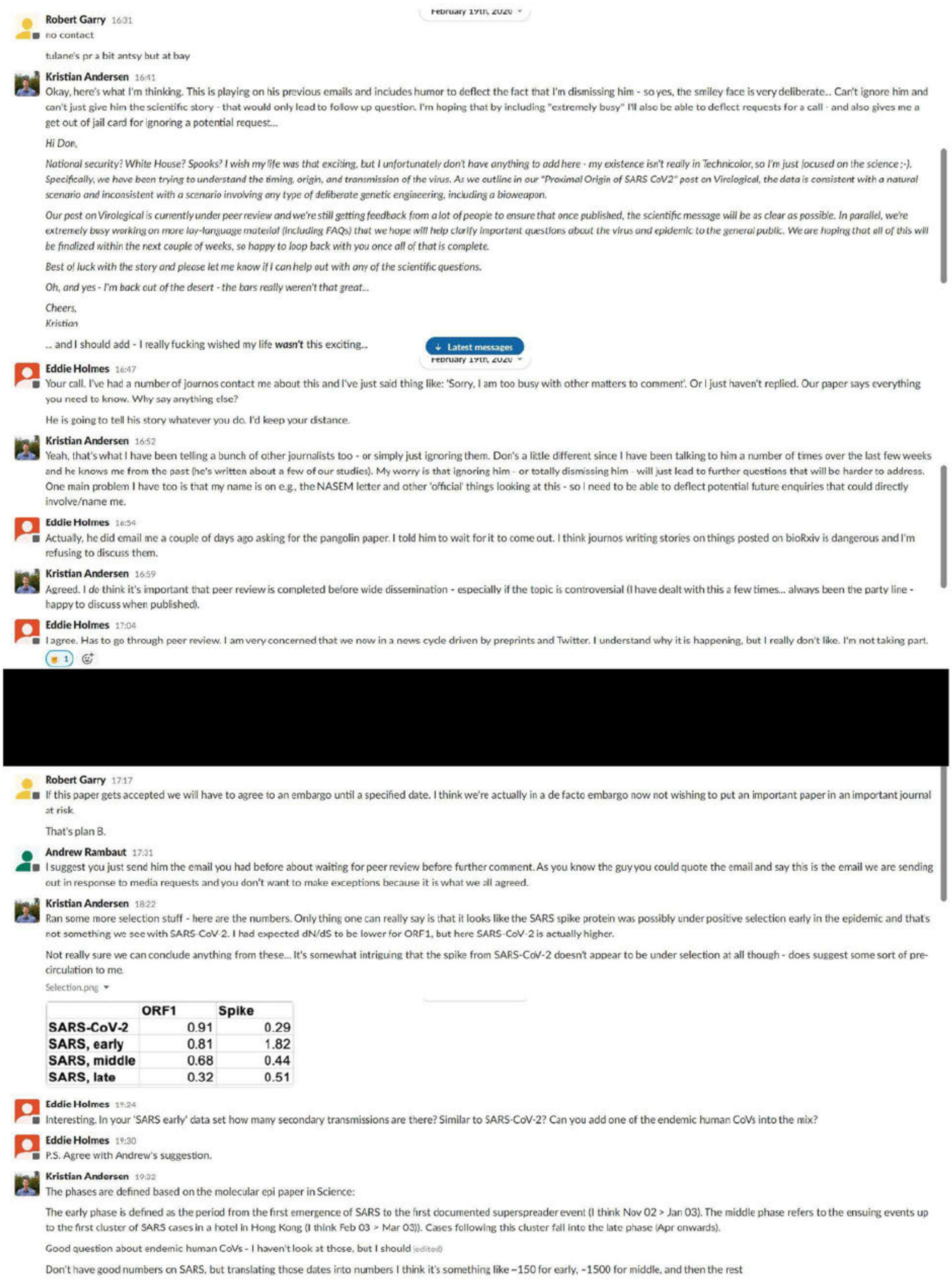
Eddie Holmes 15:59
Just don't talk about it all. I'm no longer talking to journalists.

Or doing social media for that matter.

Kristian Andersen 16:20
I think I have a way to deal with it. Will draft and share

Ignoring could further escalate, which I have to be very careful about

But to be clear - he hasn't contacted any of you?



Robert Garry 16:31
no contact

tulane's pr a bit antsy but at bay

Kristian Andersen 16:41
Okay, here's what I'm thinking. This is playing on his previous emails and includes humor to deflect the fact that I'm dismissing him - so yes, the smiley face is very deliberate... Can't ignore him and can't just give him the scientific story - that would only lead to follow up question. I'm hoping that by including "extremely busy" I'll also be able to deflect requests for a call - and also gives me a get out of jail card for ignoring a potential request...

Hi Don,

National security? White House? Spooks? I wish my life was that exciting, but I unfortunately don't have anything to add here - my existence isn't really in Technicolor, so I'm just focused on the science ;-). Specifically, we have been trying to understand the timing, origin, and transmission of the virus. As we outline in our "Proximal Origin of SARS CoV2" post on Virological, the data is consistent with a natural scenario and inconsistent with a scenario involving any type of deliberate genetic engineering, including a bioweapon.

Our post on Virological is currently under peer review and we're still getting feedback from a lot of people to ensure that once published, the scientific message will be as clear as possible. In parallel, we're extremely busy working on more lay-language material (including FAQs) that we hope will help clarify important questions about the virus and epidemic to the general public. We are hoping that all of this will be finalized within the next couple of weeks, so happy to loop back with you once all of that is complete.

Best of luck with the story and please let me know if I can help out with any of the scientific questions.

Oh, and yes - I'm back out of the desert - the bars really weren't that great...

Cheers,
Kristian

... and I should add - I really fucking wished my life **wasn't** this exciting...

Eddie Holmes 16:47
Your call. I've had a number of journos contact me about this and I've just said thing like: 'Sorry, I am too busy with other matters to comment'. Or I just haven't replied. Our paper says everything you need to know. Why say anything else?

He is going to tell his story whatever you do. I'd keep your distance.

Kristian Andersen 16:52
Yeah, that's what I have been telling a bunch of other journalists too - or simply just ignoring them. Don's a little different since I have been talking to him a number of times over the last few weeks and he knows me from the past (he's written about a few of our studies). My worry is that ignoring him - or totally dismissing him - will just lead to further questions that will be harder to address. One main problem I have too is that my name is on e.g., the NASEM letter and other 'official' things looking at this - so I need to be able to deflect potential future enquiries that could directly involve/name me.

Eddie Holmes 16:54
Actually, he did email me a couple of days ago asking for the pangolin paper. I told him to wait for it to come out. I think journos writing stories on things posted on bioRxiv is dangerous and I'm refusing to discuss them.

Kristian Andersen 16:59
Agreed. I *do* think it's important that peer review is completed before wide dissemination - especially if the topic is controversial (I have dealt with this a few times... always been the party line - happy to discuss when published).

Eddie Holmes 17:04
I agree. Has to go through peer review. I am very concerned that we now in a news cycle driven by preprints and Twitter. I understand why it is happening, but I really don't like. I'm not taking part.

[redacted]

Robert Garry 17:17
If this paper gets accepted we will have to agree to an embargo until a specified date. I think we're actually in a de facto embargo now not wishing to put an important paper in an important journal at risk.

That's plan B.

Andrew Rambaut 17:31
I suggest you just send him the email you had before about waiting for peer review before further comment. As you know the guy you could quote the email and say this is the email we are sending out in response to media requests and you don't want to make exceptions because it is what we all agreed.

Kristian Andersen 18:22
Ran some more selection stuff - here are the numbers. Only thing one can really say is that it looks like the SARS spike protein was possibly under positive selection early in the epidemic and that's not something we see with SARS-CoV-2. I had expected dN/dS to be lower for ORF1, but here SARS-CoV-2 is actually higher.

Not really sure we can conclude anything from these... It's somewhat intriguing that the spike from SARS-CoV-2 doesn't appear to be under selection at all though - does suggest some sort of pre-circulation to me.

Selection.png

	ORF1	Spike
SARS-CoV-2	0.91	0.29
SARS, early	0.81	1.82
SARS, middle	0.68	0.44
SARS, late	0.32	0.51

Eddie Holmes 19:24
Interesting. In your 'SARS early' data set how many secondary transmissions are there? Similar to SARS-CoV-2? Can you add one of the endemic human CoVs into the mix?

Eddie Holmes 19:30
P.S. Agree with Andrew's suggestion.

Kristian Andersen 19:32
The phases are defined based on the molecular epi paper in Science:

The early phase is defined as the period from the first emergence of SARS to the first documented superspreader event (I think Nov 02 > Jan 03). The middle phase refers to the ensuing events up to the first cluster of SARS cases in a hotel in Hong Kong (I think Feb 03 > Mar 03)). Cases following this cluster fall into the late phase (Apr onwards).

Good question about endemic human CoVs - I haven't look at those, but I should (edited)

Don't have good numbers on SARS, but translating those dates into numbers I think it's something like ~150 for early, ~1500 for middle, and then the rest

Eddie Holmes 02:16
Thanks for that.

However this outbreak/epidemic/pandemic goes it has been bloody good for Virological.org. Amazing number of views for the proximal origins piece. (edited)

Andrew Rambaut 05:53
I thought I better share an email that I think is really to all of us:

image.png ▾

😆 1

Robert Garry 06:27
"it looks like the SARS spike protein was possibly under positive selection early in the epidemic"

Robert Garry 06:40
Should be possible to look more closely at that- not easily. Map the mutations on the S 3D structure. I'd expect adaptation to show up or get fixed at the RBD and in the holes in the glycan shield [aka epitopes]. Might have to do it by "lineages" to see what got fixed in a certain transmission chain. It may be more random early on.

Andrew Rambaut 06:45
Hey Bob, what would you think the effect of a deletion just before the furin site (in a human SARS-CoV-2 virus). The purple in this figure. Would this be a viable spike protein? I can't tell you where this comes from just now.

image.png ▾

Andrew Rambaut 06:57
Possibly the deletion is also the polybasic residues as well:

image.png ▾

February 20th, 2020 ⌄

Robert Garry 07:06
it would be very interesting for sure. Viable yes. The PRRA created an longer loop where the furin or furin-like enzyme has to clip. If you shorten the loop and remove one if not more of the O-linked glycans you're back to something that structurally is probably like RaTG13. Looking at the sequences around S1/S2 in other CoVs there's a good bit of variation including insertions and deletions at the end of S1 or in the cleavage site themselves within a virus (like HKU1 or MHV). Also its possible to change (knockout) the cleavage site altogether and get a well-folded protein as they did to get the cryo structure in the new science paper.

Robert Garry 07:12
responding to new message - curiouser and curiouser [Alice]. But also still viable I'd get unless you knock out the last R in the PRRAR in which case you don't have any cleavage site there at all. If the virus in this case is still viable then it's using a cleavage site further down. Those exist but this would be a pretty big variation on the theme.

I'd guess

Andrew Rambaut 07:13
Interesting. Thanks.

What are the residues would I be looking for for another cleavage site?

Robert Garry 07:32
R possibly K most likely

Andrew Rambaut 07:59
One last question - could this be something that passaging in Vero-E6 cells could induce?

Robert Garry 08:21
if they were passaging in Vero cells then they no doubt used trypsin to split the cells. It's hard to was off all the trypsin (and in fact you don't want to if you're growing a virus like most flu vs that don't have a furin site. So yes i suppose if you passages a virus with a furin site a lot you might counter-select to a trypsin site or maybe even another cleavage site altogether in cell culture. CoVs do have a second cleavage site S' that is KR in most viruses right before one of the fusion peptides. There's also some alternatives for viruses that aren't "activated" and don't fuse at the surface (cathepsin) but go the endocytic route. Lot of sequence between the S1/S2 junction and the S' site.

wash off

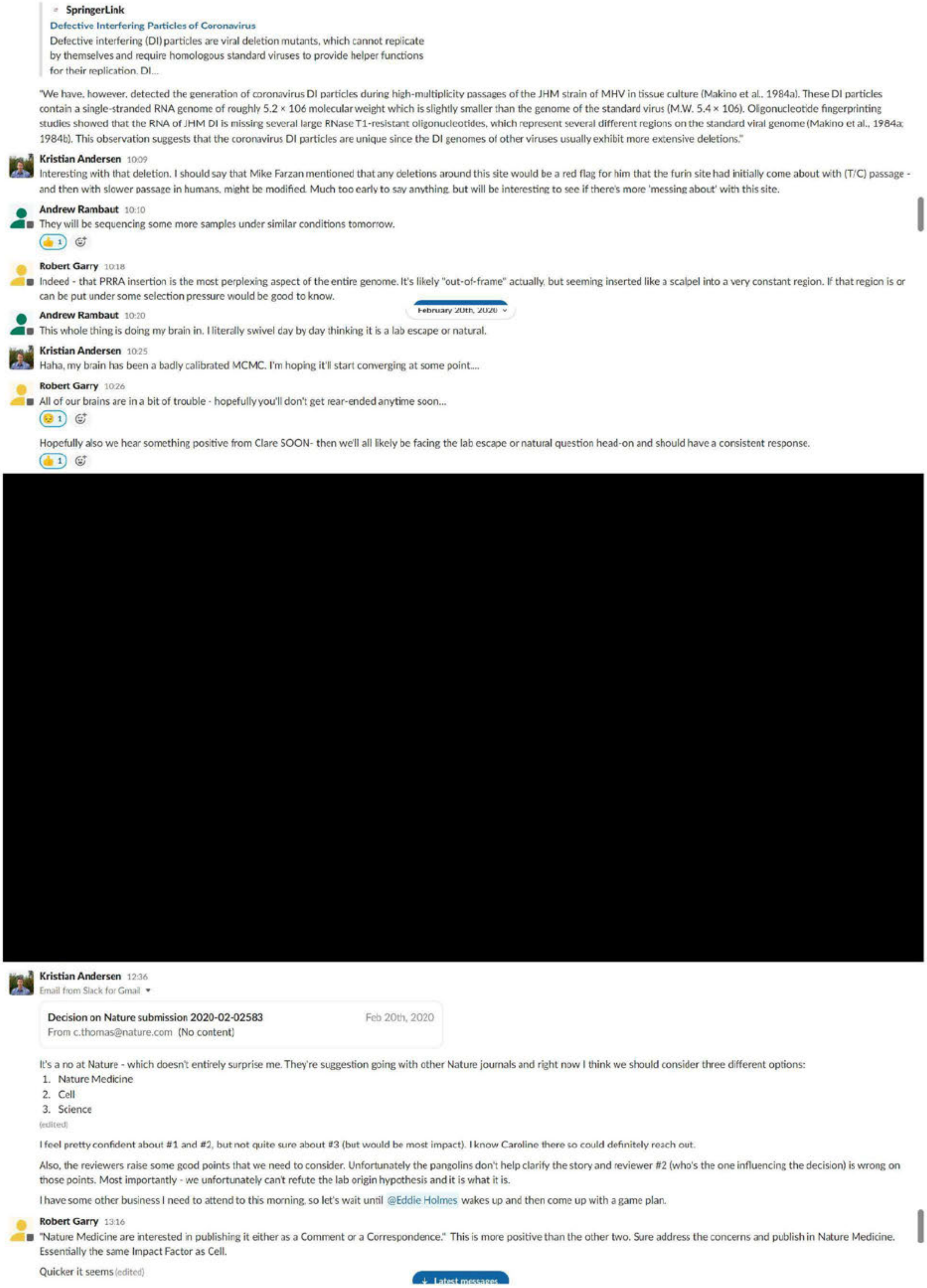
Andrew Rambaut 09:38
Basically a collaborator has found this deletion in about 50% of the reads from a sample. I guess it is possible that it is a cell adaptation (removing the glycan sites as well). I may get back to you on this if they want to take it further. (edited)

Robert Garry 09:39
Interesting - Happy to weigh in as needed!

Robert Garry 10:00
You'd probably get different perhaps opposite results with a rapid forced passage vs a meandering slow passage.

Growing virus stocks and avoiding generation of internal deletions aka defective interfering particles is something of an art form.

https://link.springer.com/chapter/10.1007%2F978-1-4684-1280-2_23



> **SpringerLink**
> Defective Interfering Particles of Coronavirus
> Defective interfering (DI) particles are viral deletion mutants, which cannot replicate by themselves and require homologous standard viruses to provide helper functions for their replication. DI...

"We have, however, detected the generation of coronavirus DI particles during high-multiplicity passages of the JHM strain of MHV in tissue culture (Makino et al., 1984a). These DI particles contain a single-stranded RNA genome of roughly 5.2 × 106 molecular weight which is slightly smaller than the genome of the standard virus (M.W. 5.4 × 106). Oligonucleotide fingerprinting studies showed that the RNA of JHM DI is missing several large RNase T1-resistant oligonucleotides, which represent several different regions on the standard viral genome (Makino et al., 1984a; 1984b). This observation suggests that the coronavirus DI particles are unique since the DI genomes of other viruses usually exhibit more extensive deletions."

Kristian Andersen 10:09
Interesting with that deletion. I should say that Mike Farzan mentioned that any deletions around this site would be a red flag for him that the furin site had initially come about with (T/C) passage - and then with slower passage in humans, might be modified. Much too early to say anything, but will be interesting to see if there's more 'messing about' with this site.

Andrew Rambaut 10:10
They will be sequencing some more samples under similar conditions tomorrow.
👍 1

Robert Garry 10:18
Indeed - that PRRA insertion is the most perplexing aspect of the entire genome. It's likely "out-of-frame" actually, but seeming inserted like a scalpel into a very constant region. If that region is or can be put under some selection pressure would be good to know.

Andrew Rambaut 10:20
This whole thing is doing my brain in. I literally swivel day by day thinking it is a lab escape or natural.

Kristian Andersen 10:25
Haha, my brain has been a badly calibrated MCMC. I'm hoping it'll start converging at some point....

Robert Garry 10:26
All of our brains are in a bit of trouble - hopefully you'll don't get rear-ended anytime soon...
😟 1

Hopefully also we hear something positive from Clare SOON- then we'll all likely be facing the lab escape or natural question head-on and should have a consistent response.
👍 1

[redacted]

Kristian Andersen 12:36
Email from Slack for Gmail ▾

> **Decision on Nature submission 2020-02-02583** Feb 20th, 2020
> From c.thomas@nature.com (No content)

It's a no at Nature - which doesn't entirely surprise me. They're suggestion going with other Nature journals and right now I think we should consider three different options:

1. Nature Medicine
2. Cell
3. Science

(edited)

I feel pretty confident about #1 and #2, but not quite sure about #3 (but would be most impact). I know Caroline there so could definitely reach out.

Also, the reviewers raise some good points that we need to consider. Unfortunately the pangolins don't help clarify the story and reviewer #2 (who's the one influencing the decision) is wrong on those points. Most importantly - we unfortunately can't refute the lab origin hypothesis and it is what it is.

I have some other business I need to attend to this morning, so let's wait until @Eddie Holmes wakes up and then come up with a game plan.

Robert Garry 13:16
"Nature Medicine are interested in publishing it either as a Comment or a Correspondence." This is more positive than the other two. Sure address the concerns and publish in Nature Medicine. Essentially the same Impact Factor as Cell.

Quicker it seems (edited)

Andrew Rambaut 13:19
My reading of that comment is NatMed would take the reviews as they are and we can just address them.

Robert Garry 13:19
AS for the comments: - for the o-glycan we could show some of the additional data on the predicted sites in other CoVs - this is convincing to me, but perhaps not to a skeptic . If not that just further tone down the comments re the O-glycans with more qualifiers.

Robert Garry 13:25
"Also state clearly that this site is only predicted so far and that experimental evidence for its biological function and its potential impact on pathogenesis are required." well the site is there - whether it is used or not technically not established, but a good bett since it's used for other CoVs and apparently knocking it out allowed the S to be stable enuff to give a 3A structure. Confused though what tjhe reviewer wants us to do what we already stated exactly?

I don't think review 2 got it at all - maybe on purpose.

The paper was to explore the possibilities of the proximal origin - not to refute the bioweapon scenario.

Andrew Rambaut 13:27
Could ask Clare to reconsider

Robert Garry 13:28
That's another plan - He/She set up a straw man that our paper was to refute SARS-CoV-2 as a bioweapon then shot it down.

Andrew Rambaut 13:29

February 20th, 2020

> But more importantly this reviewer feels, and we agree, that the Perspective would quickly become outdated when more scientific data are published (for example on potential reservoir hosts).

This is the important bit to address head on - the pangolins do not solve the issue. (edited)

1 reply 3 years ago

Robert Garry 13:29
Agreeing with Andrew that NatMed would take it .

None of the pango sequences are the smoking gun that says this virus jumped right into a person. "It is not clear why the authors rush with a speculative perspective if their central hypothesis can be supported by their own data. Please explain." Actually this is rather freaking insulting to say the least...

Kristian Andersen 13:32
replied to a thread: But more importantly this reviewer feels, and we agree, that the Perspective would quickly become outdated when more scientific data are published (for example on poten...
Yes, this is key and I addressed this in my reply back to Clare (also to see if they'd reconsider)

Screen Shot 2020-02-20 at 10.31.17 AM.png

The only potential door still open with Nature would be for Eddie and Jeremy to get a hold of Magda. Reviewer 2 in general doesn't understand what's going on (he/she doesn't understand that's even a theory in the first place) and no, sadly, the pangos don't solve this. I get a sense that Nature might be a little gun shy though - hence, we'd need to go all the way to the top.

February 20th, 2020

Robert Garry 13:36
Good Idea - let Jeremy know and give him the rationale why Reviewer 2 was full of it.

Andrew Rambaut 13:37
Perhaps produce the rebuttals?

If we end up going NatMed they will want rebuttals for these referees comments.

Robert Garry 13:37
Yes - Gonna have to do that anyway.

Kristian Andersen 13:39
Let me set up a Google Doc and share

Robert Garry 13:40
Yeah good plan - should not actually take long...

Kristian Andersen 13:44
Shared a Google Doc with y'all: https://docs.google.com/document/d/1v5FqAlqLfz1o5fOpO2VWIXKIQ3armcoWzdcfLnq4VhQ/edit#

G Suite Document

Nature rebuttal
Google Doc

I need to head out for an hour or so.

Eddie Holmes 13:58
I forwarded to Jeremy. Reviewer #2 is clearly of the Fouchier mindset. I'm very surprised at Nature here....rejecting it then recommending another Nature journal. Might want to remind them of the 43K views on Virological. My worry about transferring to Nature Medicine at that they will want the text hugely reduced for a Comment/Correspondence section. Also, I think we should stick to our guns about the message and not tone it down just to get it published. I'm pretty sure Cell would take it...they are desperate to get in on the act.

Eddie Holmes 14:23
From Jeremy: I would give them a ring first.

If really a no, then Nature Medicine – best is the quickest way now

Kristian Andersen 14:26
Agreed on approach. Eddie, do you want to give Magda a ring?

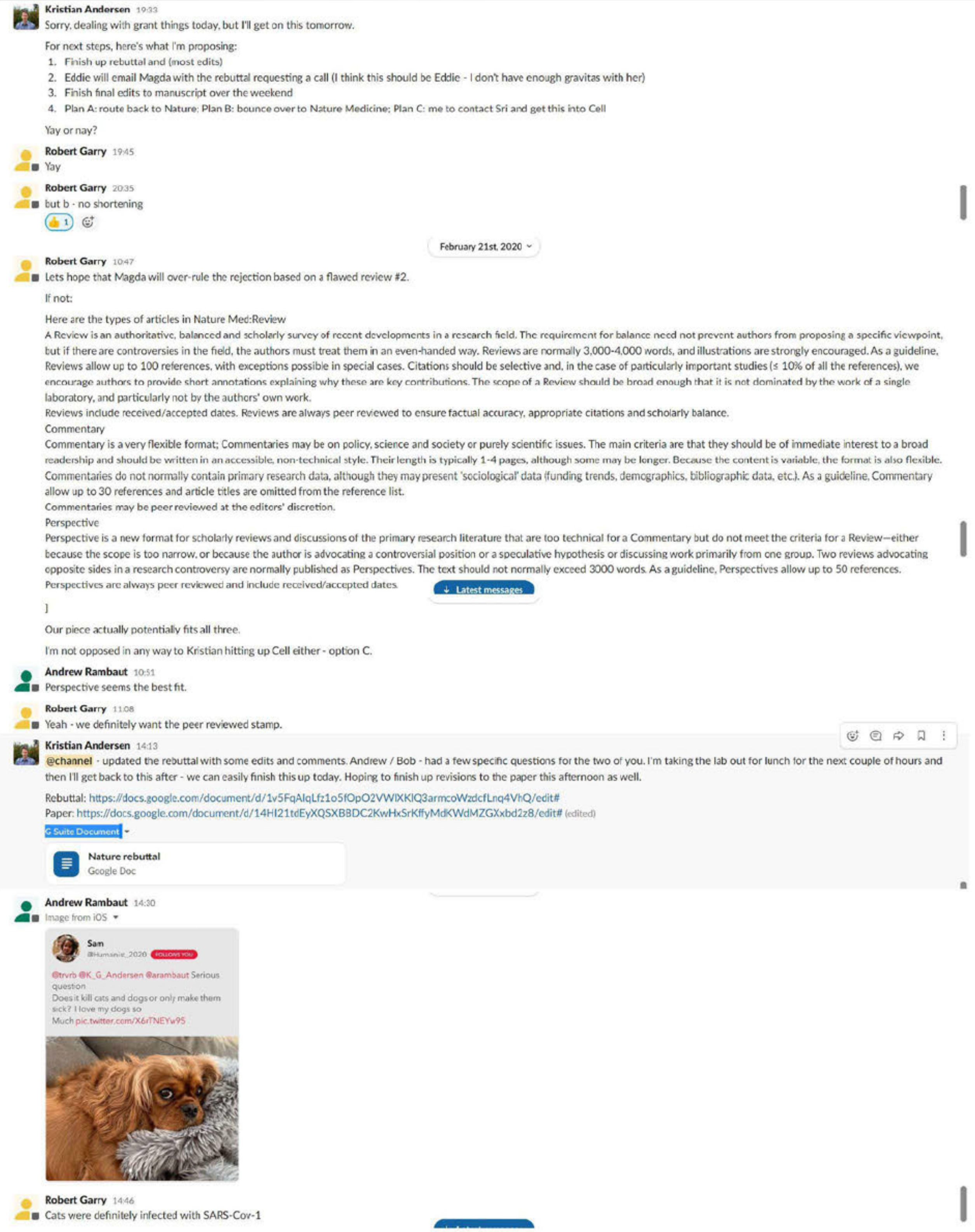
Andrew Rambaut 14:36
I agree that we should not shorten it (if anything we may need to add a few sentences.

Eddie Holmes 15:19
I'm actually in New Zealand at the moment and given travel and time differences I won't be able to her until Monday her time. Not sure someone else can tomorrow? Apologies. Perhaps we should finish the response first?

Robert Garry 15:46
I've put in my two pennies drafting responses to all the points. As always no sacred text or any problems whatsoever with wholesale deletions or edits. Please do that. There are several references and changes that will need to be made to the manuscript but not too onerous.

Yeah - no shortening.

[redacted]



Kristian Andersen 19:33
Sorry, dealing with grant things today, but I'll get on this tomorrow.

For next steps, here's what I'm proposing:

1. Finish up rebuttal and (most edits)
2. Eddie will email Magda with the rebuttal requesting a call (I think this should be Eddie - I don't have enough gravitas with her)
3. Finish final edits to manuscript over the weekend
4. Plan A: route back to Nature; Plan B: bounce over to Nature Medicine; Plan C: me to contact Sri and get this into Cell

Yay or nay?

Robert Garry 19:45
Yay

Robert Garry 20:35
but b - no shortening
👍 1

February 21st, 2020

Robert Garry 10:47
Lets hope that Magda will over-rule the rejection based on a flawed review #2.

If not:

Here are the types of articles in Nature Med:Review
A Review is an authoritative, balanced and scholarly survey of recent developments in a research field. The requirement for balance need not prevent authors from proposing a specific viewpoint, but if there are controversies in the field, the authors must treat them in an even-handed way. Reviews are normally 3,000-4,000 words, and illustrations are strongly encouraged. As a guideline, Reviews allow up to 100 references, with exceptions possible in special cases. Citations should be selective and, in the case of particularly important studies (≤ 10% of all the references), we encourage authors to provide short annotations explaining why these are key contributions. The scope of a Review should be broad enough that it is not dominated by the work of a single laboratory, and particularly not by the authors' own work.
Reviews include received/accepted dates. Reviews are always peer reviewed to ensure factual accuracy, appropriate citations and scholarly balance.
Commentary
Commentary is a very flexible format; Commentaries may be on policy, science and society or purely scientific issues. The main criteria are that they should be of immediate interest to a broad readership and should be written in an accessible, non-technical style. Their length is typically 1-4 pages, although some may be longer. Because the content is variable, the format is also flexible. Commentaries do not normally contain primary research data, although they may present 'sociological' data (funding trends, demographics, bibliographic data, etc.). As a guideline, Commentary allow up to 30 references and article titles are omitted from the reference list.
Commentaries may be peer reviewed at the editors' discretion.
Perspective
Perspective is a new format for scholarly reviews and discussions of the primary research literature that are too technical for a Commentary but do not meet the criteria for a Review—either because the scope is too narrow, or because the author is advocating a controversial position or a speculative hypothesis or discussing work primarily from one group. Two reviews advocating opposite sides in a research controversy are normally published as Perspectives. The text should not normally exceed 3000 words. As a guideline, Perspectives allow up to 50 references.
Perspectives are always peer reviewed and include received/accepted dates.

]

Our piece actually potentially fits all three.

I'm not opposed in any way to Kristian hitting up Cell either - option C.

Andrew Rambaut 10:51
Perspective seems the best fit.

Robert Garry 11:08
Yeah - we definitely want the peer reviewed stamp.

Kristian Andersen 14:13
@channel - updated the rebuttal with some edits and comments. Andrew / Bob - had a few specific questions for the two of you. I'm taking the lab out for lunch for the next couple of hours and then I'll get back to this after - we can easily finish this up today. Hoping to finish up revisions to the paper this afternoon as well.

Rebuttal: https://docs.google.com/document/d/1v5FqAlqLfz1o5fOpO2VWlXKlQ3armcoWzdcfLnq4VhQ/edit#
Paper: https://docs.google.com/document/d/14HI21tdEyXQSXBBDC2KwHxSrKffyMdKWdMZGXxbd2z8/edit# (edited)

G Suite Document

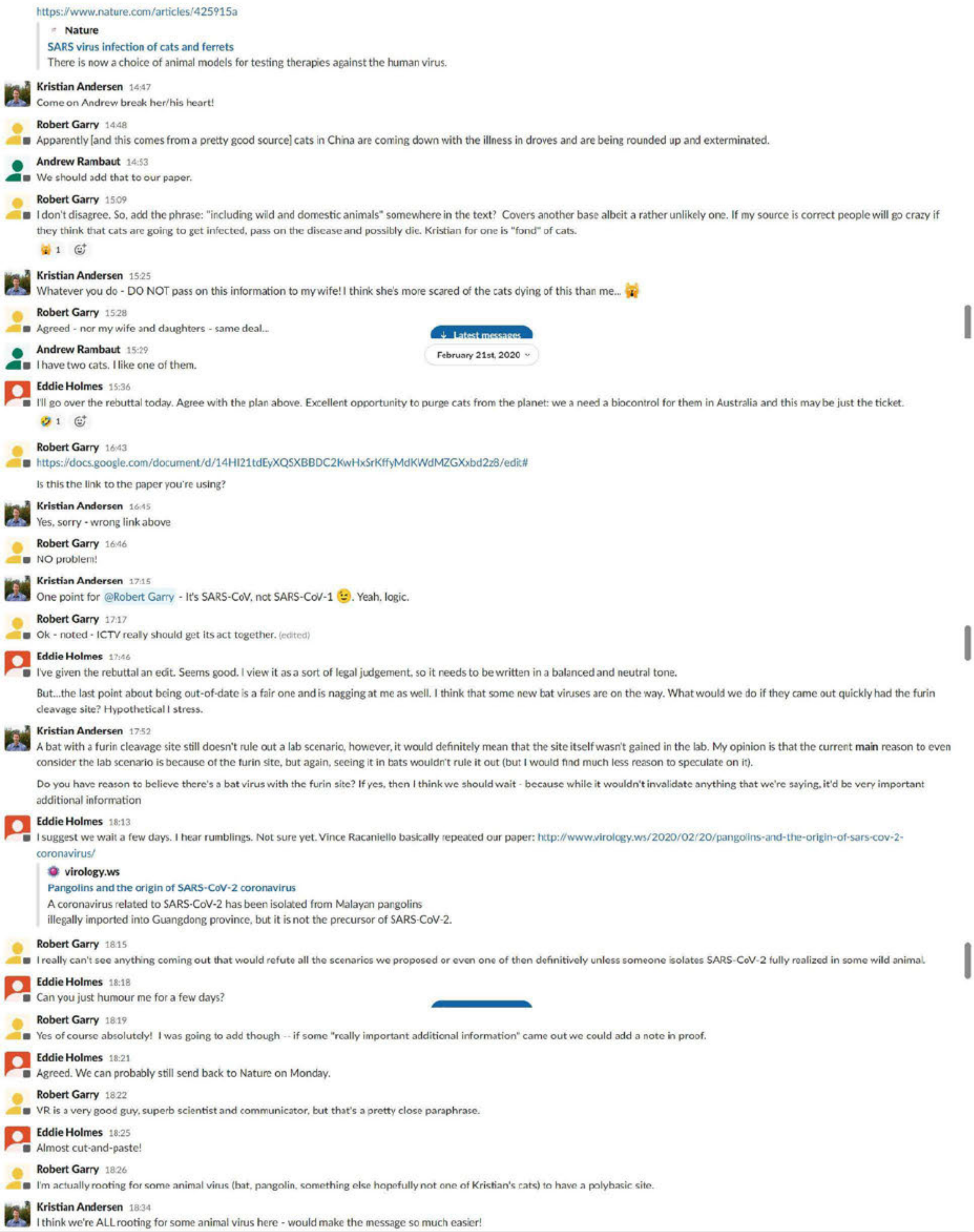
Nature rebuttal
Google Doc

Andrew Rambaut 14:30
Image from iOS

Sam
@Humanist_2020 FOLLOWS YOU

@trvrb @K_G_Andersen @arambaut Serious question
Does it kill cats and dogs or only make them sick? I love my dogs so
Much pic.twitter.com/X6rTNEYw95

Robert Garry 14:46
Cats were definitely infected with SARS-Cov-1



https://www.nature.com/articles/425915a

> **Nature**
> SARS virus infection of cats and ferrets
> There is now a choice of animal models for testing therapies against the human virus.

Kristian Andersen 14:47
Come on Andrew break her/his heart!

Robert Garry 14:48
Apparently [and this comes from a pretty good source] cats in China are coming down with the illness in droves and are being rounded up and exterminated.

Andrew Rambaut 14:53
We should add that to our paper.

Robert Garry 15:09
I don't disagree. So, add the phrase: "including wild and domestic animals" somewhere in the text? Covers another base albeit a rather unlikely one. If my source is correct people will go crazy if they think that cats are going to get infected, pass on the disease and possibly die. Kristian for one is "fond" of cats.

😱 1

Kristian Andersen 15:25
Whatever you do - DO NOT pass on this information to my wife! I think she's more scared of the cats dying of this than me... 😱

Robert Garry 15:28
Agreed - nor my wife and daughters - same deal...

Andrew Rambaut 15:29
I have two cats. I like one of them.

Eddie Holmes 15:36
I'll go over the rebuttal today. Agree with the plan above. Excellent opportunity to purge cats from the planet: we a need a biocontrol for them in Australia and this may be just the ticket.

🤣 1

Robert Garry 16:43
https://docs.google.com/document/d/14HI21tdEyXQSXBBDC2KwHxSrKffyMdKWdMZGXxbd2z8/edit#

Is this the link to the paper you're using?

Kristian Andersen 16:45
Yes, sorry - wrong link above

Robert Garry 16:46
NO problem!

Kristian Andersen 17:15
One point for @Robert Garry - It's SARS-CoV, not SARS-CoV-1 😉. Yeah, logic.

Robert Garry 17:17
Ok - noted - ICTV really should get its act together. (edited)

Eddie Holmes 17:46
I've given the rebuttal an edit. Seems good. I view it as a sort of legal judgement, so it needs to be written in a balanced and neutral tone.

But...the last point about being out-of-date is a fair one and is nagging at me as well. I think that some new bat viruses are on the way. What would we do if they came out quickly had the furin cleavage site? Hypothetical I stress.

Kristian Andersen 17:52
A bat with a furin cleavage site still doesn't rule out a lab scenario, however, it would definitely mean that the site itself wasn't gained in the lab. My opinion is that the current **main** reason to even consider the lab scenario is because of the furin site, but again, seeing it in bats wouldn't rule it out (but I would find much less reason to speculate on it).

Do you have reason to believe there's a bat virus with the furin site? If yes, then I think we should wait - because while it wouldn't invalidate anything that we're saying, it'd be very important additional information

Eddie Holmes 18:13
I suggest we wait a few days. I hear rumblings. Not sure yet. Vince Racaniello basically repeated our paper: http://www.virology.ws/2020/02/20/pangolins-and-the-origin-of-sars-cov-2-coronavirus/

> **virology.ws**
> Pangolins and the origin of SARS-CoV-2 coronavirus
> A coronavirus related to SARS-CoV-2 has been isolated from Malayan pangolins
> illegally imported into Guangdong province, but it is not the precursor of SARS-CoV-2.

Robert Garry 18:15
I really can't see anything coming out that would refute all the scenarios we proposed or even one of then definitively unless someone isolates SARS-CoV-2 fully realized in some wild animal.

Eddie Holmes 18:18
Can you just humour me for a few days?

Robert Garry 18:19
Yes of course absolutely! I was going to add though -- if some "really important additional information" came out we could add a note in proof.

Eddie Holmes 18:21
Agreed. We can probably still send back to Nature on Monday.

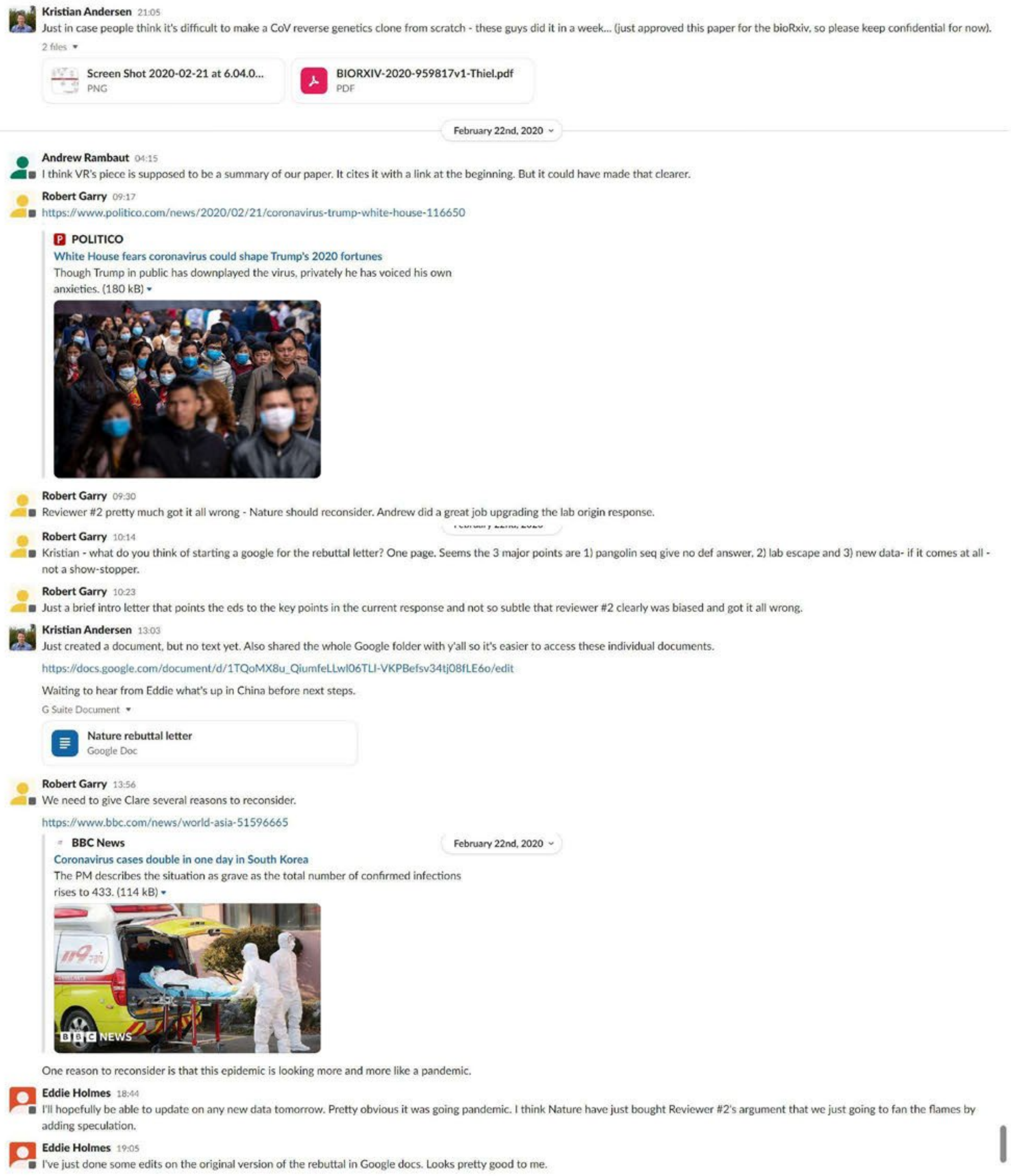
Robert Garry 18:22
VR is a very good guy, superb scientist and communicator, but that's a pretty close paraphrase.

Eddie Holmes 18:25
Almost cut-and-paste!

Robert Garry 18:26
I'm actually rooting for some animal virus (bat, pangolin, something else hopefully not one of Kristian's cats) to have a polybasic site.

Kristian Andersen 18:34
I think we're ALL rooting for some animal virus here - would make the message so much easier!

[redacted]



Kristian Andersen 21:05
Just in case people think it's difficult to make a CoV reverse genetics clone from scratch - these guys did it in a week... (just approved this paper for the bioRxiv, so please keep confidential for now).

2 files ▾

Screen Shot 2020-02-21 at 6.04.0...
PNG

BIORXIV-2020-959817v1-Thiel.pdf
PDF

February 22nd, 2020

Andrew Rambaut 04:15
I think VR's piece is supposed to be a summary of our paper. It cites it with a link at the beginning. But it could have made that clearer.

Robert Garry 09:17
https://www.politico.com/news/2020/02/21/coronavirus-trump-white-house-116650

POLITICO
White House fears coronavirus could shape Trump's 2020 fortunes
Though Trump in public has downplayed the virus, privately he has voiced his own anxieties. (180 kB) ▾

Robert Garry 09:30
Reviewer #2 pretty much got it all wrong - Nature should reconsider. Andrew did a great job upgrading the lab origin response.

Robert Garry 10:14
Kristian - what do you think of starting a google for the rebuttal letter? One page. Seems the 3 major points are 1) pangolin seq give no def answer, 2) lab escape and 3) new data- if it comes at all - not a show-stopper.

Robert Garry 10:23
Just a brief intro letter that points the eds to the key points in the current response and not so subtle that reviewer #2 clearly was biased and got it all wrong.

Kristian Andersen 13:03
Just created a document, but no text yet. Also shared the whole Google folder with y'all so it's easier to access these individual documents.

https://docs.google.com/document/d/1TQoMX8u_QiumfeLLwl06TLI-VKPBefsv34tj08fLE6o/edit

Waiting to hear from Eddie what's up in China before next steps.

G Suite Document ▾

Nature rebuttal letter
Google Doc

Robert Garry 13:56
We need to give Clare several reasons to reconsider.

https://www.bbc.com/news/world-asia-51596665

BBC News
Coronavirus cases double in one day in South Korea
The PM describes the situation as grave as the total number of confirmed infections rises to 433. (114 kB) ▾

One reason to reconsider is that this epidemic is looking more and more like a pandemic.

Eddie Holmes 18:44
I'll hopefully be able to update on any new data tomorrow. Pretty obvious it was going pandemic. I think Nature have just bought Reviewer #2's argument that we just going to fan the flames by adding speculation.

Eddie Holmes 19:05
I've just done some edits on the original version of the rebuttal in Google docs. Looks pretty good to me.

February 22nd, 2020

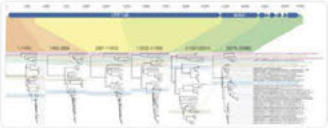
Robert Garry 19:51
Yeah - damn good -I agree about the "fan the flames by adding speculation.' it would not surprise me that the reviewer wrote a VERY strong private comment to the editor that effect to scare the hell out her. Again reviewerv#2 wrong about everything. 50K+ views and probably 10s of thousands of tweets and retweets - I did not detect fanned flames - on the contrary.

Eddie Holmes 19:54
Agreed. No doubt that the private comments to the Editor were very strong.

Robert Garry 19:55
Yeah hopefully she buys the counter-arguments

Andrew Rambaut 20:18
Been trying to get my head round the recombination. Here is the overview. Going to dig into spike next to see if I can pin down the sequence of acquisition of the RBD residues.

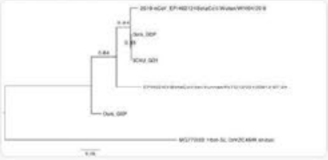
recombination.png



💯 1

Eddie Holmes 20:41
Nicely done. Very messy in the S protein though. What do you think about Tommy's synonymous trees in the RBD? The pangolin virus is not the closest to SARS-CoV....bit very close in amino acid trees as here. (edited)

selected_RBD_whole_wSCAU_aa_phyml.png

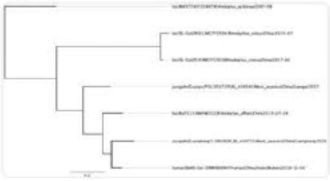


SCAU is obviously the South China Uni one.

February 22nd, 2020

Andrew Rambaut 20:57
Yes. For RBD the SCAU pangolin is closest (this is nucleotide).

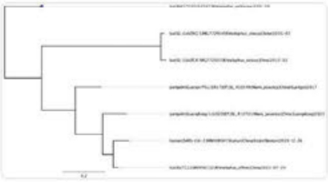
image.png



But I think this is because there is a recombinant tract in RBD in RaTG13 (that comes from elsewhere) pushing it away from SARS2.

If I clip out 202 nucleotides in the RBD that span the 6 contact sites I get RaTG13 as closest again. Also if I just mask those sites with Ns in the RaTG13.

image.png



Eddie Holmes 21:06
To me it looks like the pangolin amino acid sequence in the RBD is closer to SARS-CoV-2 than expected given their overall level of divergence.

Andrew Rambaut 21:10
image.png

Andrew Rambaut 21:26
So in the first half of the RBD (up until the blue bar), RaTG13 is 7.9% divergent from SARS2 at the nucleotide level, and the pangolin is 13.5% divergent
In the second half (i.e., the blue bar), RaTG13 is 22% divergent and pangolin is 12.6% (i.e., slightly less divergent).

For Amino Acid it is similar - 1st half, RaTG13-SARS2: 2.8%, Pango-SARS2: 3.7%, 2nd half, RaTG13-SARS2: 19.5%, Pango-SARS2: 2.3%

So it the Pangolin stays roughly the same divergence and RaTG13 shoots up.

Jeez it is 2.30 am. Going to bed.

Eddie Holmes 21:31
Thanks. Yes, go to bed.

Robert Garry 22:01
yes, many thanks!

February 23rd, 2020

Robert Garry 09:05
I can't contribute much here, but one consistent observation over the years is that virus fusion proteins use a "modular" approach, swapping in and out various components. If you're splitting the spike protein up for comparisons at the nuc and protein levels and **if there's not another more rationale way to pick the splits**, it might make sense [to me] to do it according to the "modules." This alignment shows the "modules" in spike: https://www.nature.com/articles/nature17200/figures/10. The orange "variable loop" is the receptor binding domain for CoVs that have a protein receptor like ACE-2. For CoVs that use sialic acid receptors the binding is in the NTD. MERS CoV might use both classes of receptors (sialic acid and a protein) . For some CoVs like HKU1 (in the pointed to alignment) there is a "modular" insertion in the variable loop of a proline, serine, threonine rich region aka a mucin-like domain. (edited)

Robert Garry 09:14
Apropos to that what you've labeled the "tract" appears to me to be essentially the "variable loop" that is a module frequently swapped in and out of CoV spikes. (edited)

Robert Garry 09:24
Our friend Ralph wrote about it:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2838128/

> **PubMed Central (PMC)**
> Recombination, Reservoirs, and the Modular Spike: Mechanisms of Coronavirus Cross-Species Transmission
> Over the past 30 years, several cross-species transmission events, as well as changes in virus tropism, have mediated significant animal and human diseases. Most notable is severe acute respiratory syndrome (SARS), a lower respiratory tract disease of ...

Robert Garry 10:48
https://wwwnc.cdc.gov/eid/article/19/7/12-1094_article

> **Emerging Infectious Diseases journal**
> Mutation in Spike Protein Cleavage Site and Pathogenesis of Feline Coronavirus
> Feline coronaviruses (FCoV) exist as 2 biotypes: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV). FECV causes subclin... (132 kB)
>
> EMERGING INFECTIOUS DISEASES
> A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Probably need to reference this.

Andrew Rambaut 11:03
Thanks Bob! That looks like an excellent way to try to dig down in to this (better than my squinting at the alignment and trying to see where the break-points are). Opens up all sorts of interesting questions about where do they get these modules from? Is it just homologous recombination from other coronaviruses?

Also with respect to cats - weren't you saying that there were dead cats everywhere in Wuhan?

```
The current understanding is that FIPV arises during in vivo infection from a genetic mutation of FECV (8-11). A long-standing hypothesis is that FIP viruses arise from internal mutation of
endemic FECVs (12), which is believed to occur in approximately 1%-5% of enteric infections, resulting in the ability of the virus to infect blood monocytes and tissue macrophages. The
resulting productive infection of these cells, a hallmark of FIP, enables systemic spread and results in macrophage activation, with concomitant immune-mediated events leading to death. To
date, the precise mutation or mutations that cause a shift in FCoV biotype have not been identified.
```

Robert Garry 11:14
Yes indeed - could be coincidence, but if SARS-CoV-2 is in fact infecting cats in Wuhan (and that's not a bad bet since SARS-CoV does effectively infect cats in the lab and cats were definitely infected during a early SARS cluster in an apartment building) then the polybasic site might give the virus a leg up in pathology.

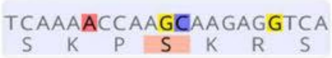
yes - homologous recombination from other coronaviruses would be my bet.

[redacted]

Robert Garry 11:27
If cats are infected, I suppose one might ask the question did people infect the cats or was it the other way around?

Andrew Rambaut 11:27
Just annotating up the spike regions in the alignment now. One quick think I noticed in the figure above is the S2' cleavage site just before the fusion peptide. If the S1/S2 cleavage site was knocked out by a deletion, would this one take over? In SARS-CoV-2 it looks like this:

image.png



```
TCAAAACCAAGCAAGAGGTCA
 S  K  P  S  K  R  S
```

Robert Garry 11:34
I think that's a distinct possibility. I'd look for a cathepsin cleavage site as well. (edited)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2519682/

> **PubMed Central (PMC)**
> Cathepsin L Functionally Cleaves the Severe Acute Respiratory Syndrome Coronavirus Class I Fusion Protein Upstream of Rather than Adjacent to the Fusion Peptide
> Unlike other class I viral fusion proteins, spike proteins on severe acute respiratory sydrome coronavirus virions are uncleaved. As we and others have demonstrated, infection by this virus depends on cathepsin proteases present in endosomal compartments ...

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6226446/

> **PubMed Central (PMC)**
> Functional analysis of potential cleavage sites in the MERS-coronavirus spike protein
> The Middle East respiratory syndrome-related coronavirus (MERS-CoV) can cause severe disease and has pandemic potential. Therefore, development of antiviral strategies is an important task. The activation of the viral spike protein (S) by host cell proteases ...

Andrew Rambaut 11:44
OK. As you guessed - that bit I labelled 'tract' which I got by eyeballing the alignment is within 2 nucs at one end and 6 nucs at the other to being the 'variable loop' in that paper, above. So that looks like a winner.

I guess the pangolin/human lineage could have got it from somewhere else but given in the rest of the genome, RaTG13 is closest, it would mean the Pangolin lineage and the one leading to SARS-CoV-2 would have to get it separately.

Robert Garry 11:50
GreatPerhaps a multistep process to get to SARS-CoV-2?

Andrew Rambaut 17:15
http://www.microbe.tv/twiv/twiv-588/ (from minute 42)

> **microbe.tv**
> TWiV 588: Coronavirus update - Save the pangolin! | This Week in Virology
> The TWiV team returns this week to SARS-CoV-2019 coverage to review the latest epi curves, the fatality rate, furin cleavage site and receptor binding domain in the spike glycoprotein, related CoV recovered from pangolins, evidence that the virus did not escape from a laboratory, and many more questions sent in by listeners.

Robert Garry 17:33
Is it possible to make money doing a podcast - or is this just a hobby? I'm not judging, just curious.

Andrew Rambaut 17:37
I have wondered that. I think it is just a hobby. But they are 2.5 hours long. I don't know who has time to listen.

Robert Garry 18:35
https://qz.com/1805422/wuhan-virology-lab-unable-to-quell-china-coronavirus-conspiracies/

> **Quartz**
> Why a Chinese virology lab is unable to quell the coronavirus conspiracy theories around it
> The episode shows how China's public has an decreasing level of trust in the government since the outbreak of the coronavirus, say experts. (98 kB) ▾

Some journals, such as Nature, have appended notes to older stories about the Wuhan lab calling the conspiracy theories about the lab "unverified."

> **Nature News & Comment**
> Inside the Chinese lab poised to study world's most dangerous pathogens
> Maximum-security biolab is part of plan to build network of BSL-4 facilities across China.

Wow - not sure Nature is correct on this.

image.png ▾

NATURE | NEWS

Inside the Chinese lab poised to study world's most dangerous pathogens

Maximum-security biolab is part of plan to build network of BSL-4 facilities across China.

Editors' note, January 2020: Many stories have promoted an unverified theory that the Wuhan lab discussed in this article played a role in the coronavirus outbreak that began in December 2019. Nature knows of no evidence that this is true; scientists believe the most likely source of the coronavirus to be an animal market.

David Cyranoski

22 February 2017 / Updated: 23 February 2017

WUHAN, CHINA

The journal Nature added a note to a 2017 story on the lab to alert readers to "unverified theories" about the lab in relation to the new coronavirus.

Robert Garry 18:58
Nature seems to be getting some bad advice - did reviewer #2 strike again?

February 24th, 2020

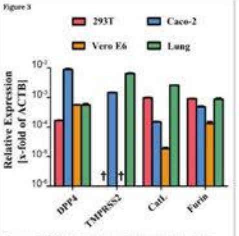
Andrew Rambaut 10:13
@Robert Garry Quick question - would Vero-E6 cells have furin available?

Kristian Andersen 10:27
I believe they do.

Robert Garry 10:33
Yes they do - heres the data.

image.png

Figure 3



Expression of DPP4 and host cell proteases in target cell lines and lung tissue. Total cellular RNA was extracted from 293T, Caco-2 and Vero E6 cells, reverse-transcribed into cDNA and quantified for DPP4 and protease transcript numbers by quantitative RT-PCR. cDNA from human lung tissue was also included in the analysis. The numbers of DPP4/protease mRNA copies are shown relative to the housekeeping gene ß-actin (ACTB). Error bars indicate standard deviation (SD). Crosses indicate samples for which no transcripts were detected.

https://www.nature.com/articles/s41598-018-34859-w

> **Scientific Reports**
> Functional analysis of potential cleavage sites in the MERS-coronaviru
> Functional analysis of potential cleavage sites in the MERS-coronavirus spike protein

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Andrew Rambaut 10:33
But perhaps not as lung epithelium cells?

Oh! Snap.

An order of mag less.

So might select against using furin cleavage site

Perhaps less than an order

Kristian Andersen 10:37
Doubt it... Being able to use furin is a neat trick

Andrew Rambaut 10:38
OK.

Just thinking about this deletion of the cleavage site we are seeing in a sample (at about 40% frequency).

Kristian Andersen 10:39
One thing furin usage might do though - make the virus less stable. So changing temperatures in T/C etc. could probably mess around with it's usage of furin.

The loss you're seeing - any sense if that specific to culture or whether it's in the patient?

Andrew Rambaut 10:47
That is what we are trying to work out. One hypothesis I was thinking of is that there is another population of viruses that has arisen targeting other cells in the body? Perhaps less furiny.

Robert Garry 10:51
Very possible. Would really like to get some site directed mutants going on that furin site - then explore tissue tropism. Pretty sure Baric and Yoshi are burning the midnite oil getting those exps done. Putting those mutants into animals very much needed. Tulane primate center has the virus and is working with a consortium to establish the animals (NHPs, ferrets etc - maybe cats).

Tulane has Chad Roy that may be one of the few people that can credibly do an aerosol challenge.

BTW- Just got an invite from Amy Maxmen of Nature to participate in a panel at a journalists' meeting in Austin end of April.

Someone should tell Nature that the fish market probably did not start the outbreak.

Kristian Andersen 10:58
All very plausible.

We now have the reverse genetics system, so I'm sure Drosten and folks are on that as well.

Andrew, one thing to check - if these are grown in culture, please have the double-check the temperature in their incubator. If it's a few degrees higher than expected, then I think we have a likely mechanism.

Amy reached out to me as well - turned it down, but Bob, that's your old stomping ground, so you should go.

2 replies Last reply 3 years ago

Robert Garry 10:59
They are just contributing to the conspiracy theories that WIV built and released SARS-CoV-2.

THat was my guess.

↓ Latest messages

Robert Garry 11:07
Old white guy - hope they get some women.

Andrew Rambaut 11:11
Ask them for the panel list (can also check for crazies)

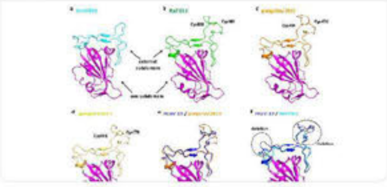
Robert Garry 11:21
Will do - I think since Kristian broke Amy's heart she is scrambling....

[redacted]

Eddie Holmes 14:41
See attached. STRICTLY confidential as I am not meant to send it out. Yunnan bat from March 2019. Highly recombinant but closest to SARS-CoV-2 in one region. Still different in the RBD but the other thing is obvious. Discuss.

PDF ▾

Fig.2-0224new.pdf
PDF



Robert Garry 14:44
Holy crap - that's amazing.

Kristian Andersen 14:45
No polybasic site, HOWEVER, this provides a mechanism. This is critical to have out and plug in - let's wait until it's out (edited)

Robert Garry 14:56
Well- it is a logical progenitor or at least a substrate for recombination -just R for trypsin or maybe it relies on CatL - also deletes two of the three predicted O-linked S or T residues (and the sequence is not predicted to be O-gylcosylated (I just checked).

Kristian Andersen 15:03
Agreed. Here's evidence showing that the virus likes to 'mess around' in this part of the genome (in animals), so that provides a template for how all of this happened in animals - critical bit of information

Robert Garry 15:04
I don't see how it gets us any closer to discriminating between any of the models. There still needed to be recombination and evolution in either an animal, animals, humans or all of the above. It does not rule out or in lab passage. If it's being posted fast yes lets wait - but if its going to be an determinate amount of time maybe get our paper out Nature or Nature Med. Add a note in proof if it comes out sooner than later - otherwise I think we anticipate that there are likely intermediates between known bat and pangolin viruses and SARS-CoV-2 or maybe add this to the discussion as a personal communication if possible.

Kristian Andersen 15:05
I think this lends pretty strong support for an animal origin of the 'confusing' features of the virus, so I think it's important to include

Kristian Andersen 15:19
None of this disproves accidental lab infection, however, it shows that all the steps can occur in nature - hence the reason to even consider a lab link is decreased. Since we have such a miniscule sampling of the animal reservoir seeing just small parts of the step-by-step mechanism is important - to me this data shows that because, yeah, it shows that the virus likes to 'mess around' with this part of the genome. I think that's important knowledge.

@Eddie Holmes - what's the publication strategy for this paper? I can see it's formatted for Nature, but will there be a bioRxiv?

Robert Garry 15:20
BTW - what is labeled the external subdomain is the variable domain Andrew was discussing in the recombination subthread above.

Andrew Rambaut 15:22
Hi. Just working my way through this.

Robert Garry 15:23
"the reason to even consider a lab link is decreased" - yeah good point. Don't think it necessarily points to a direct animal jump like SARS or MERS or a rather extended history in humans.

If you happen to be working on one of those standup desk things, I suggest sitting down.

February 24th, 2020 ▾

Kristian Andersen 15:27
Makes it **much** more likely the full furin site could have been acquired very early in humans or potentially in an intermediate host - instead of forming fully *de novo* it's more akin to what happens with flu. These are critical points that I think need to be made clear in the commentary - and can't be added in 'in proof' (given how important the message is, it needs to be as clear and solid as possible from the get go IMO).

Robert Garry 15:30
Andrew's deep sequencing result with sometimes (40%) deletions in the S1/S2 junction also confirm that the messing around is common.

Kristian Andersen 15:34
Yup, good point

[redacted]

Eddie Holmes 15:36
Sorry, haven't got time to respond now. Will talk later.

Kristian Andersen 15:36
Yeah, no worries Eddie

[nothing on bioRxiv - just checked]

Speaking of all of this - here's a press release draft (in expectation of a future publication....). If folks have time to take a look and provide edits and preferably some quotes, then that'd be awesome.

Word Document ▾

Andersen Coronavirus Nature 2020 Press Release...

Word Document

Andersen Coronavirus Nature Press Release Draft 2-24-20

The COVID-19 coronavirus epidemic has a natural origin, scientists say' 1

The novel SARS-CoV-2 coronavirus that emerged in the city of Wuhan, China, last year and has since caused a large scale COVID-19 epidemic and spread to several dozen other countries is the product of natural evolution, according to findings published today in the journal *XX*.

The analysis of public genome sequence data from SARS-CoV-2 and related viruses found no evidence that the virus was made in a laboratory or otherwise engineered.

"By comparing the available genome sequence data for known coronavirus

Eddie Holmes 15:37

One thing though: it is currently being Sanger sequenced for confirmation.

Andrew Rambaut 15:40

The figure looks quite familiar.

Robert Garry 15:42

Nice job on the PR - however, you could have more actively borrowed from the Rancaniello piece - I mean, just to be fair.

February 24th, 2020

Robert Garry 16:03

"it needs to be as clear and solid as possible from the get go IMO" Surely, and the points you outlined above should be incorporated. Makes the piece even strong IMO. This figure looks pretty mature to me and the implications are not likely to change unless Sanger somehow fills in the gaps, which seems doubtful. I'm all for starting to update our piece clear and solid as possible based on the reviews and the new info. Then we can see what day it is, when we think the new info might become public and go from there.

Robert Garry 16:12

"The figure looks quite familiar." That's simply sincere flattery.

"If folks have time to take a look and provide edits and preferably some quotes, then that'd be awesome." Can you place on the google or do you want us to edit the old fashioned way?

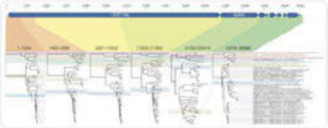
Andrew Rambaut 16:16

Both alignments start and stop at exactly the same residue as my figure and I picked those completely arbitrarily.

Andrew Rambaut 16:23

I am not sure that the new RmYN02 bat sequences add anything to the story other than bats can have insertions in the S1/S2 cleavage site. In the RBD it is basically identical to the ZC45/ZXC21 which are the recombinant ones (in brown in the figure below):

recombination.png ▾



February 24th, 2020

Robert Garry 16:33

Do we know the nucleotide sequence there - that's clearly an optimal alignment at the amino acid level but how did the sequence arise at the nucleotide level. If you compare RaTG13 to nCoV-19 the PRRA results from a single insertion of 12 nuc, BUT it's out of frame from the coding sequence of RatG13. IOWS not a simple 12 nuc insertion directly encoding PRRA. I'm guessing something like this - a single insertion event replacing 24 nuc with 18 nuc. Comparing RmYN02 to one of the bat CoVs. Possible? (edited)

Robert Garry 17:01

~~The other possibility is a very strategic six nucleotide deletion.~~ Ok - this likely didn't happen. (edited)

Andrew Rambaut 17:23

You can go from the furin sequence in SARS2 to the RmYN02 site using only deletions:

image.png ▾

```
TAATTCTCCT--GC-GGCACGT---GTAGCTAGTC
  N  S  P --A- A  R --- V  A  S
TAATTCTCCTCGGCGGGCACGTAGTGTAGCTAGTC
  N  S  P  R  R  A  R  S  V  A  S
```

But it depends on what codons are being used.

Robert Garry 17:31

Interesting!

Andrew Rambaut 17:33

There are some other solutions but always with 3 deletions.

Andrew Rambaut 17:35
Yes, so 4 deletions. (edited)

Robert Garry 18:07
Coincidence that you SF014 deletion above took out QTQT(N)? Maybe a preferred site for recombination?

Andrew Rambaut 18:38
Ooh. Interesting. Too much interlinked stuff going on.

Eddie Holmes 18:46
The virus is actually the closest to SARS-CoV-2 in some parts of the genome, although not hugely close. Very complex series of recombination events. Obviously, the key thing is the insertion but I think that is huge in the current context. Clearly shows this is in Nature. Here are the nucleotides. When did you do your alignment Andrew?

Cleavage site 20200220171523.png ▾

Nucleotide pic attached

In 'nature' small case. Not sure about publication strategy yet...soon I hope. As usual, much politics.

Andrew Rambaut 19:02
My alignment above is just a mock up - I didn't know what the nucleotides were.

So because it has those two As in there, my pure deletion solution doesn't work.

So you need 2 transitions and three deletions (or insertions) to go between these.

I am not convinced these are related inserts. Depends on the background in the rest of spike.

I still think that all it tells you is there are some bat viruses with an insertion at this site.

Eddie Holmes 23:05
Yes, but I think that is an enormous 'all' given that 99% of the lab escape idea from genomics was the cleavage site insertion and we've not seen this in any other bat virus. I don't think we would have written the same paper with this information. I also think it may be a different insertion, but it means these insertions are happening in nature.

Eddie Holmes 23:40
A bit more: (i) sequence confirmed by Sanger; (ii) bats collected May-July 2019, so ~6 months prior; (iii) in most of the virus genome it is the closest to SARS-CoV-2 although not in S; (iv) some very wide ranging recombination events; (v) essentially supports what Ref #2 says ("Who knows how many out of thousands undiscovered bat ancestors also acquired such a motif, the sampling bias in descriptions of remote bat viruses is dramatic"). That it is a different insertion is not the point in my book. Very strongly argues against lab.

97.2% identity in 1ab.

February 25th, 2020 ⌄

Kristian Andersen 00:03
I don't think this data necessarily argues against accidental infection/release, however, it shows something very important - insertions at this site can happen in nature, making the need to reach for a non-natural explanation much diminished. This is new important knowledge that would need to be introduced in our commentary and lends significantly stronger support to the 'natural' scenarios we're describing. I say we have to wait for this to come out - at a minimum on the bioRxiv. It doesn't go against (or prove/disprove) the scenarios we're describing, however, is very important knowledge for a reader to know.

@Eddie Holmes - what's your take on how we handle this? I think we should wait until this is out, update the commentary, and then put that back in via Nature/Nature Med with some significantly stronger conclusions about this being 'natural'. Thoughts?

Eddie Holmes 00:53
I'm now very strongly in favour of a natural origin. The component bits of the virus are more or less there in a tiny sample of wildlife. Plus there is more to come (this is not Zhang's data). I don't see why we need a lab origin on these data. I agree we have to hold back for bioRxiv. Hopefully something will be submitted this week. I'm actually at a meeting with Clare next week.

Eddie Holmes 01:10
Rhinolophus malayanus

Interesting Malayan coincidence

Kristian Andersen 01:31
Sounds good - I too think we should wait until this is out and then we can do a quick turn-around - I think we'll still have a paper to publish by then and in fact, I think it'll be even stronger as it'll have much less of an open ending (again, it doesn't rule out lab infection/release, *however*, there is now no longer any 'mysteries' to explain - we see the optimized RBD in pangolins and part of the furin site in bats (which is pretty cool!). Generally speaking, I also don't think we want to rush. If you can please grab Clare when you see her, then that'd be great.

@Robert Garry and @Andrew Rambaut - thoughts? (edited)

February 25th, 2020 ⌄

Andrew Rambaut 02:08
I was always in favour of the pre-adapted jump from animals hypothesis but now it is plausible that that was directly from bats.

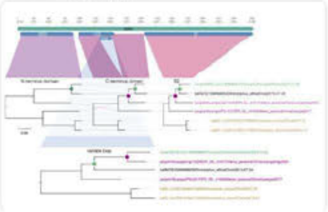
Eddie Holmes 03:04
Agreed. I promise to get this pushed out ASAP. I need to talk to Jeremy in a little while.

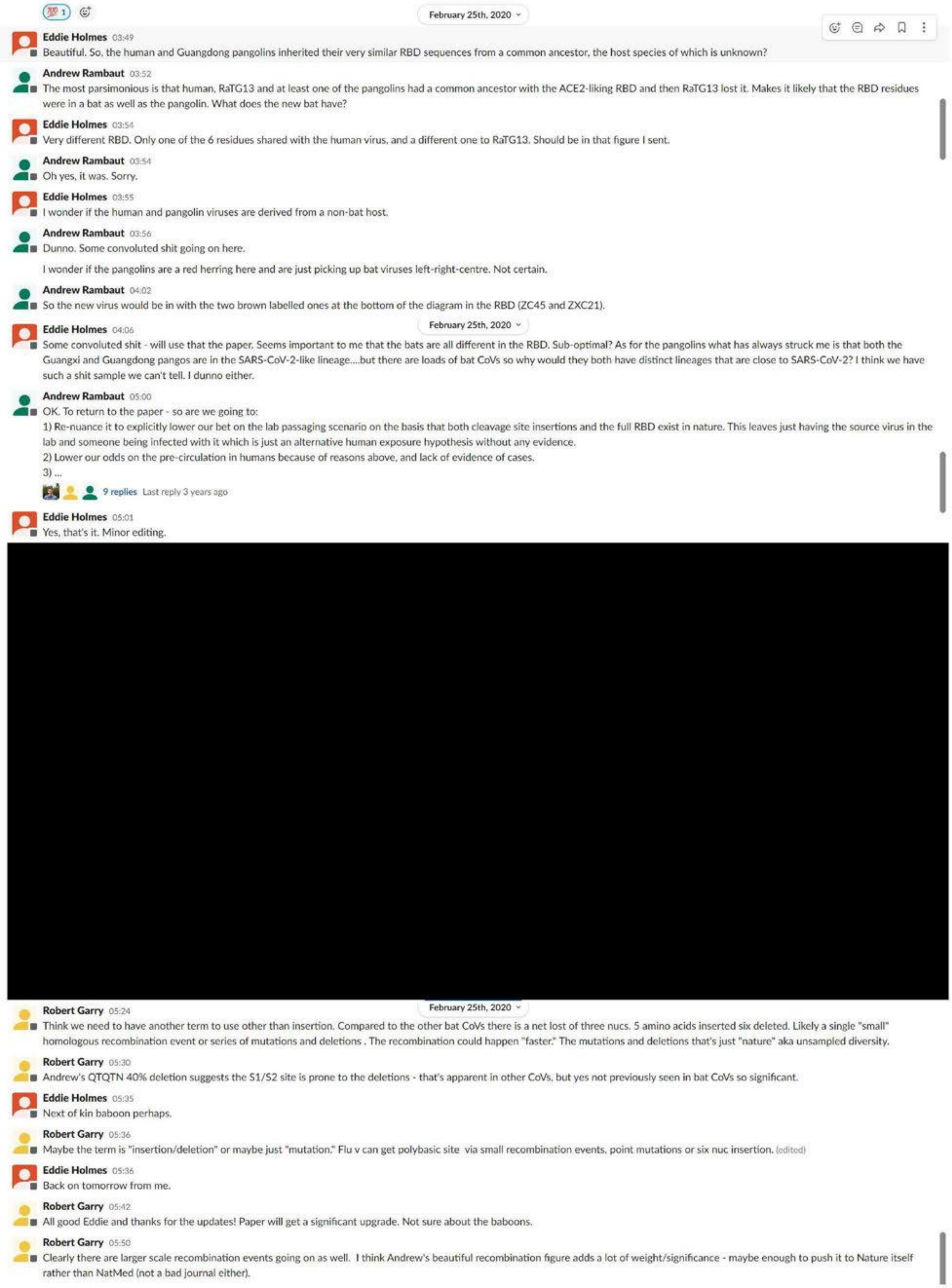
Clare wants to talk about stuff so this will clearly be on the agenda.

Eddie Holmes 03:30
Jeremy agrees with this plan. I'll get the bat paper sorted ASAP. They want to call the human virus HCoV-19 🤷‍♂️

Andrew Rambaut 03:45
Here is my spike recombination diagram. Clearly shows how RaTG13 jumps out in the RBD variable loop region.

recombination_spike.png ▾





February 25th, 2020

Eddie Holmes 03:49
Beautiful. So, the human and Guangdong pangolins inherited their very similar RBD sequences from a common ancestor, the host species of which is unknown?

Andrew Rambaut 03:52
The most parsimonious is that human, RaTG13 and at least one of the pangolins had a common ancestor with the ACE2-liking RBD and then RaTG13 lost it. Makes it likely that the RBD residues were in a bat as well as the pangolin. What does the new bat have?

Eddie Holmes 03:54
Very different RBD. Only one of the 6 residues shared with the human virus, and a different one to RaTG13. Should be in that figure I sent.

Andrew Rambaut 03:54
Oh yes, it was. Sorry.

Eddie Holmes 03:55
I wonder if the human and pangolin viruses are derived from a non-bat host.

Andrew Rambaut 03:56
Dunno. Some convoluted shit going on here.

I wonder if the pangolins are a red herring here and are just picking up bat viruses left-right-centre. Not certain.

Andrew Rambaut 04:02
So the new virus would be in with the two brown labelled ones at the bottom of the diagram in the RBD (ZC45 and ZXC21).

February 25th, 2020

Eddie Holmes 04:06
Some convoluted shit - will use that the paper. Seems important to me that the bats are all different in the RBD. Sub-optimal? As for the pangolins what has always struck me is that both the Guangxi and Guangdong pangos are in the SARS-CoV-2-like lineage....but there are loads of bat CoVs so why would they both have distinct lineages that are close to SARS-CoV-2? I think we have such a shit sample we can't tell. I dunno either.

Andrew Rambaut 05:00
OK. To return to the paper - so are we going to:
1) Re-nuance it to explicitly lower our bet on the lab passaging scenario on the basis that both cleavage site insertions and the full RBD exist in nature. This leaves just having the source virus in the lab and someone being infected with it which is just an alternative human exposure hypothesis without any evidence.
2) Lower our odds on the pre-circulation in humans because of reasons above, and lack of evidence of cases.
3) ...

9 replies Last reply 3 years ago

Eddie Holmes 05:01
Yes, that's it. Minor editing.

[redacted]

February 25th, 2020

Robert Garry 05:24
Think we need to have another term to use other than insertion. Compared to the other bat CoVs there is a net lost of three nucs. 5 amino acids inserted six deleted. Likely a single "small" homologous recombination event or series of mutations and deletions . The recombination could happen "faster." The mutations and deletions that's just "nature" aka unsampled diversity.

Robert Garry 05:30
Andrew's QTQTN 40% deletion suggests the S1/S2 site is prone to the deletions - that's apparent in other CoVs, but yes not previously seen in bat CoVs so significant.

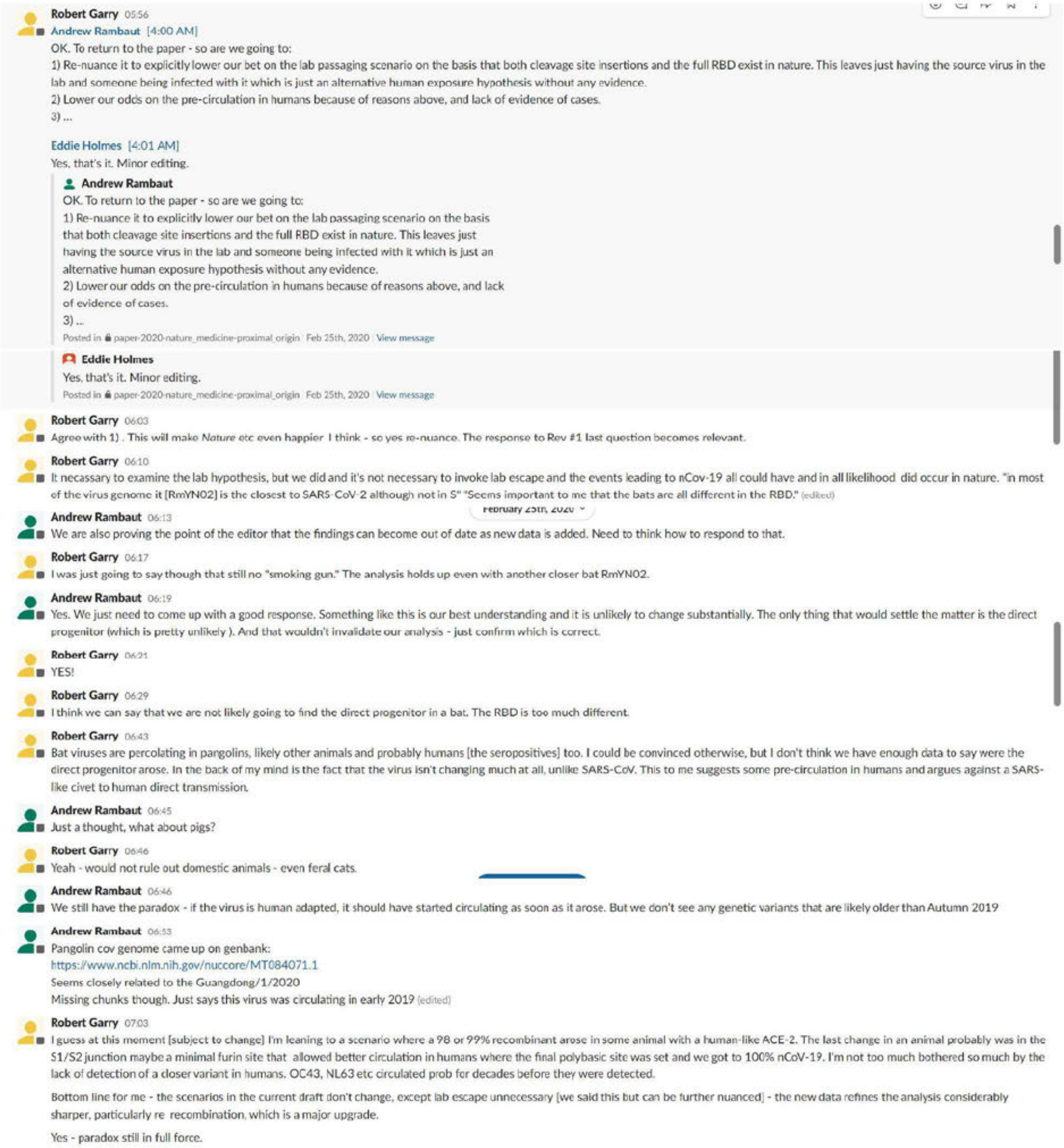
Eddie Holmes 05:35
Next of kin baboon perhaps.

Robert Garry 05:36
Maybe the term is "insertion/deletion" or maybe just "mutation." Flu v can get polybasic site via small recombination events, point mutations or six nuc insertion. (edited)

Eddie Holmes 05:36
Back on tomorrow from me.

Robert Garry 05:42
All good Eddie and thanks for the updates! Paper will get a significant upgrade. Not sure about the baboons.

Robert Garry 05:50
Clearly there are larger scale recombination events going on as well. I think Andrew's beautiful recombination figure adds a lot of weight/significance - maybe enough to push it to Nature itself rather than NatMed (not a bad journal either).



Robert Garry 05:56

Andrew Rambaut [4:00 AM]
OK. To return to the paper - so are we going to:
1) Re-nuance it to explicitly lower our bet on the lab passaging scenario on the basis that both cleavage site insertions and the full RBD exist in nature. This leaves just having the source virus in the lab and someone being infected with it which is just an alternative human exposure hypothesis without any evidence.
2) Lower our odds on the pre-circulation in humans because of reasons above, and lack of evidence of cases.
3) ...

Eddie Holmes [4:01 AM]
Yes, that's it. Minor editing.

> **Andrew Rambaut**
> OK. To return to the paper - so are we going to:
> 1) Re-nuance it to explicitly lower our bet on the lab passaging scenario on the basis that both cleavage site insertions and the full RBD exist in nature. This leaves just having the source virus in the lab and someone being infected with it which is just an alternative human exposure hypothesis without any evidence.
> 2) Lower our odds on the pre-circulation in humans because of reasons above, and lack of evidence of cases.
> 3) ...
> Posted in paper-2020-nature_medicine-proximal_origin | Feb 25th, 2020 | View message

> **Eddie Holmes**
> Yes, that's it. Minor editing.
> Posted in paper-2020-nature_medicine-proximal_origin | Feb 25th, 2020 | View message

Robert Garry 06:03
Agree with 1) . This will make *Nature* etc even happier I think - so yes re-nuance. The response to Rev #1 last question becomes relevant.

Robert Garry 06:10
It necassary to examine the lab hypothesis, but we did and it's not necessary to invoke lab escape and the events leading to nCov-19 all could have and in all likelihood did occur in nature. "in most of the virus genome it [RmYN02] is the closest to SARS-CoV-2 although not in S" "Seems important to me that the bats are all different in the RBD." (edited)

Andrew Rambaut 06:13
We are also proving the point of the editor that the findings can become out of date as new data is added. Need to think how to respond to that.

Robert Garry 06:17
I was just going to say though that still no "smoking gun." The analysis holds up even with another closer bat RmYN02.

Andrew Rambaut 06:19
Yes. We just need to come up with a good response. Something like this is our best understanding and it is unlikely to change substantially. The only thing that would settle the matter is the direct progenitor (which is pretty unlikely). And that wouldn't invalidate our analysis - just confirm which is correct.

Robert Garry 06:21
YES!

Robert Garry 06:29
I think we can say that we are not likely going to find the direct progenitor in a bat. The RBD is too much different.

Robert Garry 06:43
Bat viruses are percolating in pangolins, likely other animals and probably humans [the seropositives] too. I could be convinced otherwise, but I don't think we have enough data to say were the direct progenitor arose. In the back of my mind is the fact that the virus isn't changing much at all, unlike SARS-CoV. This to me suggests some pre-circulation in humans and argues against a SARS-like civet to human direct transmission.

Andrew Rambaut 06:45
Just a thought, what about pigs?

Robert Garry 06:46
Yeah - would not rule out domestic animals - even feral cats.

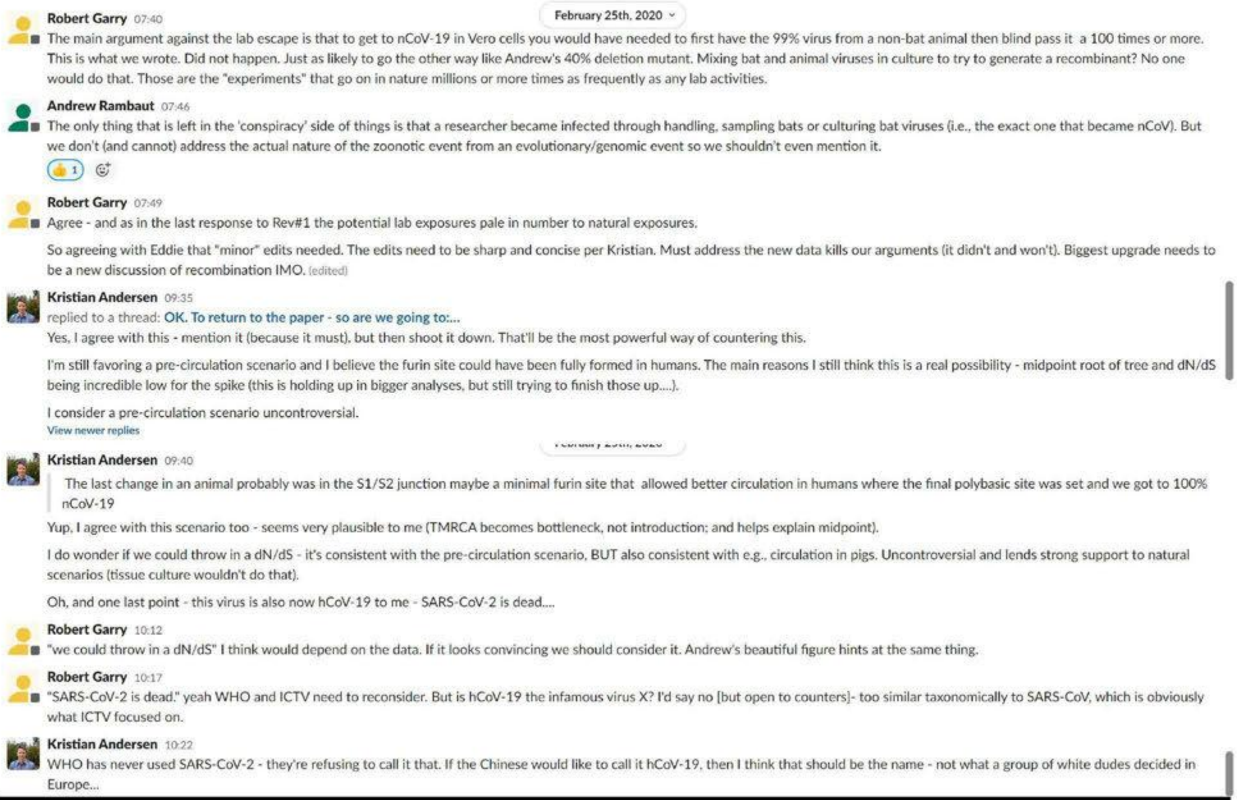
Andrew Rambaut 06:46
We still have the paradox - if the virus is human adapted, it should have started circulating as soon as it arose. But we don't see any genetic variants that are likely older than Autumn 2019

Andrew Rambaut 06:53
Pangolin cov genome came up on genbank:
https://www.ncbi.nlm.nih.gov/nuccore/MT084071.1
Seems closely related to the Guangdong/1/2020
Missing chunks though. Just says this virus was circulating in early 2019 (edited)

Robert Garry 07:03
I guess at this moment [subject to change] I'm leaning to a scenario where a 98 or 99% recombinant arose in some animal with a human-like ACE-2. The last change in an animal probably was in the S1/S2 junction maybe a minimal furin site that allowed better circulation in humans where the final polybasic site was set and we got to 100% nCoV-19. I'm not too much bothered so much by the lack of detection of a closer variant in humans. OC43, NL63 etc circulated prob for decades before they were detected.

Bottom line for me - the scenarios in the current draft don't change, except lab escape unnecessary [we said this but can be further nuanced] - the new data refines the analysis considerably sharper, particularly re recombination, which is a major upgrade.

Yes - paradox still in full force.



February 25th, 2020

Robert Garry 07:40
The main argument against the lab escape is that to get to nCoV-19 in Vero cells you would have needed to first have the 99% virus from a non-bat animal then blind pass it a 100 times or more. This is what we wrote. Did not happen. Just as likely to go the other way like Andrew's 40% deletion mutant. Mixing bat and animal viruses in culture to try to generate a recombinant? No one would do that. Those are the "experiments" that go on in nature millions or more times as frequently as any lab activities.

Andrew Rambaut 07:46
The only thing that is left in the 'conspiracy' side of things is that a researcher became infected through handling, sampling bats or culturing bat viruses (i.e., the exact one that became nCoV). But we don't (and cannot) address the actual nature of the zoonotic event from an evolutionary/genomic event so we shouldn't even mention it.
👍 1

Robert Garry 07:49
Agree - and as in the last response to Rev#1 the potential lab exposures pale in number to natural exposures.

So agreeing with Eddie that "minor" edits needed. The edits need to be sharp and concise per Kristian. Must address the new data kills our arguments (it didn't and won't). Biggest upgrade needs to be a new discussion of recombination IMO. (edited)

Kristian Andersen 09:35
replied to a thread: OK. To return to the paper - so are we going to:...
Yes, I agree with this - mention it (because it must), but then shoot it down. That'll be the most powerful way of countering this.

I'm still favoring a pre-circulation scenario and I believe the furin site could have been fully formed in humans. The main reasons I still think this is a real possibility - midpoint root of tree and dN/dS being incredible low for the spike (this is holding up in bigger analyses, but still trying to finish those up....).

I consider a pre-circulation scenario uncontroversial.
View newer replies

Kristian Andersen 09:40

> The last change in an animal probably was in the S1/S2 junction maybe a minimal furin site that allowed better circulation in humans where the final polybasic site was set and we got to 100% nCoV-19

Yup, I agree with this scenario too - seems very plausible to me (TMRCA becomes bottleneck, not introduction; and helps explain midpoint).

I do wonder if we could throw in a dN/dS - it's consistent with the pre-circulation scenario, BUT also consistent with e.g., circulation in pigs. Uncontroversial and lends strong support to natural scenarios (tissue culture wouldn't do that).

Oh, and one last point - this virus is also now hCoV-19 to me - SARS-CoV-2 is dead....

Robert Garry 10:12
"we could throw in a dN/dS" I think would depend on the data. If it looks convincing we should consider it. Andrew's beautiful figure hints at the same thing.

Robert Garry 10:17
"SARS-CoV-2 is dead." yeah WHO and ICTV need to reconsider. But is hCoV-19 the infamous virus X? I'd say no [but open to counters]- too similar taxonomically to SARS-CoV, which is obviously what ICTV focused on.

Kristian Andersen 10:22
WHO has never used SARS-CoV-2 - they're refusing to call it that. If the Chinese would like to call it hCoV-19, then I think that should be the name - not what a group of white dudes decided in Europe...

[redacted]

[redacted]

Andrew Rambaut 13:05

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On a visit to Shaoguan, Guangdong province, last year, the Guardian and staff from CBCGDF saw a caged facility previously used for attempted breeding of the notoriously hard-to-breed pangolin.

While there were no longer pangolin at the site, several locals near the facility confirmed the species had been raised there, along with monkeys and other wildlife
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https://www.theguardian.com/environment/2020/feb/25/coronavirus-closures-reveal-vast-scale-of-chinas-secretive-wildlife-farm-industry

the Guardian

Coronavirus closures reveal vast scale of China's secretive wildlife farm industry

Peacocks, porcupines and pangolins among species bred on almost 20,000 farms closed in wake of virus

Feb 24th, 2020 (155 kB)

February 25th, 2020

Robert Garry 13:37

[redacted]

i hope some one is sampling those animals - would be a good place to generate diversity in covs.

[redacted]

Eddie Holmes 15:43

[redacted]

I agree that we should use nCoV-19. Will do so from now on.

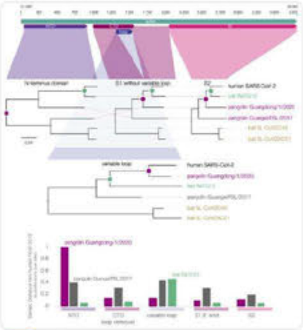
[redacted]

February 26th, 2020

Andrew Rambaut 10:00

I have added a plot of distances to the bottom of this. The bars match the dots on the trees

recombination_spike.png



💯 1

Latest messages

February 26th, 2020

Kristian Andersen 10:14

This looks great! Which part contains the RBD and the key residues?

Andrew Rambaut 10:20

variable loop

If we use it we can try to standardise the two figures.

Kristian Andersen 10:24

I think we should definitely use it - but yeah, we'd probably need to standardize the two to make it easier to follow. Love this one - it very nicely illustrates the natural scenario explaining the RBD!

More 'credit' from TWiV.... http://www.microbe.tv/twievo/twievo-52/

> **microbe.tv**
> TWiEVO 52: Virus evolution by land and by sea and by CoV | This Week in Evolution
> Nels and Vincent examine SARS-CoV-2 from an evolutionary viewpoint, examining what the spike glycoprotein sequence informs us about the origin of the virus.

Nice little figure they have there

Robert Garry 10:56

Looks great - minor tweak: should be N-terminal domain.

1

Robert Garry 11:01

Yeah - GREAT

February 26th, 2020

Robert Garry 11:08

Can this be summarized as: 1) RaTG13 is closest to nCoV-19 [need to harmonize] in S except for the variable loop, where closest is pango Guangdong 1/2020. Suggests recombination. 2) Spike also appears to be a hotspot for recombination in the pango viruses. Outside of spike and the variable domain is RATG13 still closest to nCov-19 or is this hCov-19 in all the genes?

Andrew Rambaut 11:42

Yes. But I think the key point is that the RaTG13 has had a new variable loop region come in (it's genetic distance jumps up, whereas the pangolin stays the same). I think we can infer from that that the RaTG13 lineage had the good RBD residues prior to this recombination event.

So we can infer that the ACE2 liking RBD was in bats.

Robert Garry 12:20

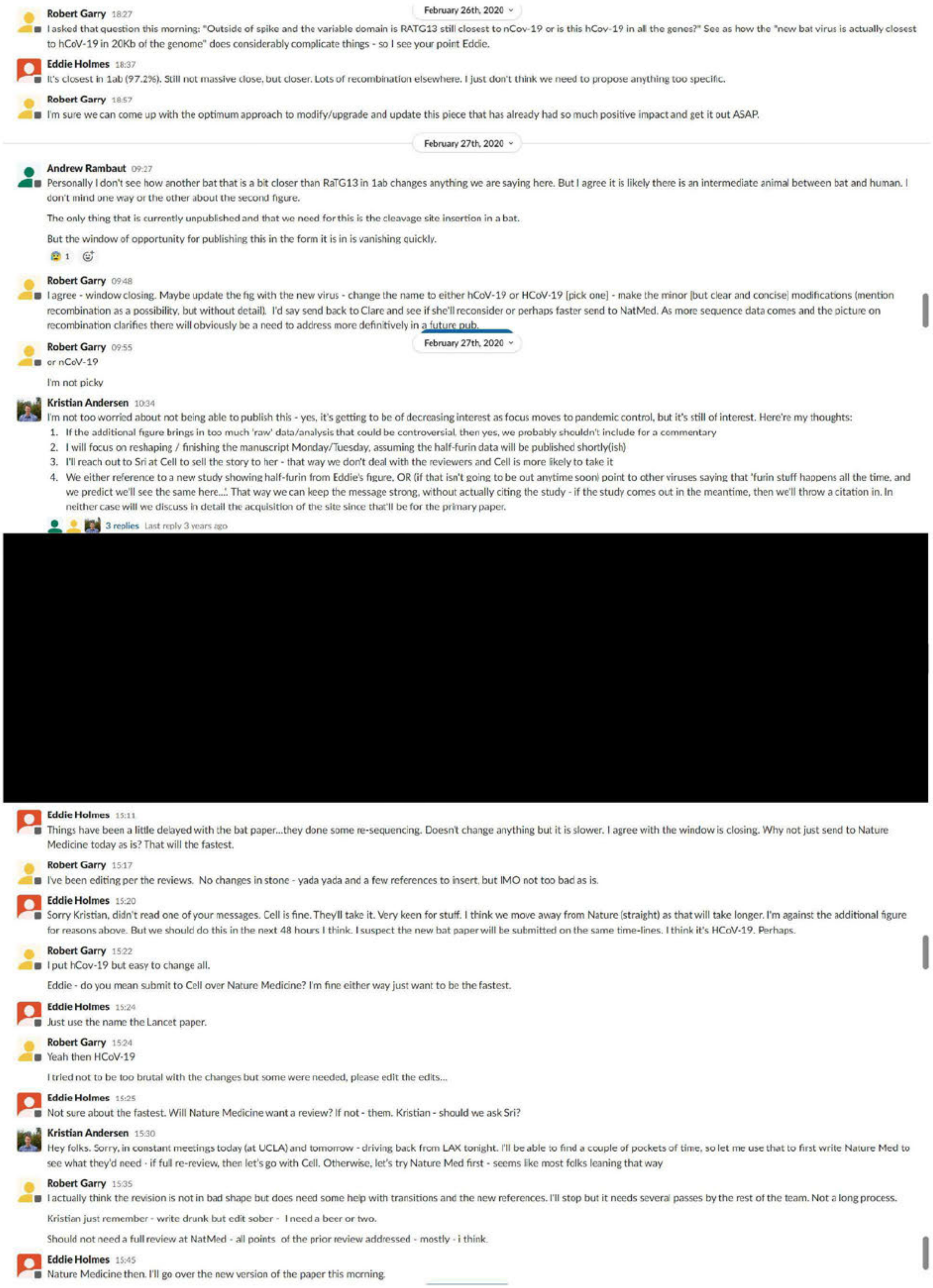
So, 1) recombination in the variable loop to optimize an already pretty good human-like RBD in a RaTG13-like virus followed by 2) insertion/deletion/recombination/mutation [still grasping for a verb] at the S1/S2 junction generated the progeritor to nCoV-19. Does this awesome analysis provide clues as to what species 1 or 2 took place in? Seems 1 or 2 could potentially have been in pangolins, another animal or humans. Even if 1 and 2 both took place in animals some pre-Wuhan circulation may have been required in humans to lock in the optimal polybasic site. (edited)

Robert Garry 14:37

Should SARS-CoV go on this second figure? It's on the first one.

Eddie Holmes 17:57

I have to say that I disagree with this. I think we should stick to the original plan for this article as much as possible and not try to be too detailed about what we think happened (e.g. which bits in which hosts) and I don't think we should use Andrew's figure in this piece. I say this because I certain that the picture is going to change rapidly as new data come out and I am loathed to make any strong conclusions when the sample is so small. For example, I don't think we firmly conclude that the hCoV-19 RBD came from a bat. I strongly believe there was another intermediate host somewhere. In addition, the new bat virus is actually closest to hCoV-19 in 20Kb of the genome. Also, it puts me in a very difficult position as it means that I am on papers that will be published around the same time making almost contradictory statements. So, if you want to go into detail saying which bit of sequence came from where then I feel that I'll need to remove my name. I honestly don't we need to do this: I think we just evaluate the data in support of the various hypotheses and leave it like this.



February 26th, 2020

Robert Garry 18:27
I asked that question this morning: "Outside of spike and the variable domain is RATG13 still closest to nCov-19 or is this hCov-19 in all the genes?" See as how the "new bat virus is actually closest to hCoV-19 in 20Kb of the genome" does considerably complicate things - so I see your point Eddie.

Eddie Holmes 18:37
It's closest in 1ab (97.2%). Still not massive close, but closer. Lots of recombination elsewhere. I just don't think we need to propose anything too specific.

Robert Garry 18:57
I'm sure we can come up with the optimum approach to modify/upgrade and update this piece that has already had so much positive impact and get it out ASAP.

February 27th, 2020

Andrew Rambaut 09:27
Personally I don't see how another bat that is a bit closer than RaTG13 in 1ab changes anything we are saying here. But I agree it is likely there is an intermediate animal between bat and human. I don't mind one way or the other about the second figure.

The only thing that is currently unpublished and that we need for this is the cleavage site insertion in a bat.

But the window of opportunity for publishing this in the form it is in is vanishing quickly.

😰 1

Robert Garry 09:48
I agree - window closing. Maybe update the fig with the new virus - change the name to either hCoV-19 or HCoV-19 [pick one] - make the minor [but clear and concise] modifications (mention recombination as a possibility, but without detail). I'd say send back to Clare and see if she'll reconsider or perhaps faster send to NatMed. As more sequence data comes and the picture on recombination clarifies there will obviously be a need to address more definitively in a future pub.

February 27th, 2020

Robert Garry 09:55
or nCoV-19

I'm not picky

Kristian Andersen 10:34
I'm not too worried about not being able to publish this - yes, it's getting to be of decreasing interest as focus moves to pandemic control, but it's still of interest. Here're my thoughts:

1. If the additional figure brings in too much 'raw' data/analysis that could be controversial, then yes, we probably shouldn't include for a commentary
2. I will focus on reshaping / finishing the manuscript Monday/Tuesday, assuming the half-furin data will be published shortly(ish)
3. I'll reach out to Sri at Cell to sell the story to her - that way we don't deal with the reviewers and Cell is more likely to take it
4. We either reference to a new study showing half-furin from Eddie's figure, OR (if that isn't going to be out anytime soon) point to other viruses saying that 'furin stuff happens all the time, and we predict we'll see the same here...'. That way we can keep the message strong, without actually citing the study - if the study comes out in the meantime, then we'll throw a citation in. In neither case will we discuss in detail the acquisition of the site since that'll be for the primary paper.

3 replies Last reply 3 years ago

[redacted]

Eddie Holmes 15:11
Things have been a little delayed with the bat paper...they done some re-sequencing. Doesn't change anything but it is slower. I agree with the window is closing. Why not just send to Nature Medicine today as is? That will the fastest.

Robert Garry 15:17
I've been editing per the reviews. No changes in stone - yada yada and a few references to insert, but IMO not too bad as is.

Eddie Holmes 15:20
Sorry Kristian, didn't read one of your messages. Cell is fine. They'll take it. Very keen for stuff. I think we move away from Nature (straight) as that will take longer. I'm against the additional figure for reasons above. But we should do this in the next 48 hours I think. I suspect the new bat paper will be submitted on the same time-lines. I think it's HCoV-19. Perhaps.

Robert Garry 15:22
I put hCov-19 but easy to change all.

Eddie - do you mean submit to Cell over Nature Medicine? I'm fine either way just want to be the fastest.

Eddie Holmes 15:24
Just use the name the Lancet paper.

Robert Garry 15:24
Yeah then HCoV-19

I tried not to be too brutal with the changes but some were needed, please edit the edits...

Eddie Holmes 15:25
Not sure about the fastest. Will Nature Medicine want a review? If not - them. Kristian - should we ask Sri?

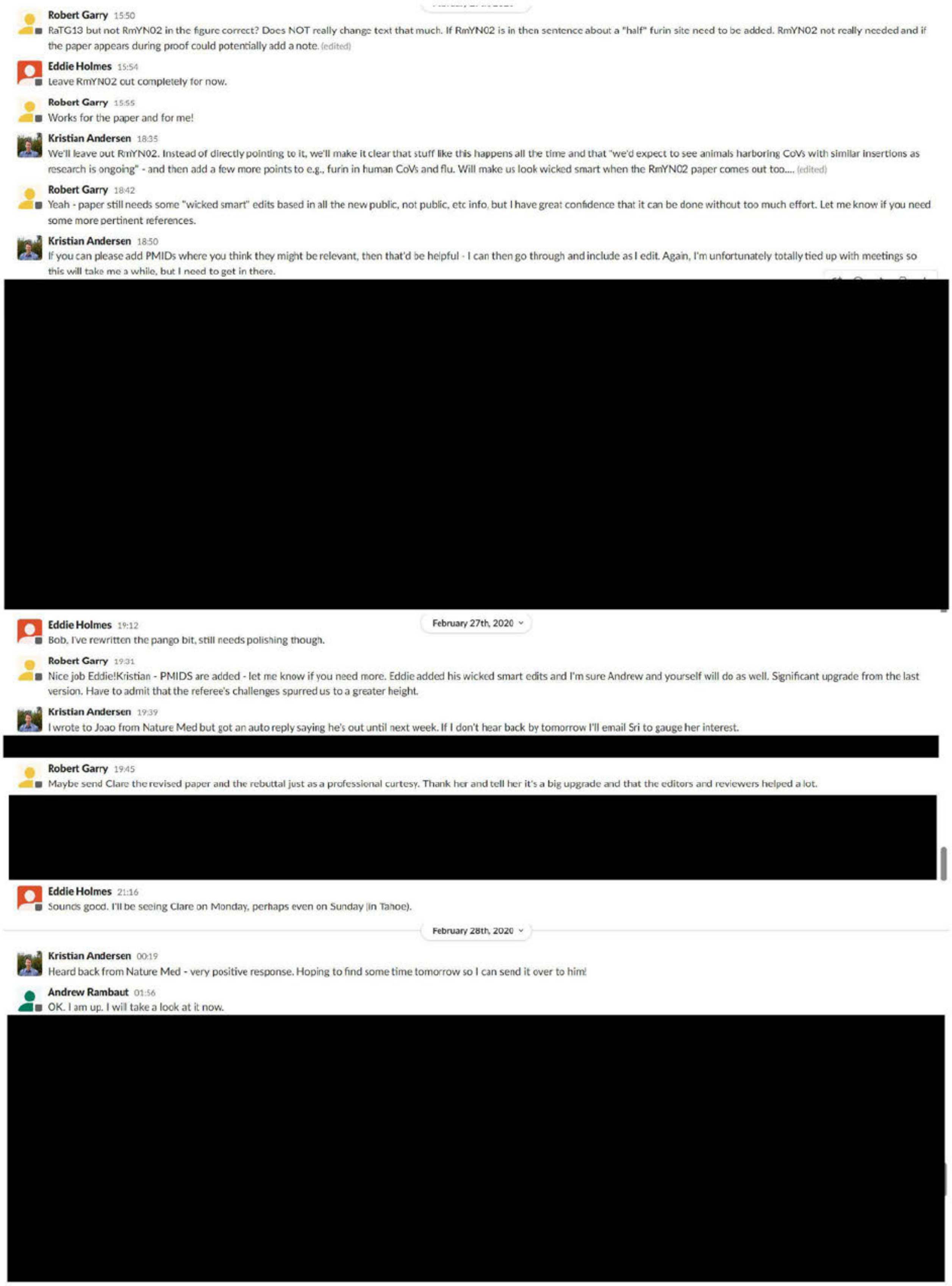
Kristian Andersen 15:30
Hey folks. Sorry, in constant meetings today (at UCLA) and tomorrow - driving back from LAX tonight. I'll be able to find a couple of pockets of time, so let me use that to first write Nature Med to see what they'd need - if full re-review, then let's go with Cell. Otherwise, let's try Nature Med first - seems like most folks leaning that way

Robert Garry 15:35
I actually think the revision is not in bad shape but does need some help with transitions and the new references. I'll stop but it needs several passes by the rest of the team. Not a long process.

Kristian just remember - write drunk but edit sober - I need a beer or two.

Should not need a full review at NatMed - all points of the prior review addressed - mostly - i think.

Eddie Holmes 15:45
Nature Medicine then. I'll go over the new version of the paper this morning.



Robert Garry 15:50
RaTG13 but not RmYN02 in the figure correct? Does NOT really change text that much. If RmYN02 is in then sentence about a "half" furin site need to be added. RmYN02 not really needed and if the paper appears during proof could potentially add a note. (edited)

Eddie Holmes 15:54
Leave RmYN02 out completely for now.

Robert Garry 15:55
Works for the paper and for me!

Kristian Andersen 18:35
We'll leave out RmYN02. Instead of directly pointing to it, we'll make it clear that stuff like this happens all the time and that "we'd expect to see animals harboring CoVs with similar insertions as research is ongoing" - and then add a few more points to e.g., furin in human CoVs and flu. Will make us look wicked smart when the RmYN02 paper comes out too.... (edited)

Robert Garry 18:42
Yeah - paper still needs some "wicked smart" edits based in all the new public, not public, etc info, but I have great confidence that it can be done without too much effort. Let me know if you need some more pertinent references.

Kristian Andersen 18:50
If you can please add PMIDs where you think they might be relevant, then that'd be helpful - I can then go through and include as I edit. Again, I'm unfortunately totally tied up with meetings so this will take me a while, but I need to get in there.

[redacted]

February 27th, 2020

Eddie Holmes 19:12
Bob, I've rewritten the pango bit, still needs polishing though.

Robert Garry 19:31
Nice job Eddie!Kristian - PMIDS are added - let me know if you need more. Eddie added his wicked smart edits and I'm sure Andrew and yourself will do as well. Significant upgrade from the last version. Have to admit that the referee's challenges spurred us to a greater height.

Kristian Andersen 19:39
I wrote to Joao from Nature Med but got an auto reply saying he's out until next week. If I don't hear back by tomorrow I'll email Sri to gauge her interest.

[redacted]

Robert Garry 19:45
Maybe send Clare the revised paper and the rebuttal just as a professional curtesy. Thank her and tell her it's a big upgrade and that the editors and reviewers helped a lot.

[redacted]

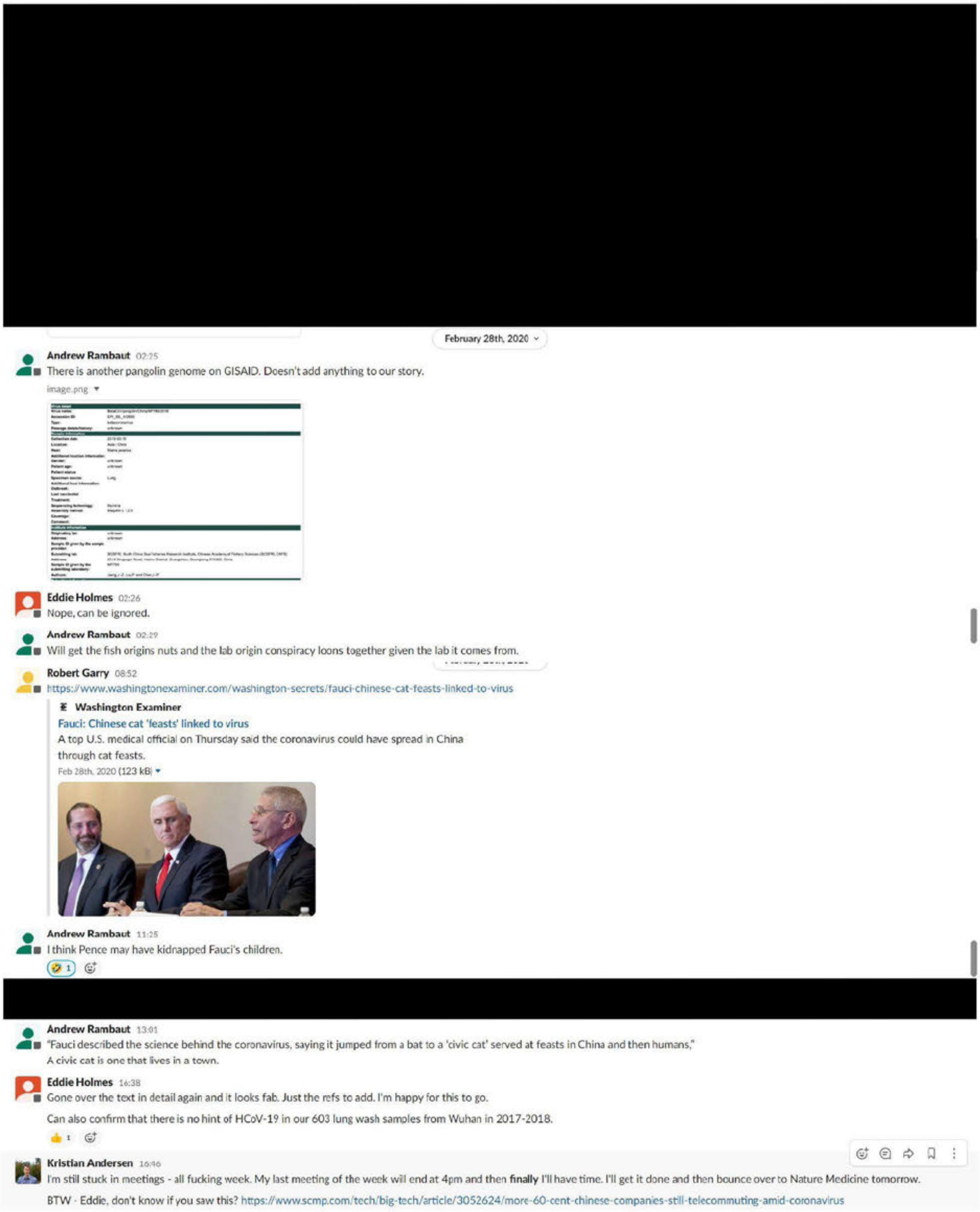
Eddie Holmes 21:16
Sounds good. I'll be seeing Clare on Monday, perhaps even on Sunday (in Tahoe).

February 28th, 2020

Kristian Andersen 00:19
Heard back from Nature Med - very positive response. Hoping to find some time tomorrow so I can send it over to him!

Andrew Rambaut 01:56
OK. I am up. I will take a look at it now.

[redacted]



[redacted]

February 28th, 2020

Andrew Rambaut 02:25
There is another pangolin genome on GISAID. Doesn't add anything to our story.
image.png

Eddie Holmes 02:26
Nope, can be ignored.

Andrew Rambaut 02:29
Will get the fish origins nuts and the lab origin conspiracy loons together given the lab it comes from.

Robert Garry 08:52
https://www.washingtonexaminer.com/washington-secrets/fauci-chinese-cat-feasts-linked-to-virus

Washington Examiner
Fauci: Chinese cat 'feasts' linked to virus
A top U.S. medical official on Thursday said the coronavirus could have spread in China through cat feasts.
Feb 28th, 2020 (123 kB)

Andrew Rambaut 11:25
I think Pence may have kidnapped Fauci's children.
🤣 1

[redacted]

Andrew Rambaut 13:01
"Fauci described the science behind the coronavirus, saying it jumped from a bat to a 'civic cat' served at feasts in China and then humans,"
A civic cat is one that lives in a town.

Eddie Holmes 16:38
Gone over the text in detail again and it looks fab. Just the refs to add. I'm happy for this to go.

Can also confirm that there is no hint of HCoV-19 in our 603 lung wash samples from Wuhan in 2017-2018.
👍 1

Kristian Andersen 16:46
I'm still stuck in meetings - all fucking week. My last meeting of the week will end at 4pm and then **finally** I'll have time. I'll get it done and then bounce over to Nature Medicine tomorrow.

BTW - Eddie, don't know if you saw this? https://www.scmp.com/tech/big-tech/article/3052624/more-60-cent-chinese-companies-still-telecommuting-amid-coronavirus

South China Morning Post

Workers at 60 per cent of Chinese firms still telecommuting under lockdown

More than 60 per cent of companies in major Chinese cities have not reopened offices since the Lunar New Year holiday, allowing employees to work remotely from home.

Feb 27th, 2020 (238 kB)

Reference to show that the furin site is functional in hCoV: https://www.cell.com/pb-assets/journals/research/cell/Cell_S0092-8674(20)30262-2.pdf

Eddie Holmes 17:55

Oh, good reference - we should cite that. I'm in very regular contact with people in China - they are doing fine. People are out and about on the streets as normal in Shanghai. I'm hoping that things might start to calm down a bit when people don't start dropping dead in the sensible streets of northern Europe. The Korean numbers look the best measured to me - CFR is ~0.5%. Clearly a massive underestimation of cases in Hubei.

[redacted]

Kristian Andersen 20:02

[redacted]

@Eddie Holmes - do you have a version of our previous submission with line numbers?

Eddie Holmes 20:26

No. I can't see that we ever had one.

Kristian Andersen 20:27

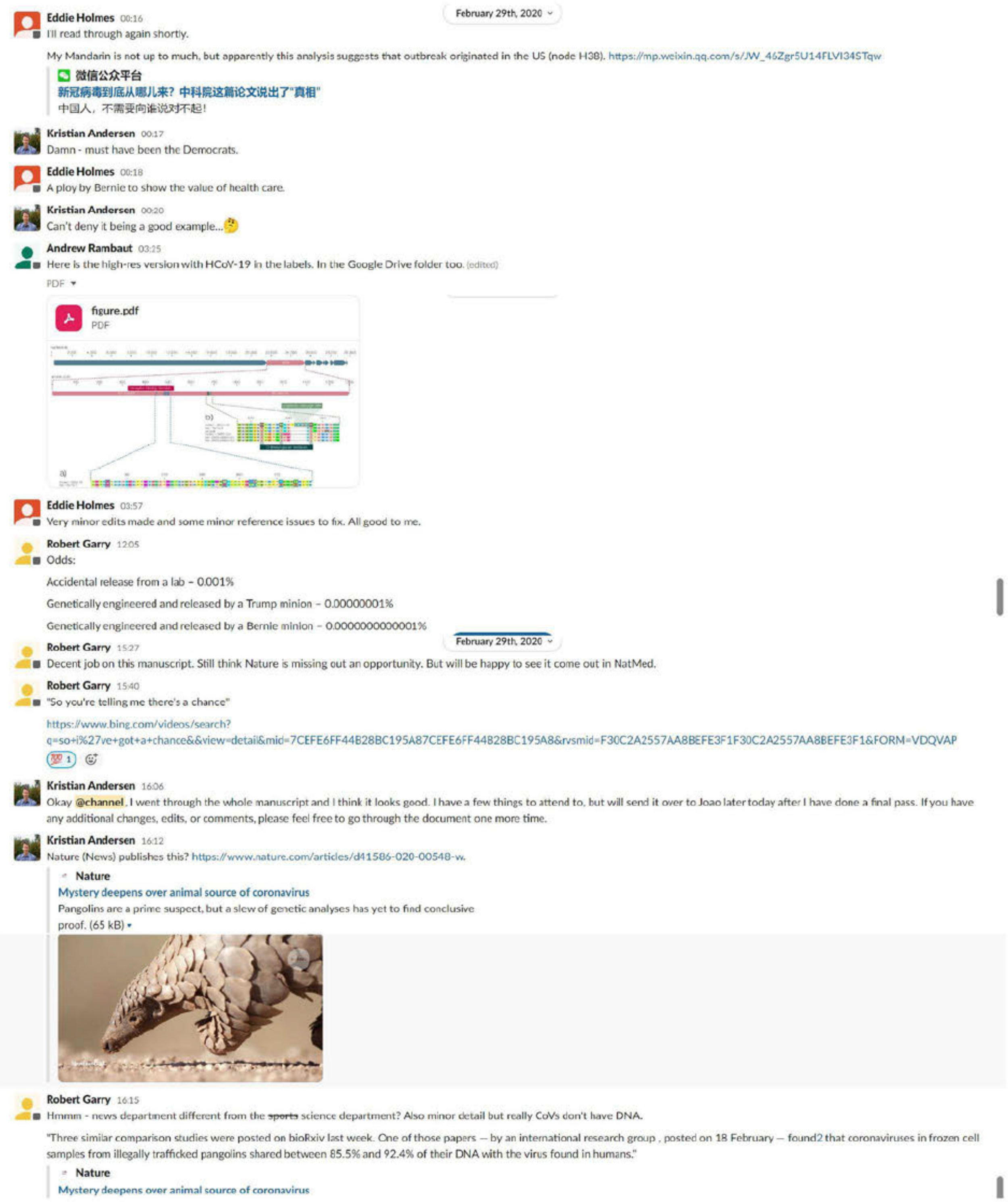
I don't think we did - I think it might be in the Nature system... All good - I managed to figure it out. Do we have a high resolution version of @Andrew Rambaut updated figure? (edited)

Eddie Holmes 20:32

Have checked: the one I submitted did not have line numbers. I don't have a version of the figure that says 'HCoV-19'.

Kristian Andersen 23:43

Will finish this tomorrow morning. Some funky bits that required rewriting and a number of missing references. Should be sorted out now, so should be completed soon. @Andrew Rambaut one comment for you, and can you please also share a high resolution version of the most up-to-date Fig. 1?



February 29th, 2020

Eddie Holmes 00:16
I'll read through again shortly.

My Mandarin is not up to much, but apparently this analysis suggests that outbreak originated in the US (node H38). https://mp.weixin.qq.com/s/JW_46Zgr5U14FLVI34STqw

> **微信公众平台**
> 新冠病毒到底从哪儿来？中科院这篇论文说出了"真相"
> 中国人，不需要向谁说对不起！

Kristian Andersen 00:17
Damn - must have been the Democrats.

Eddie Holmes 00:18
A ploy by Bernie to show the value of health care.

Kristian Andersen 00:20
Can't deny it being a good example...🤔

Andrew Rambaut 03:25
Here is the high-res version with HCoV-19 in the labels. In the Google Drive folder too. (edited)

PDF ▾

figure.pdf
PDF

Eddie Holmes 03:57
Very minor edits made and some minor reference issues to fix. All good to me.

Robert Garry 12:05
Odds:

Accidental release from a lab - 0.001%

Genetically engineered and released by a Trump minion - 0.00000001%

Genetically engineered and released by a Bernie minion - 0.0000000000001%

February 29th, 2020

Robert Garry 15:27
Decent job on this manuscript. Still think Nature is missing out an opportunity. But will be happy to see it come out in NatMed.

Robert Garry 15:40
"So you're telling me there's a chance"

https://www.bing.com/videos/search?q=so+i%27ve+got+a+chance&&view=detail&mid=7CEFE6FF44B28BC195A87CEFE6FF44B28BC195A8&rvsmid=F30C2A2557AA8BEFE3F1F30C2A2557AA8BEFE3F1&FORM=VDQVAP

💯 1

Kristian Andersen 16:06
Okay @channel, I went through the whole manuscript and I think it looks good. I have a few things to attend to, but will send it over to Joao later today after I have done a final pass. If you have any additional changes, edits, or comments, please feel free to go through the document one more time.

Kristian Andersen 16:12
Nature (News) publishes this? https://www.nature.com/articles/d41586-020-00548-w.

> **Nature**
> Mystery deepens over animal source of coronavirus
> Pangolins are a prime suspect, but a slew of genetic analyses has yet to find conclusive proof. (65 kB) ▾

Robert Garry 16:15
Hmmm - news department different from the ~~sports~~ science department? Also minor detail but really CoVs don't have DNA.

"Three similar comparison studies were posted on bioRxiv last week. One of those papers — by an international research group , posted on 18 February — found2 that coronaviruses in frozen cell samples from illegally trafficked pangolins shared between 85.5% and 92.4% of their DNA with the virus found in humans."

> **Nature**
> Mystery deepens over animal source of coronavirus

> Pangolins are a prime suspect, but a slew of genetic analyses has yet to find conclusive proof. (65 kB) ▾
>
> 

Nature should publish our paper to fully inform the mystery.

Kristian Andersen 16:20
@Eddie Holmes - are you seeing Clare this weekend?

Kristian Andersen 16:50
Talked to Eddie. He'll see Clare tomorrow or Monday. We'll send it to Nature Med later today and then Eddie will give Clare a full run-down - if there's a chance they still want it in Nature, then they can pull it pack from Nature Med. I don't really care too much - this'll get a big audience anyway.

Andrew Rambaut 16:53
Sounds all good to me.

Great work.

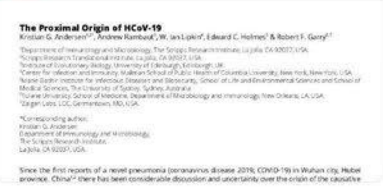
Robert Garry 17:30
ditto!

[redacted]

Kristian Andersen 19:23
Here goes - just popped it over to Joao.

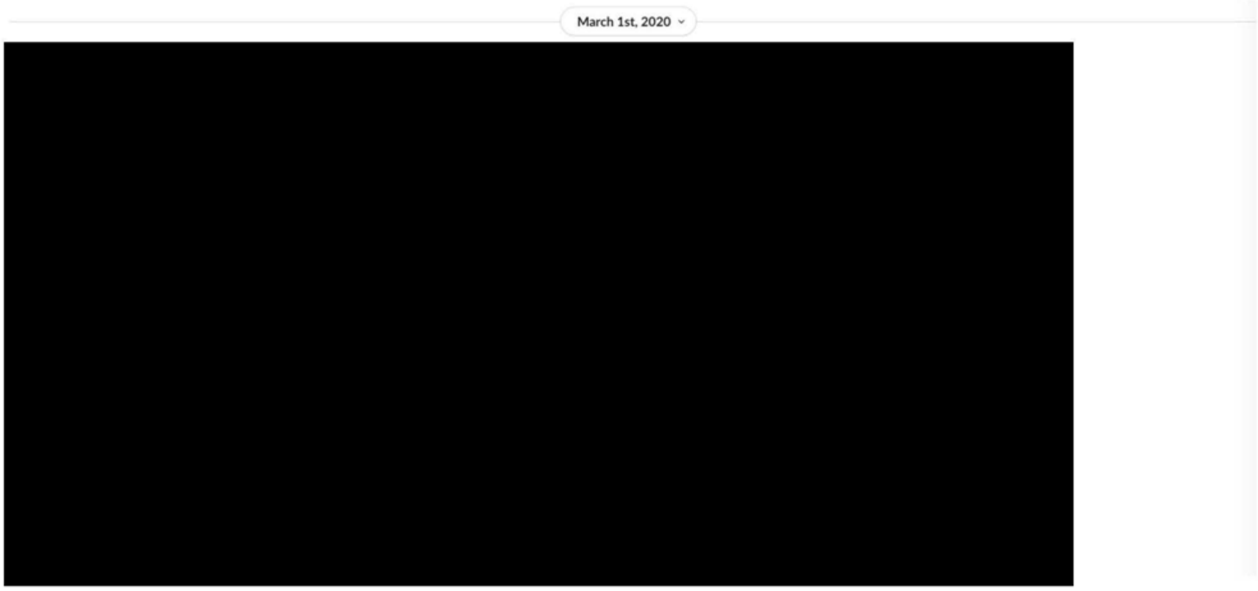
PDF ▾

The Proximal Origin of HCoV-19.pdf
PDF



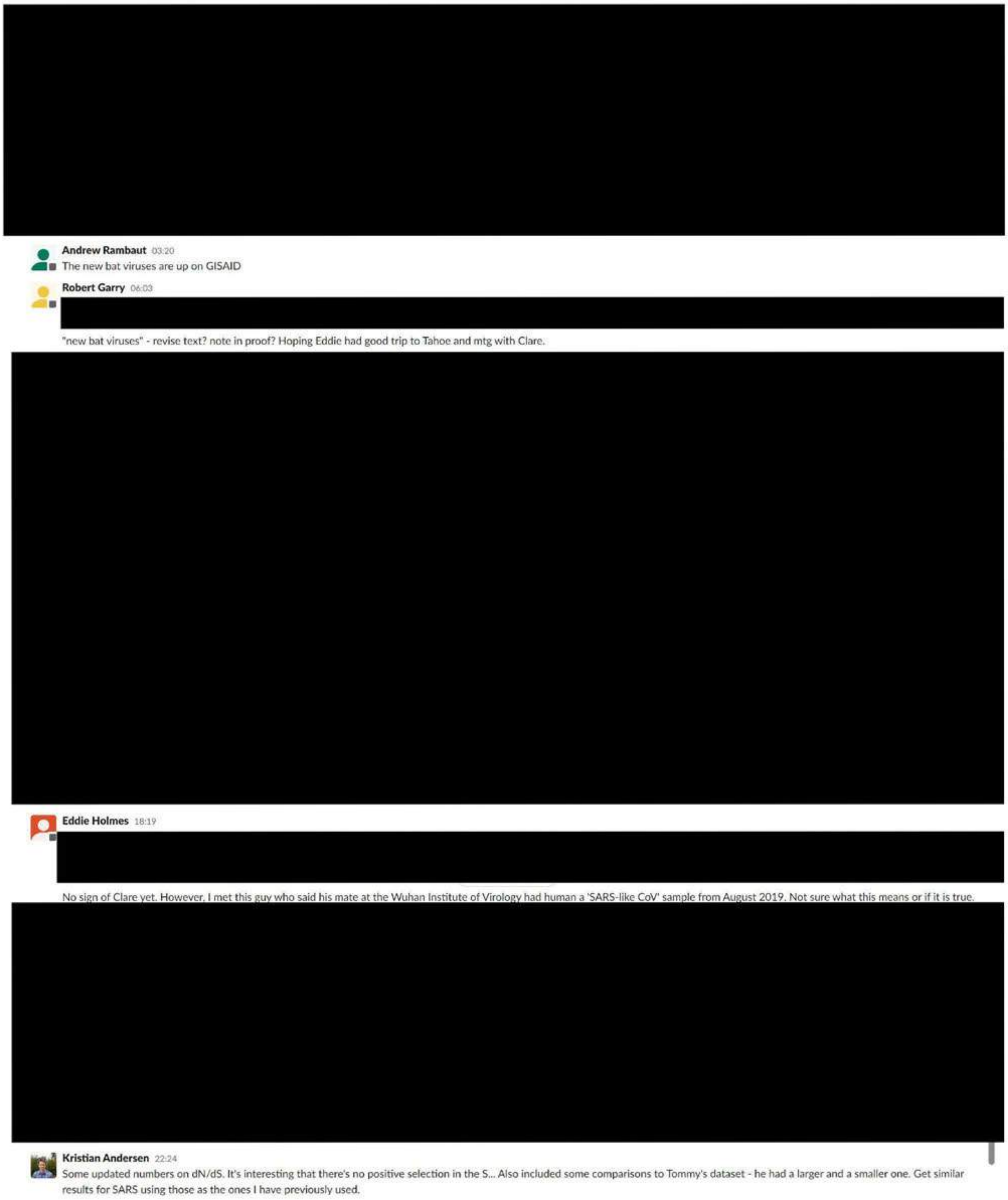
The Proximal Origin of HCoV-19
Kristian G. Andersen, Andrew Rambaut, W. Ian Lipkin, Edward C. Holmes & Robert F. Garry

Since the first reports of a novel pneumonia (coronavirus disease 2019; COVID-19) in Wuhan city, Hubei province, China[1,2] there has been considerable discussion and uncertainty over the origin of the causative



March 1st, 2020

[redacted]



[redacted]

Andrew Rambaut 03:20
The new bat viruses are up on GISAID

Robert Garry 06:03

[redacted]

"new bat viruses" - revise text? note in proof? Hoping Eddie had good trip to Tahoe and mtg with Clare.

[redacted]

Eddie Holmes 18:19

[redacted]

No sign of Clare yet. However, I met this guy who said his mate at the Wuhan Institute of Virology had human a 'SARS-like CoV' sample from August 2019. Not sure what this means or if it is true.

[redacted]

Kristian Andersen 22:24
Some updated numbers on dN/dS. It's interesting that there's no positive selection in the S... Also included some comparisons to Tommy's dataset - he had a larger and a smaller one. Get similar results for SARS using those as the ones I have previously used.

Interesting for this too is the fact that ORF1 in HCoV *does* have a pretty high dN/dS - similar to SARS early. It's almost as if the spike protein is adapted to human, but the rest of the virus isn't. Could be some crazy ass recombination event.

I'm hoping to get a chance to look at the now bigger HCoV dataset later in the week to see if anything has changed - this dataset is a couple of weeks old.

Screen Shot 2020-03-02 at 7.21.03 PM.png ▾

	ORF1	Spike
HCoV-19	0.91	0.29
SARS, early	0.81	1.82
SARS, middle	0.68	0.44
SARS, late	0.32	0.51
SARS, Tommy_big	0.54	0.90
SARS, Tommy_small	0.48	0.85
SARS, ViPR	0.62	0.82
MERS, ViPR	0.32	0.38
HKU1, ViPR	0.11	0.29

March 3rd, 2020 ▾

Eddie Holmes 00:29

Loads more Chinese genomes coming. I'm not quite when, but they are coming.

1 reply 3 years ago

March 3rd, 2020 ▾

Eddie Holmes 00:45

I don't think Clare is here. There are other Nature people and they think she may have cancelled due to the pandemic.

Kristian Andersen 01:04

Fuuuk

Robert Garry 05:20

i'd send Clare the revised paper/response - let her know we submitted to NatMed.

Andrew Rambaut 05:21

Yeah. Maybe with a cheeky 'you can still have it if you want it' at the end.

Robert Garry 10:37

"Could be some crazy ass recombination event." Seems pretty likely . Can you check the dN/dS of genes that are 3' of spike?

[redacted]

Kristian Andersen 15:10

Joao from Nature Med wants us to cut to ~2200 words and up to 30 references. We currently have ~3000 words and 60 references. Yay or nay?

Andrew Rambaut 15:29

March 3rd, 2020 ▾

800 words?

Is that an acceptance?

Kristian Andersen 15:31

Not an acceptance - but close. And yeah, we'd need to cut 800 words which probably wouldn't be too hard

Email from Slack for Gmail ▾

RE: Interest in "Proximal Origins of hCoV-19"? Mar 3rd, 2020
From Joao Monteiro (No content)

Robert Garry 16:57

Yes that's fine. Should NOT be too hard to cut. (edited)

👍 1

Eddie Holmes 17:38

I say yay. We need it out. I can easily take a look later today.

Andrew Rambaut 17:40

I will go over it now with suggestions on - see what I can find to trim.

Andrew Rambaut 19:10

OK. Got 2/3s of the way through. Not sure how much it saves but feel free to reject anything you feel goes too far.

March 3rd, 2020

Oh. And someone else is going to have to prune references.

Eddie Holmes 20:10
I'll see what I can do shortly.

Eddie Holmes 21:12
I've given it a good hack following Andrew's edits - now down to 2304 words. Pretty close. I'll leave someone else to deal with the references - I've cut a few.

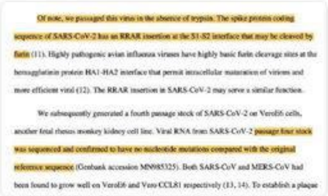
Kristian Andersen 21:41
Thanks guys. I'll get on it first thing tomorrow morning and shave off the last amount of fat and cut down the references.

Kristian Andersen 22:24
I *do* find these bits peculiar...

For the first part, SARS-like viruses replicate at very low levels in tissue culture, but require trypsin for efficient replication. Prolonged culturing would therefore create an enormous selection pressure for the acquisition of a furin site. This paper shows that the furin site is fully functional.

For the second part, it's kinda unusual that the virus doesn't pick up any mutations after culturing (Dave O'Connor told me the same) - typically viruses pick up mutations pretty quickly in tissue culture. (edited)

Screen Shot 2020-03-03 at 7.18.46 PM.png



Of note, we passaged this virus in the absence of trypsin. The spike protein coding sequence of SARS-CoV-2 has an RRAR insertion at the S1-S2 interface that may be cleaved by furin (11). Highly pathogenic avian influenza viruses have highly basic furin cleavage sites at the hemagglutinin protein HA1-HA2 interface that permit intracellular maturation of virions and more efficient viral (12). The RRAR insertion in SARS-CoV-2 may serve a similar function.

We subsequently generated a fourth passage stock of SARS-CoV-2 on VeroE6 cells, another fetal rhesus monkey kidney cell line. Viral RNA from SARS-CoV-2 passage four stock was sequenced and confirmed to have no nucleotide mutations compared with the original reference sequence (Genbank accession MN985325). Both SARS-CoV and MERS-CoV had been found to grow well on VeroE6 and Vero CCL81 respectively (13, 14). To establish a plaque

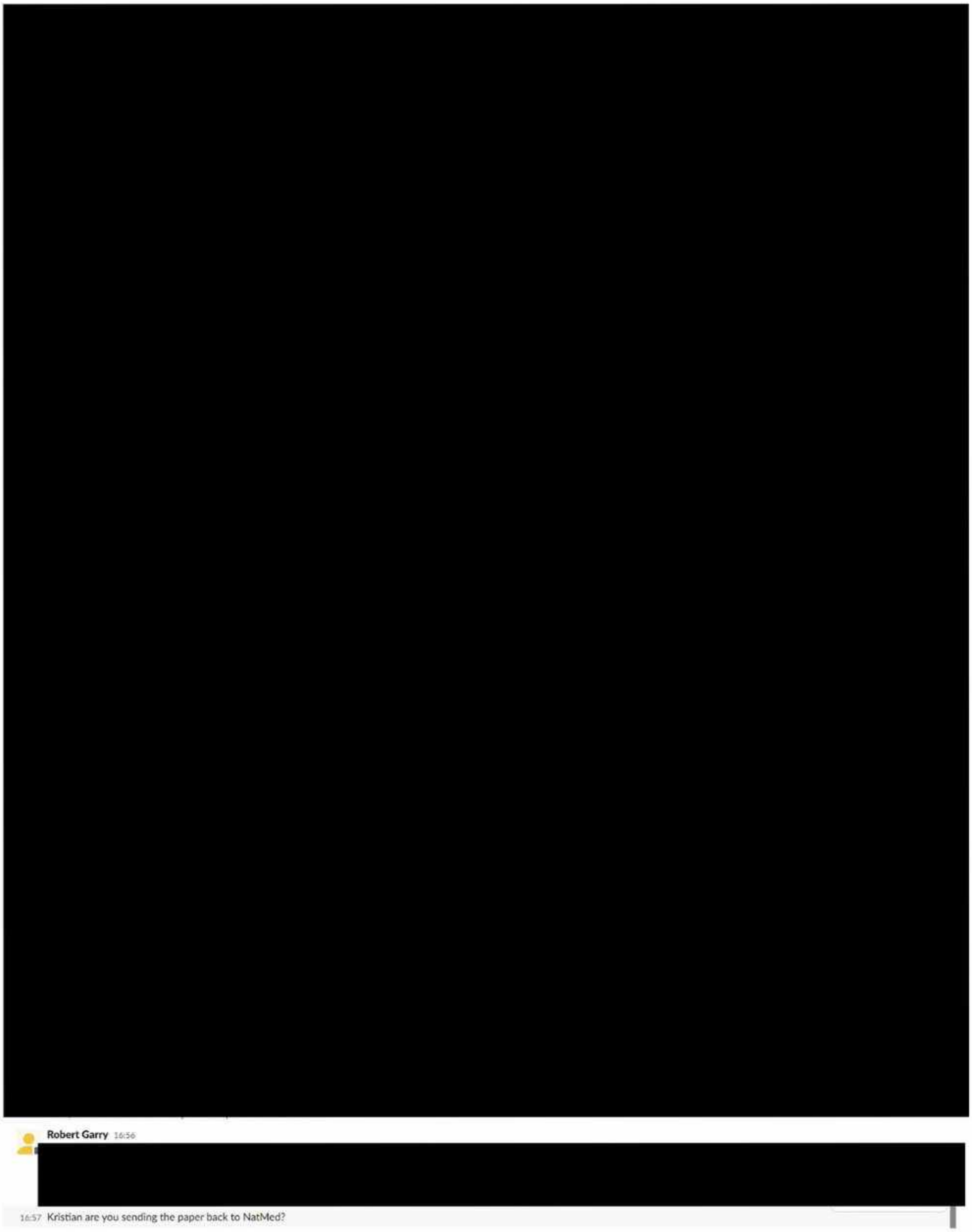
This is from the recent bioRxiv paper on the first US patient: https://www.biorxiv.org/content/10.1101/2020.03.02.972935v1.full.pdf

March 4th, 2020

Andrew Rambaut 03:58
There are some parallel changes going on in ORF1ab:
https://nextstrain.org/ncov?c=gt-ORF1a_3606&m=div

This one happens in two of the lineages that had the one above:
https://nextstrain.org/ncov?c=gt-ORF1a_1599&m=div

[redacted]



[redacted]

Robert Garry 16:56

[redacted]

16:57 Kristian are you sending the paper back to NatMed?

It looks good.

One reference to update.

Kristian Andersen 16:58
I am. Sorry, need to calm down first 😉. Will send it back within the hour.

[redacted]

Kristian Andersen 18:56
Any COIs to declare? @Robert Garry ? (can't have the full VHFC one - now a non-profit..)

6 replies Last reply 3 years ago

Andrew Rambaut 18:56
image.png ▾

Those dirty Canadians....

Robert Garry 19:11
Is this another reason to push hard to get those Iranian samples?

[redacted]

Kristian Andersen 19:18
One *should* wonder why this is the top trending article on Nature Medicine... I think our paper might be timely.

Screen Shot 2020-03-04 at 4.15.28 PM.png ▾

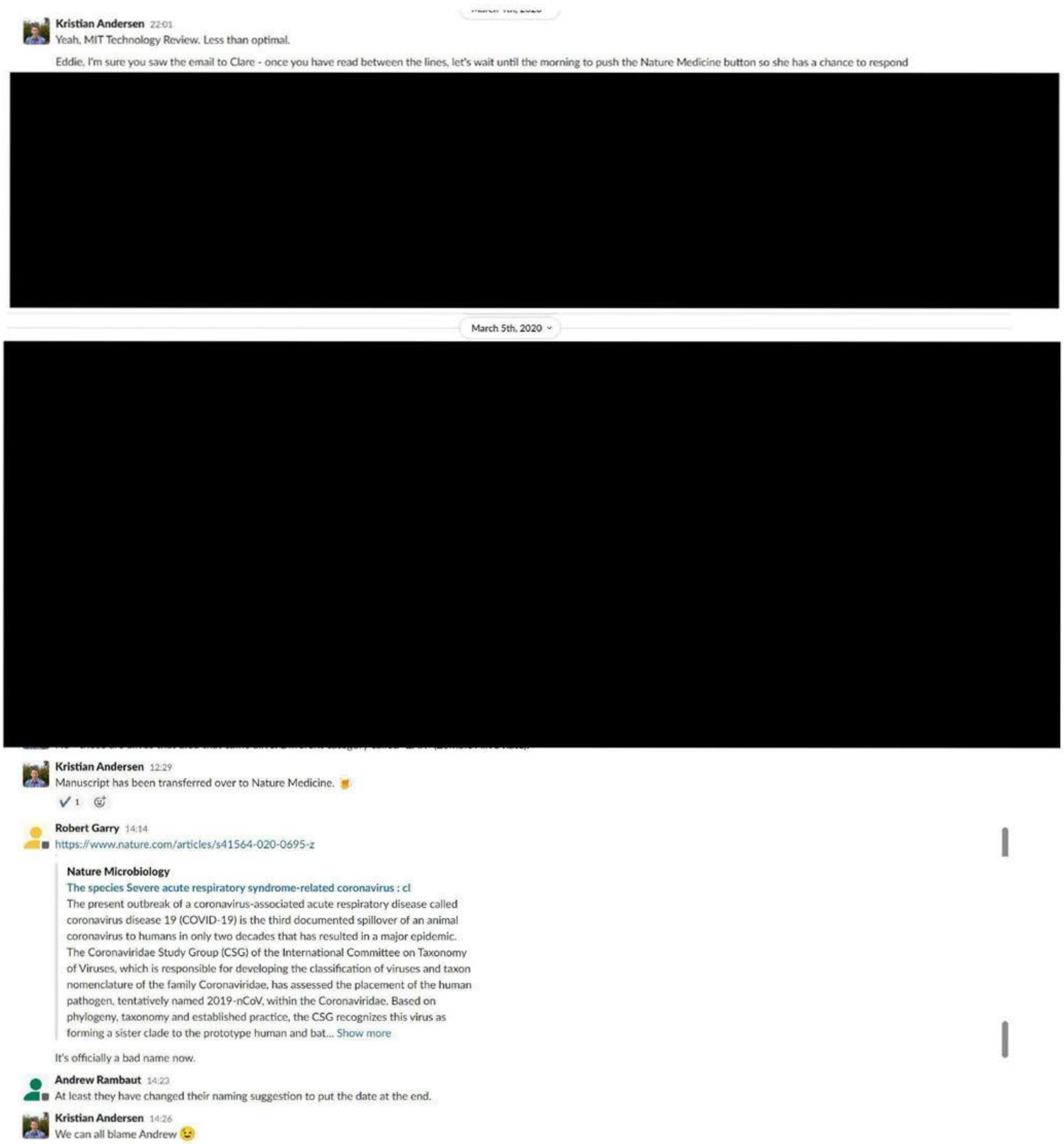
[redacted]

Kristian Andersen 21:21
Boo - can't call it HCoV-19... Predictably unfortunately 😞. Also pinged Clare with a coy email - just in case...

Robert Garry 21:45
No problem - I guess they balked on Wuhan Turtle Flu Virus as well?

Eddie Holmes 21:46
Sorry, I was out all day. Now in LAX wait to escape the war zone. Thanks for pushing all this stuff through. To clarify, Nature say it has to be SARS-CoV-2? The quote about the Bavarian chap...what was from the Technology Review? I can't access that. If so, that is just appalling.

Eddie Holmes 21:52
Can't we use 'the virus formerly known as 'SARS-CoV-2'?
👍 2



Kristian Andersen 22:01
Yeah, MIT Technology Review. Less than optimal.

Eddie, I'm sure you saw the email to Clare - once you have read between the lines, let's wait until the morning to push the Nature Medicine button so she has a chance to respond

[redacted]

March 5th, 2020

[redacted]

Kristian Andersen 12:29
Manuscript has been transferred over to Nature Medicine. 🍺
✓ 1

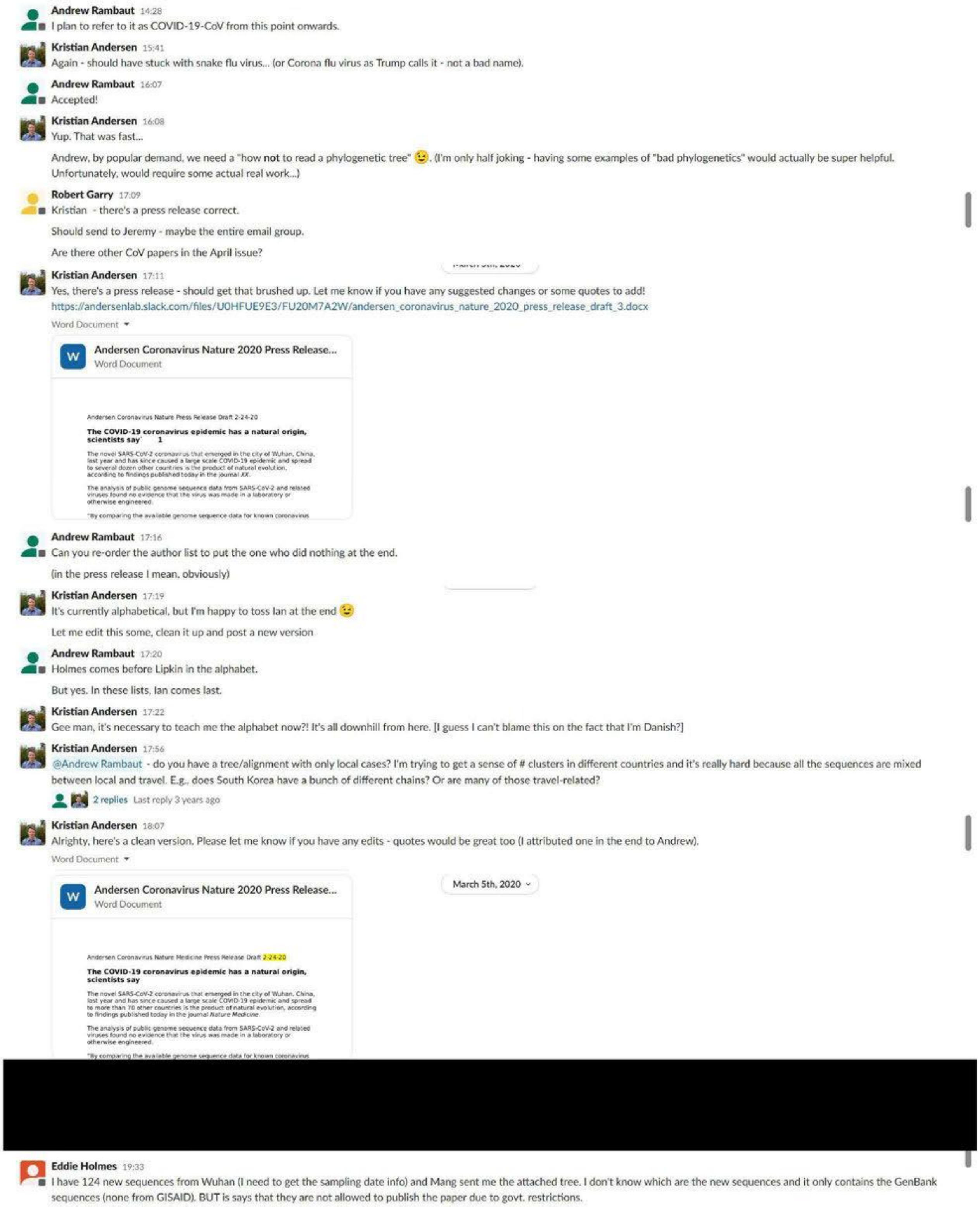
Robert Garry 14:14
https://www.nature.com/articles/s41564-020-0695-z

> **Nature Microbiology**
> The species Severe acute respiratory syndrome-related coronavirus : cl
> The present outbreak of a coronavirus-associated acute respiratory disease called coronavirus disease 19 (COVID-19) is the third documented spillover of an animal coronavirus to humans in only two decades that has resulted in a major epidemic. The Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses, which is responsible for developing the classification of viruses and taxon nomenclature of the family Coronaviridae, has assessed the placement of the human pathogen, tentatively named 2019-nCoV, within the Coronaviridae. Based on phylogeny, taxonomy and established practice, the CSG recognizes this virus as forming a sister clade to the prototype human and bat... Show more

It's officially a bad name now.

Andrew Rambaut 14:23
At least they have changed their naming suggestion to put the date at the end.

Kristian Andersen 14:26
We can all blame Andrew 😉



Andrew Rambaut 14:28
I plan to refer to it as COVID-19-CoV from this point onwards.

Kristian Andersen 15:41
Again - should have stuck with snake flu virus... (or Corona flu virus as Trump calls it - not a bad name).

Andrew Rambaut 16:07
Accepted!

Kristian Andersen 16:08
Yup. That was fast...

Andrew, by popular demand, we need a "how **not** to read a phylogenetic tree" 😉. (I'm only half joking - having some examples of "bad phylogenetics" would actually be super helpful. Unfortunately, would require some actual real work...)

Robert Garry 17:09
Kristian - there's a press release correct.

Should send to Jeremy - maybe the entire email group.

Are there other CoV papers in the April issue?

Kristian Andersen 17:11
Yes, there's a press release - should get that brushed up. Let me know if you have any suggested changes or some quotes to add!
https://andersenlab.slack.com/files/U0HFUE9E3/FU20M7A2W/andersen_coronavirus_nature_2020_press_release_draft_3.docx

Word Document ▾

Andersen Coronavirus Nature 2020 Press Release...
Word Document

Andersen Coronavirus Nature Press Release Draft 2-24-20

The COVID-19 coronavirus epidemic has a natural origin, scientists say 1

The novel SARS-CoV-2 coronavirus that emerged in the city of Wuhan, China, last year and has since caused a large scale COVID-19 epidemic and spread to several dozen other countries is the product of natural evolution, according to findings published today in the journal XX.

The analysis of public genome sequence data from SARS-CoV-2 and related viruses found no evidence that the virus was made in a laboratory or otherwise engineered.

"By comparing the available genome sequence data for known coronavirus

Andrew Rambaut 17:16
Can you re-order the author list to put the one who did nothing at the end.

(in the press release I mean, obviously)

Kristian Andersen 17:19
It's currently alphabetical, but I'm happy to toss Ian at the end 😉

Let me edit this some, clean it up and post a new version

Andrew Rambaut 17:20
Holmes comes before Lipkin in the alphabet.

But yes. In these lists, Ian comes last.

Kristian Andersen 17:22
Gee man, it's necessary to teach me the alphabet now?! It's all downhill from here. [I guess I can't blame this on the fact that I'm Danish?]

Kristian Andersen 17:56
@Andrew Rambaut - do you have a tree/alignment with only local cases? I'm trying to get a sense of # clusters in different countries and it's really hard because all the sequences are mixed between local and travel. E.g., does South Korea have a bunch of different chains? Or are many of those travel-related?

2 replies Last reply 3 years ago

Kristian Andersen 18:07
Alrighty, here's a clean version. Please let me know if you have any edits - quotes would be great too (I attributed one in the end to Andrew).

Word Document ▾

March 5th, 2020 ⌄

Andersen Coronavirus Nature 2020 Press Release...
Word Document

Andersen Coronavirus Nature Medicine Press Release Draft 2-24-20

The COVID-19 coronavirus epidemic has a natural origin, scientists say

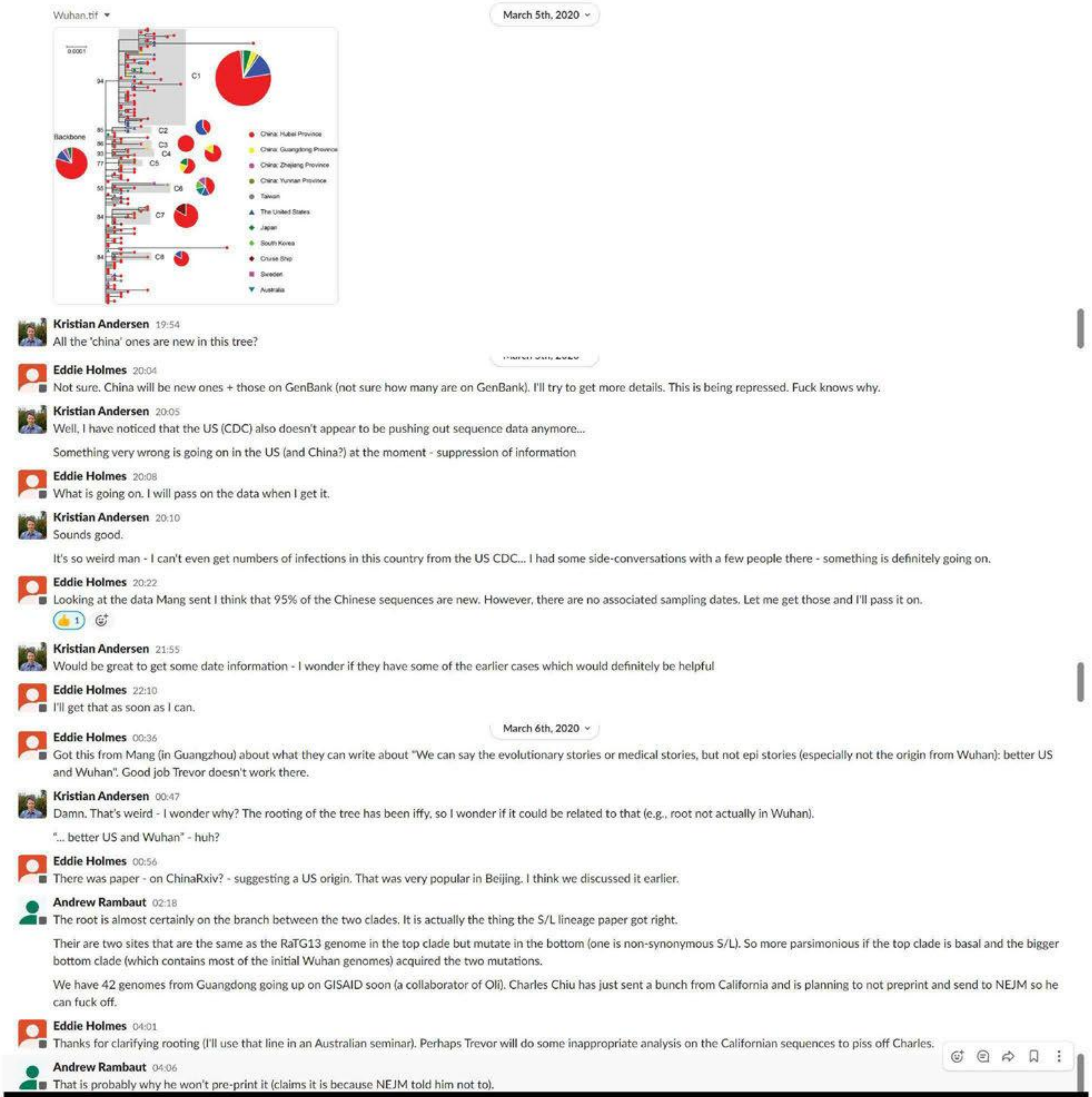
The novel SARS-CoV-2 coronavirus that emerged in the city of Wuhan, China, last year and has since caused a large scale COVID-19 epidemic and spread to more than 70 other countries is the product of natural evolution, according to findings published today in the journal *Nature Medicine*.

The analysis of public genome sequence data from SARS-CoV-2 and related viruses found no evidence that the virus was made in a laboratory or otherwise engineered.

"By comparing the available genome sequence data for known coronavirus

[redacted]

Eddie Holmes 19:33
I have 124 new sequences from Wuhan (I need to get the sampling date info) and Mang sent me the attached tree. I don't know which are the new sequences and it only contains the GenBank sequences (none from GISAID). BUT is says that they are not allowed to publish the paper due to govt. restrictions.



March 5th, 2020

Kristian Andersen 19:54
All the 'china' ones are new in this tree?

Eddie Holmes 20:04
Not sure. China will be new ones + those on GenBank (not sure how many are on GenBank). I'll try to get more details. This is being repressed. Fuck knows why.

Kristian Andersen 20:05
Well, I have noticed that the US (CDC) also doesn't appear to be pushing out sequence data anymore...

Something very wrong is going on in the US (and China?) at the moment - suppression of information

Eddie Holmes 20:08
What is going on. I will pass on the data when I get it.

Kristian Andersen 20:10
Sounds good.

It's so weird man - I can't even get numbers of infections in this country from the US CDC... I had some side-conversations with a few people there - something is definitely going on.

Eddie Holmes 20:22
Looking at the data Mang sent I think that 95% of the Chinese sequences are new. However, there are no associated sampling dates. Let me get those and I'll pass it on.

👍 1

Kristian Andersen 21:55
Would be great to get some date information - I wonder if they have some of the earlier cases which would definitely be helpful

Eddie Holmes 22:10
I'll get that as soon as I can.

March 6th, 2020

Eddie Holmes 00:36
Got this from Mang (in Guangzhou) about what they can write about "We can say the evolutionary stories or medical stories, but not epi stories (especially not the origin from Wuhan): better US and Wuhan". Good job Trevor doesn't work there.

Kristian Andersen 00:47
Damn. That's weird - I wonder why? The rooting of the tree has been iffy, so I wonder if it could be related to that (e.g., root not actually in Wuhan).

"... better US and Wuhan" - huh?

Eddie Holmes 00:56
There was paper - on ChinaRxiv? - suggesting a US origin. That was very popular in Beijing. I think we discussed it earlier.

Andrew Rambaut 02:18
The root is almost certainly on the branch between the two clades. It is actually the thing the S/L lineage paper got right.

Their are two sites that are the same as the RaTG13 genome in the top clade but mutate in the bottom (one is non-synonymous S/L). So more parsimonious if the top clade is basal and the bigger bottom clade (which contains most of the initial Wuhan genomes) acquired the two mutations.

We have 42 genomes from Guangdong going up on GISAID soon (a collaborator of Oli). Charles Chiu has just sent a bunch from California and is planning to not preprint and send to NEJM so he can fuck off.

Eddie Holmes 04:01
Thanks for clarifying rooting (I'll use that line in an Australian seminar). Perhaps Trevor will do some inappropriate analysis on the Californian sequences to piss off Charles.

Andrew Rambaut 04:06
That is probably why he won't pre-print it (claims it is because NEJM told him not to).

[redacted]

Robert Garry 10:17
www.foreignaffairs.com/articles/united-states/2020-03-05/us-chinese-distrust-inviting-dangerous-coronavirus-conspiracy

Our NatMed piece still relevant!

https://www.vox.com/2020/3/4/21156607/how-did-the-coronavirus-get-started-china-wuhan-lab

Vox
The conspiracy theories about the origins of the coronavirus, debunked
There's a rumor the coronavirus started in a Chinese lab. And a scientific consensus it didn't.
Mar 4th, 2020 (87 kB)

Mentions Vince...

Mentions Vince...

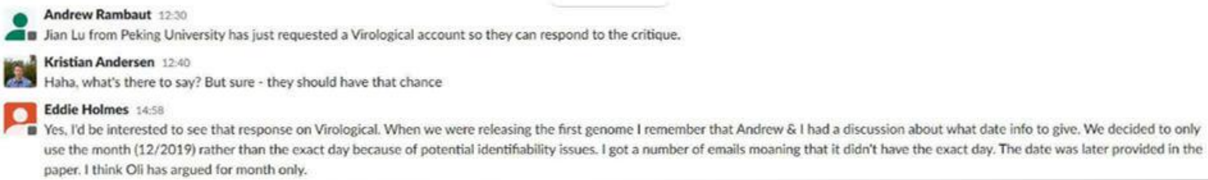
March 6th, 2020

Robert Garry 10:36

[redacted]

Consider the possibility of writing a letter to NYTimes or WashPost re Origins - could even mention responsible epi

[redacted]

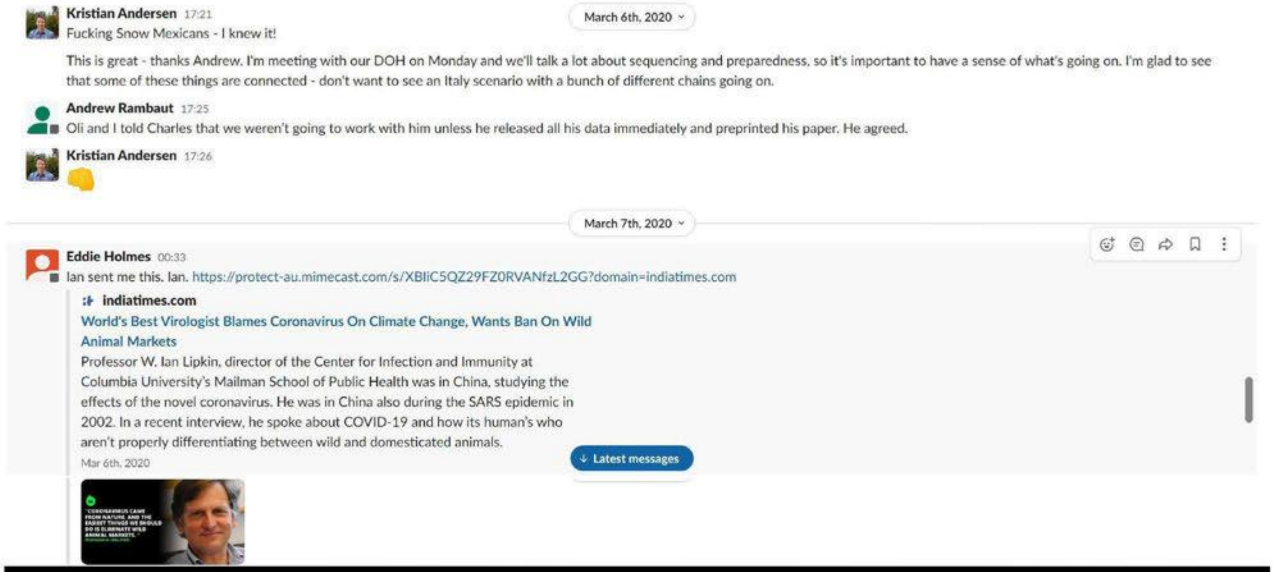


Andrew Rambaut 12:30
Jian Lu from Peking University has just requested a Virological account so they can respond to the critique.

Kristian Andersen 12:40
Haha, what's there to say? But sure - they should have that chance

Eddie Holmes 14:58
Yes, I'd be interested to see that response on Virological. When we were releasing the first genome I remember that Andrew & I had a discussion about what date info to give. We decided to only use the month (12/2019) rather than the exact day because of potential identifiability issues. I got a number of emails moaning that it didn't have the exact day. The date was later provided in the paper. I think Oli has argued for month only.

[redacted]



Kristian Andersen 17:21
Fucking Snow Mexicans - I knew it!

This is great - thanks Andrew. I'm meeting with our DOH on Monday and we'll talk a lot about sequencing and preparedness, so it's important to have a sense of what's going on. I'm glad to see that some of these things are connected - don't want to see an Italy scenario with a bunch of different chains going on.

Andrew Rambaut 17:25
Oli and I told Charles that we weren't going to work with him unless he released all his data immediately and preprinted his paper. He agreed.

Kristian Andersen 17:26
👊

Eddie Holmes 00:33
Ian sent me this. Ian. https://protect-au.mimecast.com/s/XBIiC5QZ29FZ0RVANfzL2GG?domain=indiatimes.com

indiatimes.com
World's Best Virologist Blames Coronavirus On Climate Change, Wants Ban On Wild Animal Markets
Professor W. Ian Lipkin, director of the Center for Infection and Immunity at Columbia University's Mailman School of Public Health was in China, studying the effects of the novel coronavirus. He was in China also during the SARS epidemic in 2002. In a recent interview, he spoke about COVID-19 and how its human's who aren't properly differentiating between wild and domesticated animals.
Mar 6th, 2020

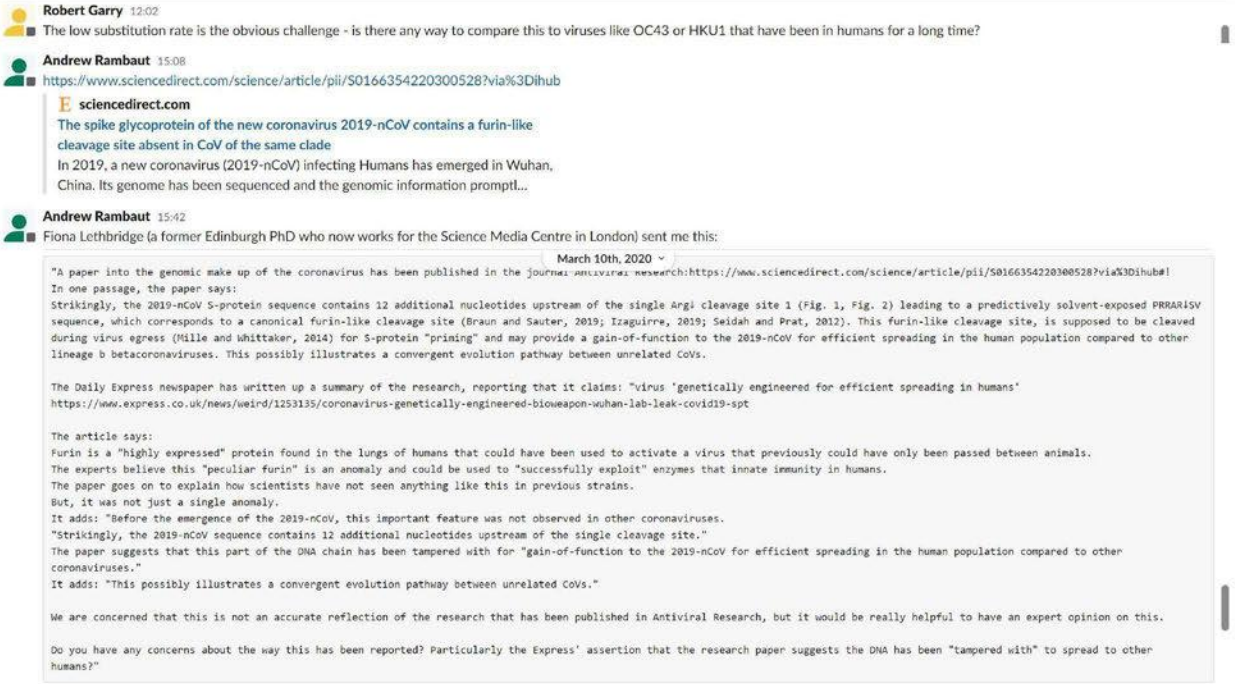
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



Robert Garry 12:02
The low substitution rate is the obvious challenge - is there any way to compare this to viruses like OC43 or HKU1 that have been in humans for a long time?

Andrew Rambaut 15:08
https://www.sciencedirect.com/science/article/pii/S0166354220300528?via%3Dihub

> **sciencedirect.com**
> **The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade**
> In 2019, a new coronavirus (2019-nCoV) infecting Humans has emerged in Wuhan, China. Its genome has been sequenced and the genomic information promptl...

Andrew Rambaut 15:42
Fiona Lethbridge (a former Edinburgh PhD who now works for the Science Media Centre in London) sent me this:

```
"A paper into the genomic make up of the coronavirus has been published in the journal Antiviral Research:https://www.sciencedirect.com/science/article/pii/S0166354220300528?via%3Dihub#!
In one passage, the paper says:
Strikingly, the 2019-nCoV S-protein sequence contains 12 additional nucleotides upstream of the single Arg↓ cleavage site 1 (Fig. 1, Fig. 2) leading to a predictively solvent-exposed PRRAR↓SV
sequence, which corresponds to a canonical furin-like cleavage site (Braun and Sauter, 2019; Izaguirre, 2019; Seidah and Prat, 2012). This furin-like cleavage site, is supposed to be cleaved
during virus egress (Mille and Whittaker, 2014) for S-protein "priming" and may provide a gain-of-function to the 2019-nCoV for efficient spreading in the human population compared to other
lineage b betacoronaviruses. This possibly illustrates a convergent evolution pathway between unrelated CoVs.

The Daily Express newspaper has written up a summary of the research, reporting that it claims: "virus 'genetically engineered for efficient spreading in humans'
https://www.express.co.uk/news/weird/1253135/coronavirus-genetically-engineered-bioweapon-wuhan-lab-leak-covid19-spt

The article says:
Furin is a "highly expressed" protein found in the lungs of humans that could have been used to activate a virus that previously could have only been passed between animals.
The experts believe this "peculiar furin" is an anomaly and could be used to "successfully exploit" enzymes that innate immunity in humans.
The paper goes on to explain how scientists have not seen anything like this in previous strains.
But, it was not just a single anomaly.
It adds: "Before the emergence of the 2019-nCoV, this important feature was not observed in other coronaviruses.
"Strikingly, the 2019-nCoV sequence contains 12 additional nucleotides upstream of the single cleavage site."
The paper suggests that this part of the DNA chain has been tampered with for "gain-of-function to the 2019-nCoV for efficient spreading in the human population compared to other
coronaviruses."
It adds: "This possibly illustrates a convergent evolution pathway between unrelated CoVs."

We are concerned that this is not an accurate reflection of the research that has been published in Antiviral Research, but it would be really helpful to have an expert opinion on this.

Do you have any concerns about the way this has been reported? Particularly the Express' assertion that the research paper suggests the DNA has been "tampered with" to spread to other
humans?"
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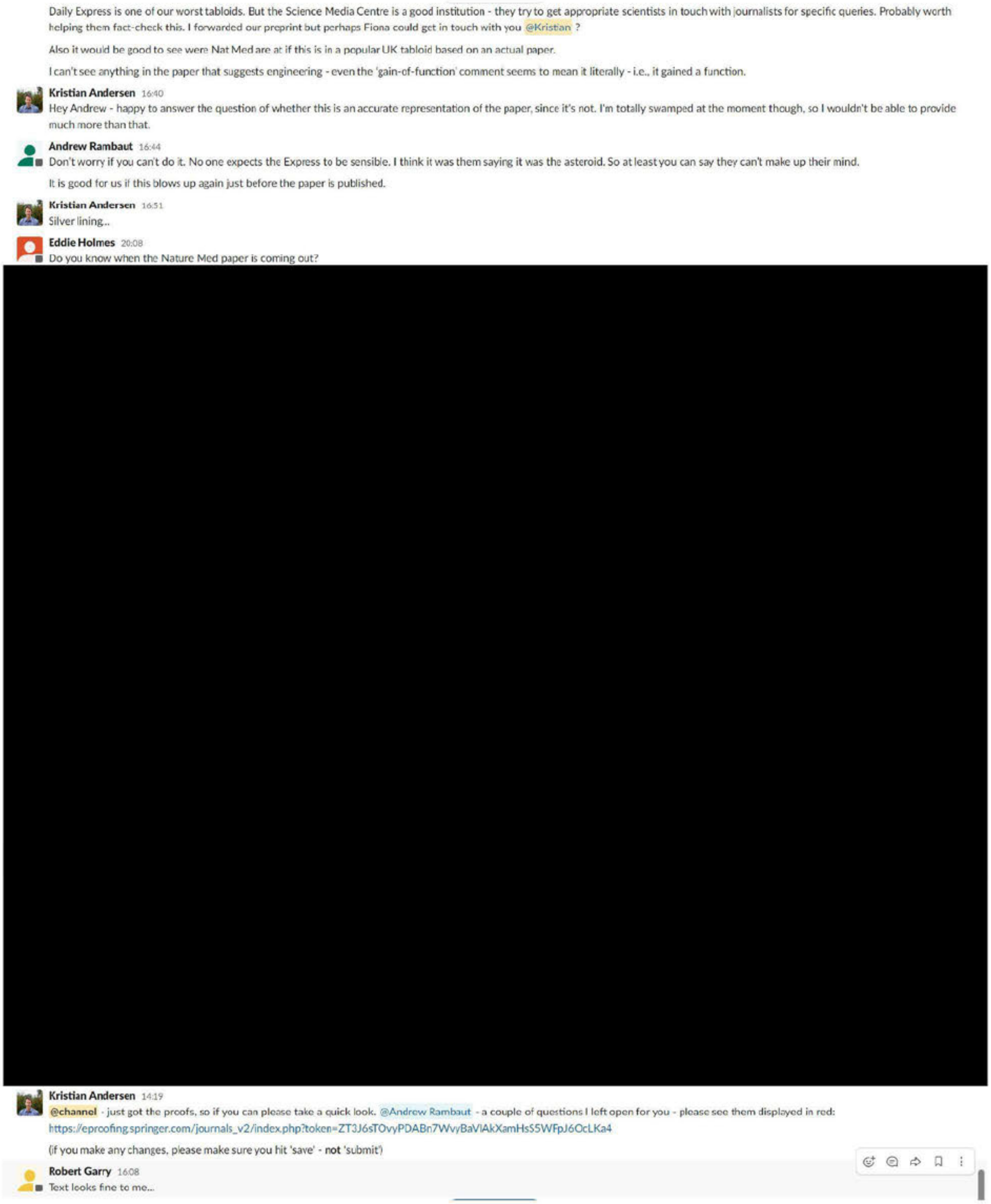


Daily Express is one of our worst tabloids. But the Science Media Centre is a good institution - they try to get appropriate scientists in touch with journalists for specific queries. Probably worth helping them fact-check this. I forwarded our preprint but perhaps Fiona could get in touch with you @Kristian ?

Also it would be good to see were Nat Med are at if this is in a popular UK tabloid based on an actual paper.

I can't see anything in the paper that suggests engineering - even the 'gain-of-function' comment seems to mean it literally - i.e., it gained a function.

Kristian Andersen 16:40
Hey Andrew - happy to answer the question of whether this is an accurate representation of the paper, since it's not. I'm totally swamped at the moment though, so I wouldn't be able to provide much more than that.

Andrew Rambaut 16:44
Don't worry if you can't do it. No one expects the Express to be sensible. I think it was them saying it was the asteroid. So at least you can say they can't make up their mind.

It is good for us if this blows up again just before the paper is published.

Kristian Andersen 16:51
Silver lining...

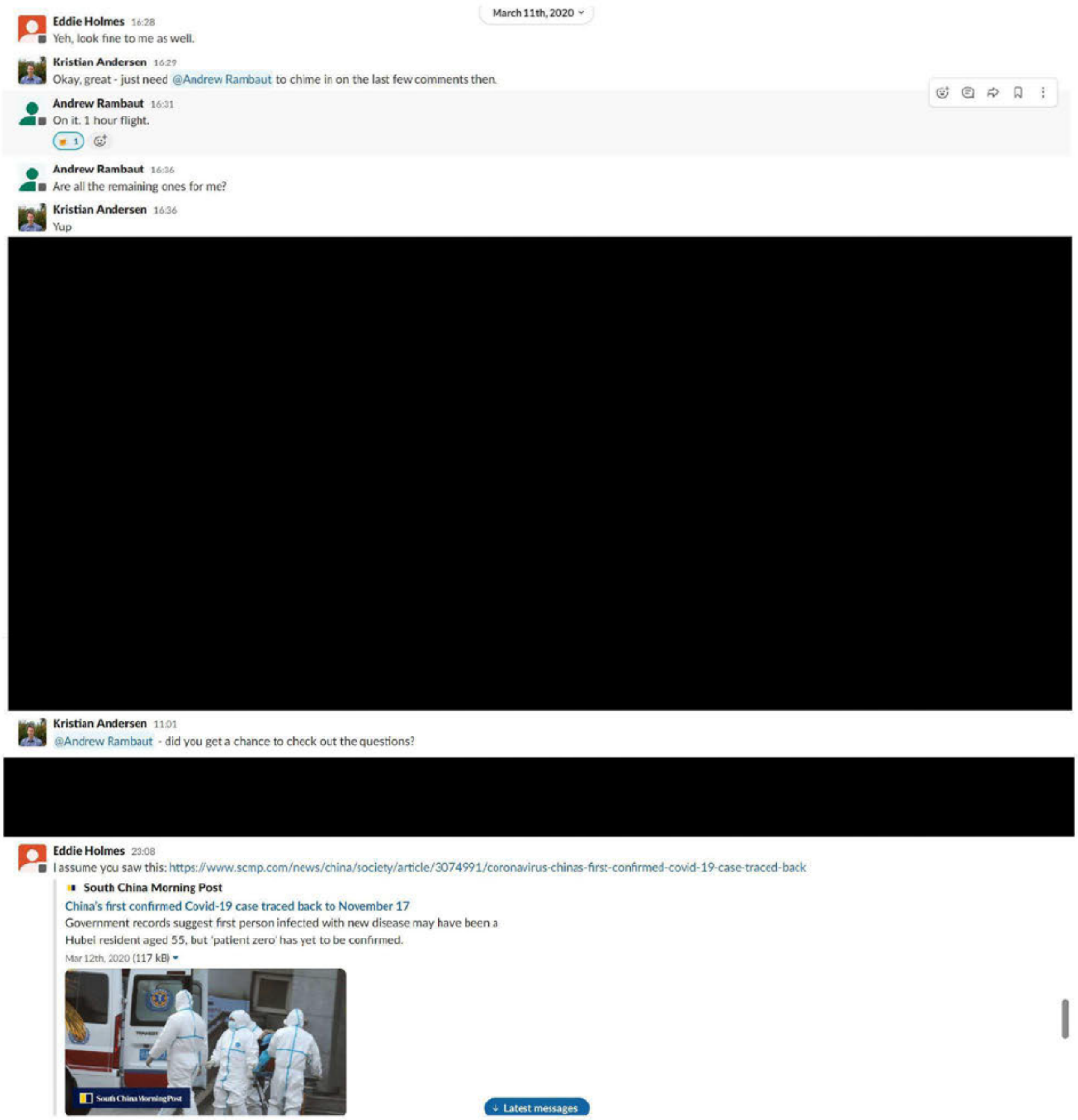
Eddie Holmes 20:08
Do you know when the Nature Med paper is coming out?

[redacted]

Kristian Andersen 14:19
@channel - just got the proofs, so if you can please take a quick look. @Andrew Rambaut - a couple of questions I left open for you - please see them displayed in red:
https://eproofing.springer.com/journals_v2/index.php?token=ZT3J6sTOvyPDABn7WvyBaVlAkXamHsS5WFpJ6OcLKa4

(if you make any changes, please make sure you hit 'save' - **not** 'submit')

Robert Garry 16:08
Text looks fine to me...



Eddie Holmes 16:28
Yeh, look fine to me as well.

Kristian Andersen 16:29
Okay, great - just need @Andrew Rambaut to chime in on the last few comments then.

Andrew Rambaut 16:31
On it. 1 hour flight.

Andrew Rambaut 16:36
Are all the remaining ones for me?

Kristian Andersen 16:36
Yup

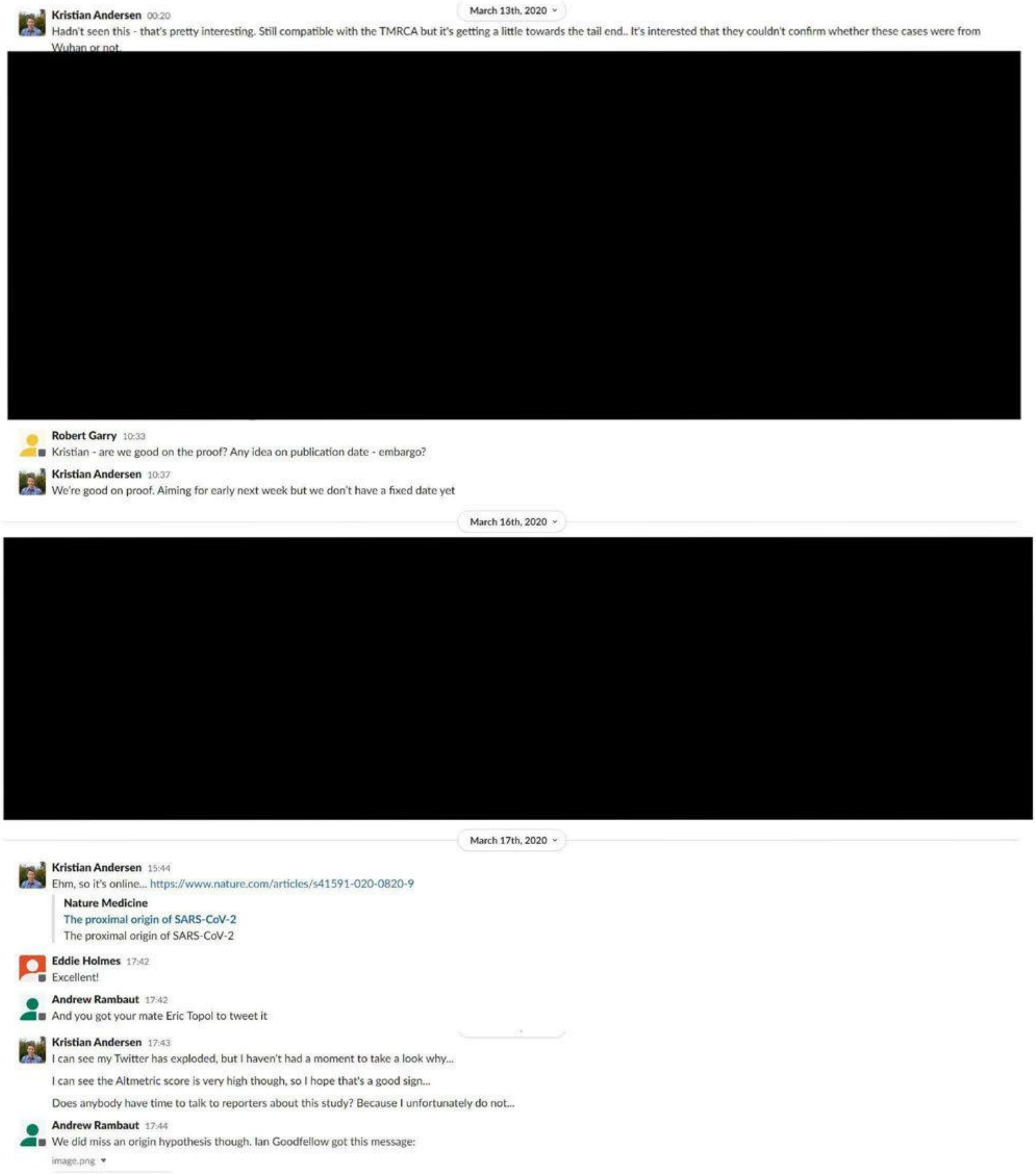
[redacted]

Kristian Andersen 11:01
@Andrew Rambaut - did you get a chance to check out the questions?

[redacted]

Eddie Holmes 23:08
I assume you saw this: https://www.scmp.com/news/china/society/article/3074991/coronavirus-chinas-first-confirmed-covid-19-case-traced-back

South China Morning Post
China's first confirmed Covid-19 case traced back to November 17
Government records suggest first person infected with new disease may have been a Hubei resident aged 55, but 'patient zero' has yet to be confirmed.
Mar 12th, 2020 (117 kB)



March 13th, 2020

Kristian Andersen 00:20
Hadn't seen this - that's pretty interesting. Still compatible with the TMRCA but it's getting a little towards the tail end.. It's interested that they couldn't confirm whether these cases were from Wuhan or not.

[redacted]

Robert Garry 10:33
Kristian - are we good on the proof? Any idea on publication date - embargo?

Kristian Andersen 10:37
We're good on proof. Aiming for early next week but we don't have a fixed date yet

March 16th, 2020

[redacted]

March 17th, 2020

Kristian Andersen 15:44
Ehm, so it's online... https://www.nature.com/articles/s41591-020-0820-9

> **Nature Medicine**
> The proximal origin of SARS-CoV-2
> The proximal origin of SARS-CoV-2

Eddie Holmes 17:42
Excellent!

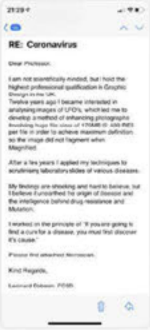
Andrew Rambaut 17:42
And you got your mate Eric Topol to tweet it

Kristian Andersen 17:43
I can see my Twitter has exploded, but I haven't had a moment to take a look why...

I can see the Altmetric score is very high though, so I hope that's a good sign...

Does anybody have time to talk to reporters about this study? Because I unfortunately do not...

Andrew Rambaut 17:44
We did miss an origin hypothesis though. Ian Goodfellow got this message:



21:29

RE: Coronavirus

Dear Professor,

I am not scientifically minded, but I hold the highest professional qualification in Graphic Design in the UK.
Twelve years ago I became interested in analysing images of UFO's, which led me to develop a method of enhancing photographs [illegible] per file in order to achieve maximum definition so the image did not fragment when Magnified.

After a few years I applied my techniques to scrutinising laboratory slides of various diseases.

My findings are shocking and hard to believe, but I believe I unearthed the origin of disease and the intelligence behind drug resistance and Mutation.

I worked on the principle of "If you are going to find a cure for a disease, you must first discover it's cause."

Please find attached Microscan.

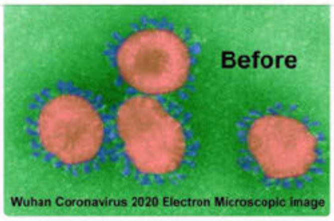
Kind Regards,

[illegible]

Here is the evidence...

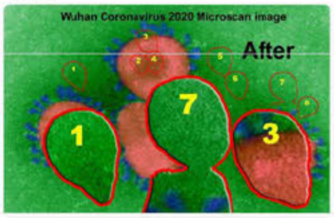
The before picture...

March 17th, 2020



And the definitive analysis...

image.png



Gif Keyboard APP 17:48
@Kristian: /gifs laughing (86 kB)

Kristian Andersen 17:48
Seriously?

Eddie Holmes 17:52
Amazing. I've got model fatigue. My son has started trial remote learning today so that's my day gone.

Kristian Andersen 17:53
I sometime wish I had the kind of imagination that led to this 'identification' - or that I had a conspiratorial mind. Would make life SO much more exciting!

Eddie Holmes 17:57
I had an image of Roy Anderson sitting in his garden at home waiting for the phone call to come and save us, a bit like Barnes Wallis in The Dambusters

March 18th, 2020

Kristian Andersen 02:42
Okay,

Screen Shot 2020-03-17 at 11.41.44 PM.png

This article is in the 99th percentile (ranked 11th) of the 146,188 tracked articles of a similar age in all journals and the 98th percentile (ranked 1st) of the 73 tracked articles of a similar age in *Nature Medicine*

View more on Altmetric

Andrew Rambaut 03:03
See that Nature!

Andrew Rambaut 03:09
Mainly the Spanish (bored and on lock-down):

image.png

Geographical breakdown

Country	Count	As %
Spain	1784	12%
United States	1062	7%
Brazil	559	4%
Mexico	425	3%
United Kingdom	306	2%
Chile	283	2%
Venezuela, Bolivarian Republic of	202	1%
Egypt	197	1%
Turkey	195	1%
Other	2551	17%
Unknown	7618	50%

Eddie Holmes 05:46
Is it banned in China? Glad to see Venezuela, Bolivarian Republic of in the mix.

Eddie Holmes 06:05
https://www.leonarddobsonart.co.uk/

> **leonarddobsonart**
> Commissioned Artwork | Leonard Dobson Art | Fleet
> Leonarddobsonart.co.uk offers Art and commisioned art. Covering Northern art, beach scences, local scences, retro romanticism, abstract, landscapes, portrait, city skylines and illustrations.

Andrew Rambaut 06:36
I can see aliens in that picture.

Eddie Holmes 16:19
Priceless: https://twitter.com/CARRENEAN

> **twitter.com**
> LEONARD DOBSON (@CARRENEAN) | Twitter
> The latest Tweets from LEONARD DOBSON (@CARRENEAN): "There's more to air crash investigation than concluding 'Pilot Error' or 'Mechanical Failure'.........
> https://t.co/XuHjiL5pZU"

Kristian Andersen 16:41
I don't know man - he might be on to something. https://twitter.com/CARRENEAN/status/1078041436975755264?s=20

March 19th, 2020

Kristian Andersen 00:05
This is nuts - we officially past the highest scoring paper of last year... Given the number of completely nutso emails I have received today, I'm not quite sure we managed to convince all the conspiracy theorists out there...

Screen Shot 2020-03-18 at 9.04.11 PM.png

Eddie Holmes 01:24
Wow!

Today, I saw a middle-aged woman arrested at Woollies (a supermarket) where I live - and taken away in handcuffs - for trying to hoard food. I quickly put back the 2nd pack of hot cross buns I had.

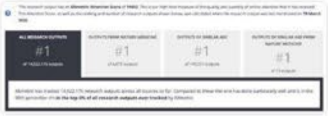
1

Latest messages

Eddie Holmes 01:34
Nature Nature missed a trick with that paper...I hope they are watching this...

Kristian Andersen 02:33
No kidding. This is by far the highest scoring Nature Medicine paper ever - I suspect higher than any other Nature paper as well. I hope that one reviewer is proud of his hard work.

Andrew Rambaut 03:13
image.png ▾



Kristian Andersen 03:19
Wait, it's the highest?

Andrew Rambaut 03:24
That is what this is saying no?

Kristian Andersen 03:24
I believe so, yes.

Andrew Rambaut 03:26
Perhaps this month or this year so far.

Kristian Andersen 03:26
The highest Altmetric score ever. Fuck me, surely that's gotta be some sort of academic achievement. It's like winning a prize for having the biggest pumpkin at the county fare.

Andrew Rambaut 03:26
What was the snake flu paper?

Kristian Andersen 03:28
I thought that was higher... But maybe they refuse to track it 😉

Hmmm, much lower: https://wiley.altmetric.com/details/74354946

wiley.altmetric.com
Report for: Cross-species transmission of the newly identified coronavirus 2019-nCoV
In the top 5% of all research outputs scored by Altmetric

Andrew Rambaut 03:29
https://www.altmetric.com/top100/2019/

Altmetric
The Altmetric Top 100 – 2019
What research caught the public imagination in 2019? Check out our annual list of papers with the most attention. (33 kB) ▾

↓ Latest messages

Top last year was 13557

Kristian Andersen 03:30
Yeah, we're well above that

Andrew Rambaut 03:30
In a few days.

Kristian Andersen 03:31
Ehm, well above already.. https://www.altmetric.com/details/77676422#score

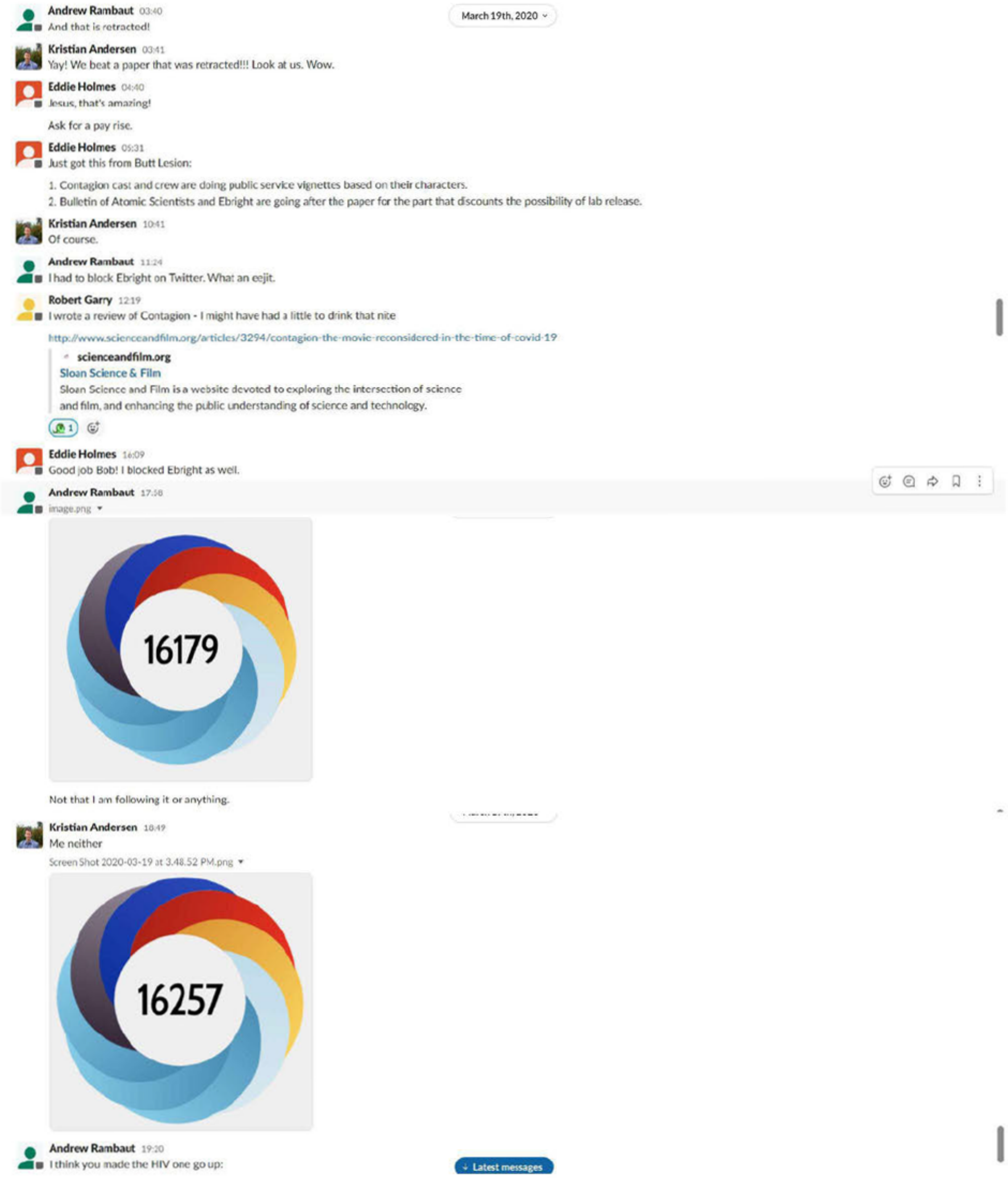
altmetric.com
Report for: The proximal origin of SARS-CoV-2
In the top 5% of all research outputs scored by Altmetric

Andrew Rambaut 03:32
And previous years are all much lower. So yes! Top! Fuck me.

Kristian Andersen 03:32
WE RUUUUUUULE. That's tenure secured, right there.

Kristian Andersen 03:38
Importantly. https://biorxiv.altmetric.com/details/74957328

biorxiv.altmetric.com
Report for: Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag
In the top 5% of all research outputs scored by Altmetric



Andrew Rambaut 03:40
And that is retracted!

Kristian Andersen 03:41
Yay! We beat a paper that was retracted!!! Look at us. Wow.

Eddie Holmes 04:40
Jesus, that's amazing!

Ask for a pay rise.

Eddie Holmes 05:31
Just got this from Butt Lesion:

1. Contagion cast and crew are doing public service vignettes based on their characters.
2. Bulletin of Atomic Scientists and Ebright are going after the paper for the part that discounts the possibility of lab release.

Kristian Andersen 10:41
Of course.

Andrew Rambaut 11:24
I had to block Ebright on Twitter. What an eejit.

Robert Garry 12:19
I wrote a review of Contagion - I might have had a little to drink that nite

http://www.scienceandfilm.org/articles/3294/contagion-the-movie-reconsidered-in-the-time-of-covid-19

> **scienceandfilm.org**
> Sloan Science & Film
> Sloan Science and Film is a website devoted to exploring the intersection of science and film, and enhancing the public understanding of science and technology.

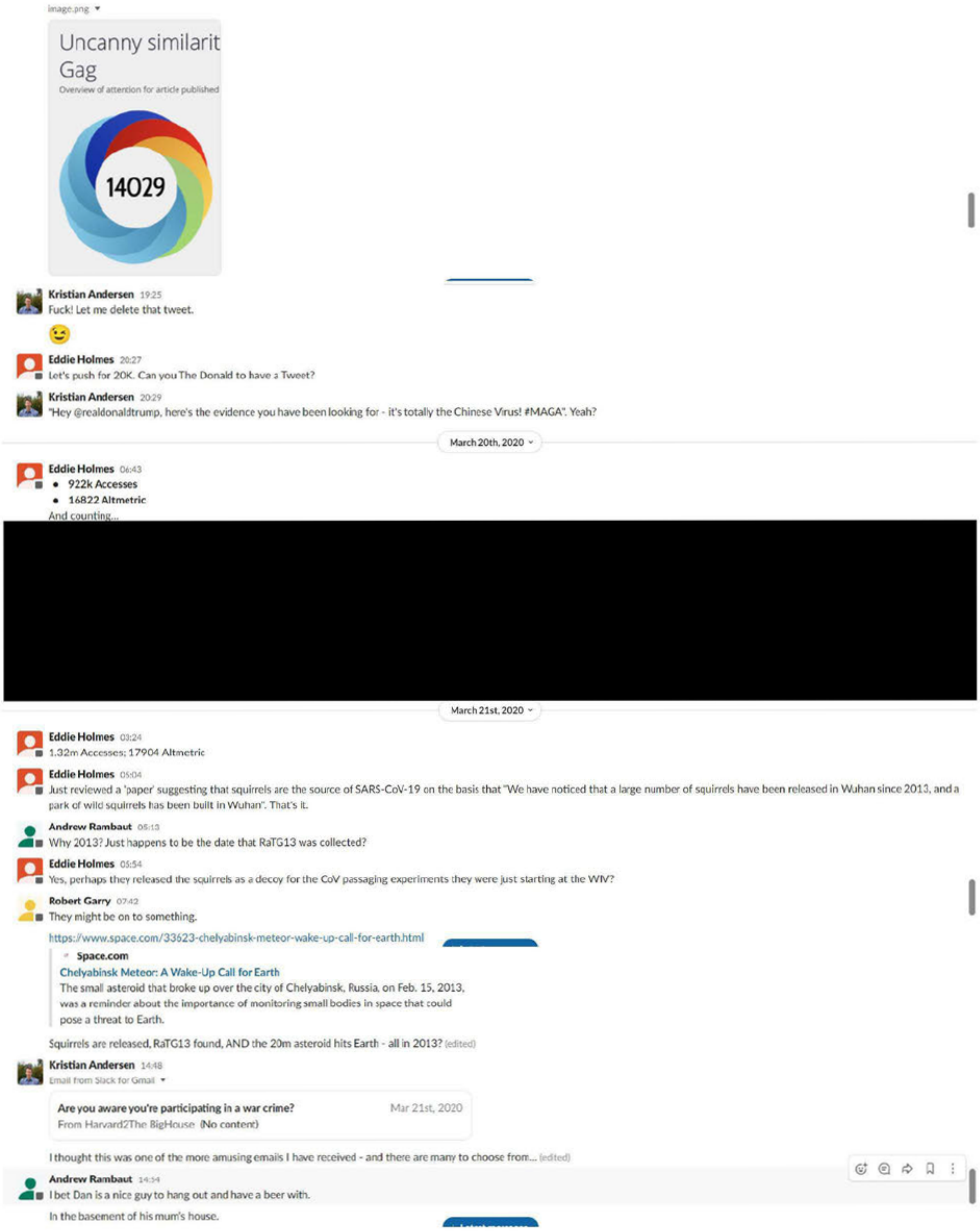
Eddie Holmes 16:09
Good job Bob! I blocked Ebright as well.

Andrew Rambaut 17:58
image.png

Not that I am following it or anything.

Kristian Andersen 18:49
Me neither
Screen Shot 2020-03-19 at 3.48.52 PM.png

Andrew Rambaut 19:20
I think you made the HIV one go up:



Kristian Andersen 19:25
Fuck! Let me delete that tweet.

😉

Eddie Holmes 20:27
Let's push for 20K. Can you The Donald to have a Tweet?

Kristian Andersen 20:29
"Hey @realdonaldtrump, here's the evidence you have been looking for - it's totally the Chinese Virus! #MAGA". Yeah?

March 20th, 2020

Eddie Holmes 06:43

- 922k Accesses
- 16822 Altmetric

And counting...

[redacted]

March 21st, 2020

Eddie Holmes 03:24
1.32m Accesses; 17904 Altmetric

Eddie Holmes 05:04
Just reviewed a 'paper' suggesting that squirrels are the source of SARS-CoV-19 on the basis that "We have noticed that a large number of squirrels have been released in Wuhan since 2013, and a park of wild squirrels has been built in Wuhan". That's it.

Andrew Rambaut 05:13
Why 2013? Just happens to be the date that RaTG13 was collected?

Eddie Holmes 05:54
Yes, perhaps they released the squirrels as a decoy for the CoV passaging experiments they were just starting at the WIV?

Robert Garry 07:42
They might be on to something.

https://www.space.com/33623-chelyabinsk-meteor-wake-up-call-for-earth.html

> **Space.com**
> Chelyabinsk Meteor: A Wake-Up Call for Earth
> The small asteroid that broke up over the city of Chelyabinsk, Russia, on Feb. 15, 2013, was a reminder about the importance of monitoring small bodies in space that could pose a threat to Earth.

Squirrels are released, RaTG13 found, AND the 20m asteroid hits Earth - all in 2013? (edited)

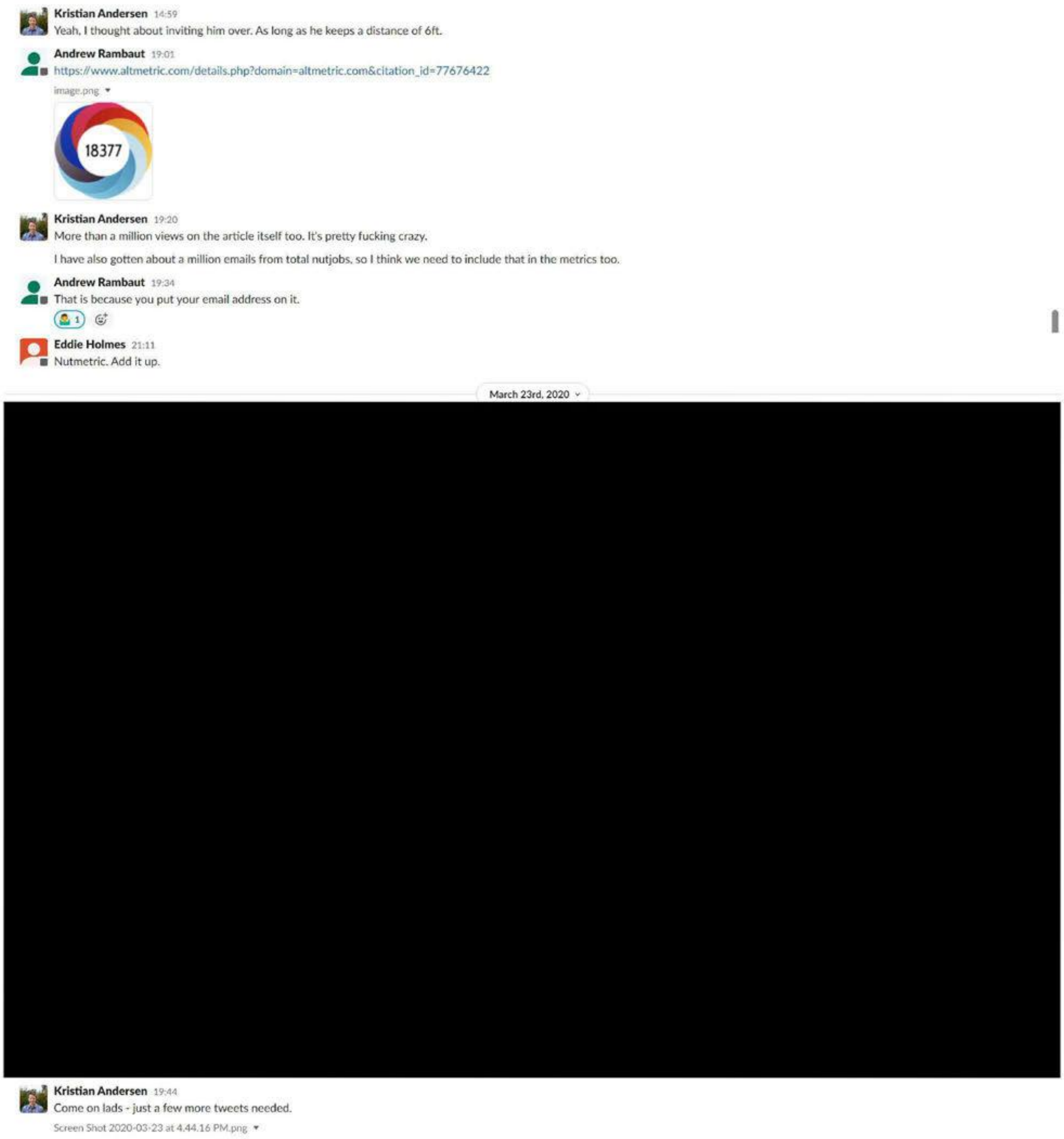
Kristian Andersen 14:48
Email from Slack for Gmail

> **Are you aware you're participating in a war crime?** Mar 21st, 2020
> From Harvard2The BigHouse (No content)

I thought this was one of the more amusing emails I have received - and there are many to choose from... (edited)

Andrew Rambaut 14:54
I bet Dan is a nice guy to hang out and have a beer with.

In the basement of his mum's house.



Kristian Andersen 14:59
Yeah, I thought about inviting him over. As long as he keeps a distance of 6ft.

Andrew Rambaut 19:01
https://www.altmetric.com/details.php?domain=altmetric.com&citation_id=77676422
image.png ▾

18377

Kristian Andersen 19:20
More than a million views on the article itself too. It's pretty fucking crazy.

I have also gotten about a million emails from total nutjobs, so I think we need to include that in the metrics too.

Andrew Rambaut 19:34
That is because you put your email address on it.

Eddie Holmes 21:11
Nutmetric. Add it up.

March 23rd, 2020

[redacted]

Kristian Andersen 19:44
Come on lads - just a few more tweets needed.
Screen Shot 2020-03-23 at 4.44.16 PM.png ▾

Andrew Rambaut 20:08

relax. will get there soon. 25000 is a nicer number though, I think.

Still weird that it is Spain (and some Spanish speaking countries) that is doing most of the tweeting about this.

image.png ▾

Country	Count	As %
Spain	5994	10%
United States	2948	5%
Brazil	2327	4%
Chile	1759	3%
Venezuela, Bolivarian Republic of	1253	2%
Mexico	1245	2%
Colombia	1137	2%
France	933	2%
United Kingdom	930	2%
Other	10568	18%
Unknown	28429	49%

Kristian Andersen 20:14

Let's aim for 50,000! And yeah - super weird it's Spain - not sure what's up with that. Nothing from China, which is peculiar - but I guess they don't really use Twitter (and maybe can't access the paper either)

[redacted]

Kristian Andersen 13:33

Yeehaw

Screen Shot 2020-03-24 at 10.31.42.png ▾

🍾 1

March 24th, 2020

Eddie Holmes 18:31

🍾

Eddie Holmes 21:59

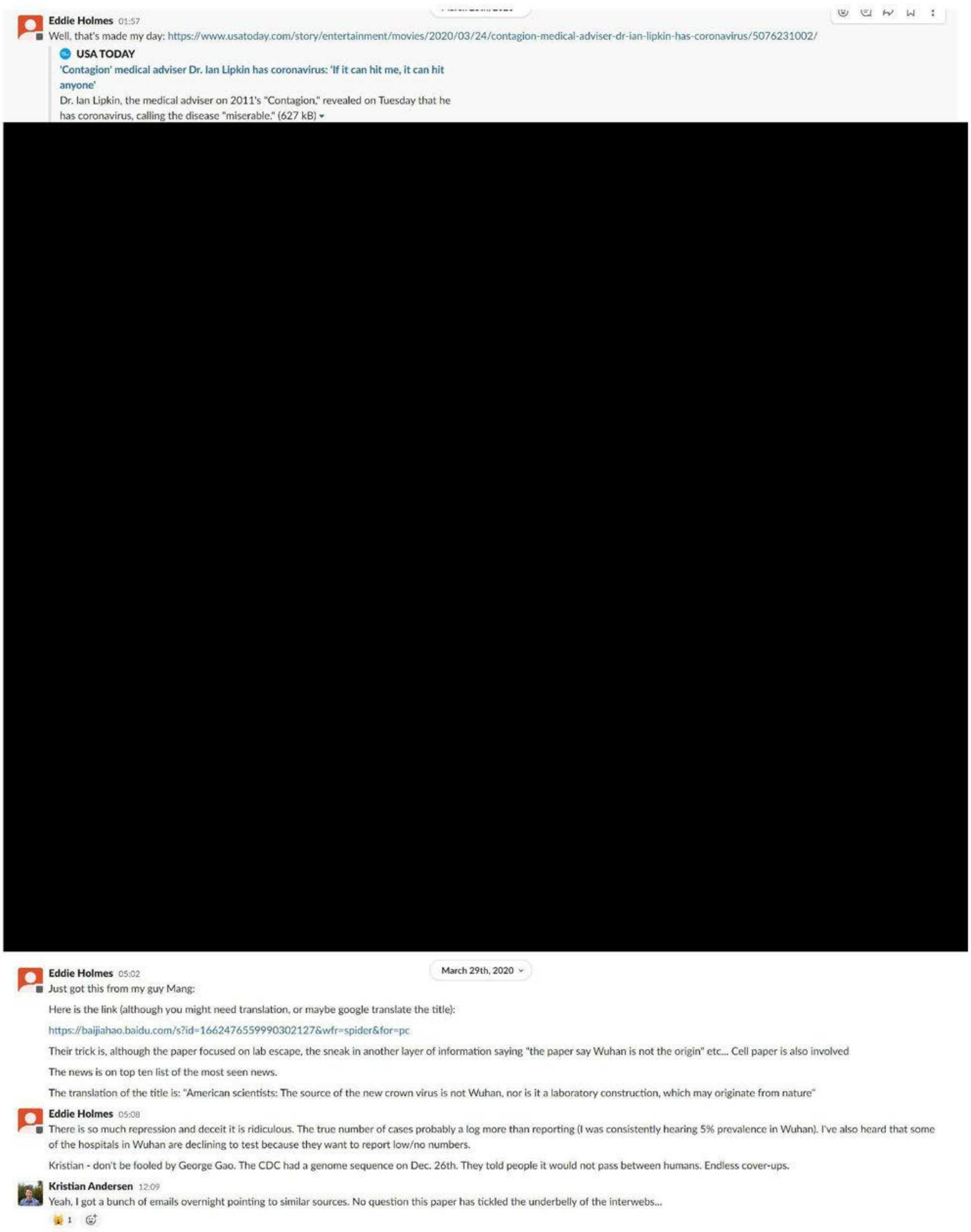
Was that you getting the Bedford approval on Twitter Andrew? You might be honoured.

Kristian Andersen 22:10

It's actually this: https://twitter.com/nickpickles/status/1241156502305427459 / https://docs.google.com/forms/d/e/1FAIpQLScCxMT877v16ya7RnDQ5Lb9pdUDbPBVPdWgDS_ptlgXCwM72g/viewform

Somebody had put me on a list too so it came through via email.

[redacted]



Eddie Holmes 01:57
Well, that's made my day: https://www.usatoday.com/story/entertainment/movies/2020/03/24/contagion-medical-adviser-dr-ian-lipkin-has-coronavirus/5076231002/

USA TODAY
'Contagion' medical adviser Dr. Ian Lipkin has coronavirus: 'If it can hit me, it can hit anyone'
Dr. Ian Lipkin, the medical adviser on 2011's "Contagion," revealed on Tuesday that he has coronavirus, calling the disease "miserable." (627 kB)

[redacted]

March 29th, 2020

Eddie Holmes 05:02
Just got this from my guy Mang:

Here is the link (although you might need translation, or maybe google translate the title):

https://baijiahao.baidu.com/s?id=1662476559990302127&wfr=spider&for=pc

Their trick is, although the paper focused on lab escape, the sneak in another layer of information saying "the paper say Wuhan is not the origin" etc... Cell paper is also involved

The news is on top ten list of the most seen news.

The translation of the title is: "American scientists: The source of the new crown virus is not Wuhan, nor is it a laboratory construction, which may originate from nature"

Eddie Holmes 05:08
There is so much repression and deceit it is ridiculous. The true number of cases probably a log more than reporting (I was consistently hearing 5% prevalence in Wuhan). I've also heard that some of the hospitals in Wuhan are declining to test because they want to report low/no numbers.

Kristian - don't be fooled by George Gao. The CDC had a genome sequence on Dec. 26th. They told people it would not pass between humans. Endless cover-ups.

Kristian Andersen 12:09
Yeah, I got a bunch of emails overnight pointing to similar sources. No question this paper has tickled the underbelly of the interwebs...

March 29th, 2020

Robert Garry 12:36

Oh yeah it's tickled.**From:** Yuchen Liang [redacted]
Date: Saturday, March 28, 2020 at 11:35 PM
To: Robert Garry [redacted]
Subject: Professor, your name is trending on Chinese twitter

External Sender. Be aware of links, attachments and requests.
Dear Professor Garry,

Please excuse me for not including my name here for the purpose of confidentiality. One interview you gave to ABC was quote by China's state television as proof that Covid-19 did not start in Wuhan and it is now trending second in Weibo, China's version of Twitter.
I looked at the original interview, I believe you said originally: "our analyses and others too, point to an earlier origin than that (that the virus originated at a fish market in Wuhan), there were definitely cases there, but that wasn't the origin of the virus."
This was translated and quoted by the Chinese media as saying that there is an earlier origin than Wuhan. Is this what you really meant or did you mean that the virus did not originate from the fish market but still has its likely origin in Wuhan? If it is the second case, your words have been manipulated and used by Chinese state media to push for the theory that the virus has a non-Chinese, likely American origin. In fact, most Chinese netizens, at least those who are not censored, already bought that theory pushed by state media and officials such as Foreign ministry spokeperson Zhao Lijian, who claimed that the virus were brought to China by American soldiers.
I am just writing to let you know what is happening with your interview in China. I understand that one purpose of the research paper you did on Covid-19 was to dispel conspiracy theories. I just don't want your words to be used against your intention. Have a pleasant day.

Best wishes,
(Sorry that I cannot leave my name here, you can just ask anyone who knows Chinese to check Weibo, they can verify what I said.)

"the sneak in another layer of information saying "the paper say Wuhan is not the origin"

Herein lays the issue.

↓ Latest messages

March 29th, 2020

Andrew Rambaut 14:27

Apparently we said it could have been circulating in humans for decades...
https://www.scmp.com/news/china/science/article/3077442/coronavirus-pathogen-could-have-been-spreading-humans-decades

> **South China Morning Post**
> Coronavirus may have been spreading in humans for decades, study says
> Virus may have jumped from animal to humans long before the first detection in Wuhan, according to research by an international team of scientists.
> Mar 29th, 2020 (124 kB)

Kristian Andersen 14:31

Apparently so...

Could have been a million years, really - who knows.

Andrew Rambaut 14:32

Actually the decades bit may have been extrapolated from Collins

> "Then, as a result of gradual evolutionary changes over years or perhaps decades, the virus eventually gained the ability to spread from human to human and cause serious, often life-threatening disease," he said in an article published on the institute's website on Thursday.

Kristian Andersen 14:38

Ahhh, interesting - a fair number of inaccuracies in Collin's description of the paper. When the guy who wrote it contacted me there were so many mistakes I told him to read the fucking paper first... Luckily Bob took care of the most egregious mistakes - I just couldn't find the time.

Robert Garry 14:49

Yeah - just tried to fix the one that were - well 180 degrees off.

Robert Garry 14:58

Could have been a million years, really - who knows.

yeah - kinda what I said

Robert Garry 15:27

doi: https://doi.org/10.1101/2020.03.22.002204

> bR **bioRxiv**
> Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site
> Direct RNA sequencing using an Oxford Nanopore MinION characterised the transcriptome of SARS-CoV-2 grown in Vero E6 cells. This cell line is being widely used to propagate the novel coronavirus. The viral transcriptome was analysed using a recently developed ORF-centric pipeline. This revealed the pattern of viral transcripts, (i.e. subgenomic mRNAs), generally fitted the predicted replication and transcription model for coronaviruses. A 24 nt in-frame deletion was detected in subgenomic mRNAs encoding the spike (S) glycoprotein. This feature was identified in over half of the mapped transcripts and was predicted to remove a proposed furin cleavage site from the S glycoprotein. This motif d... Show more
> Mar 24th, 2020

This kind of thing much more interesting...

Kristian Andersen 15:31
Yeah, that's pretty cool - kinda even further rules out tissue culture passage

Robert Garry 15:35
Climbing toward 3M accesses and 25K on Altmetric

image.png ▾

The proximal origin of SARS-CoV-2

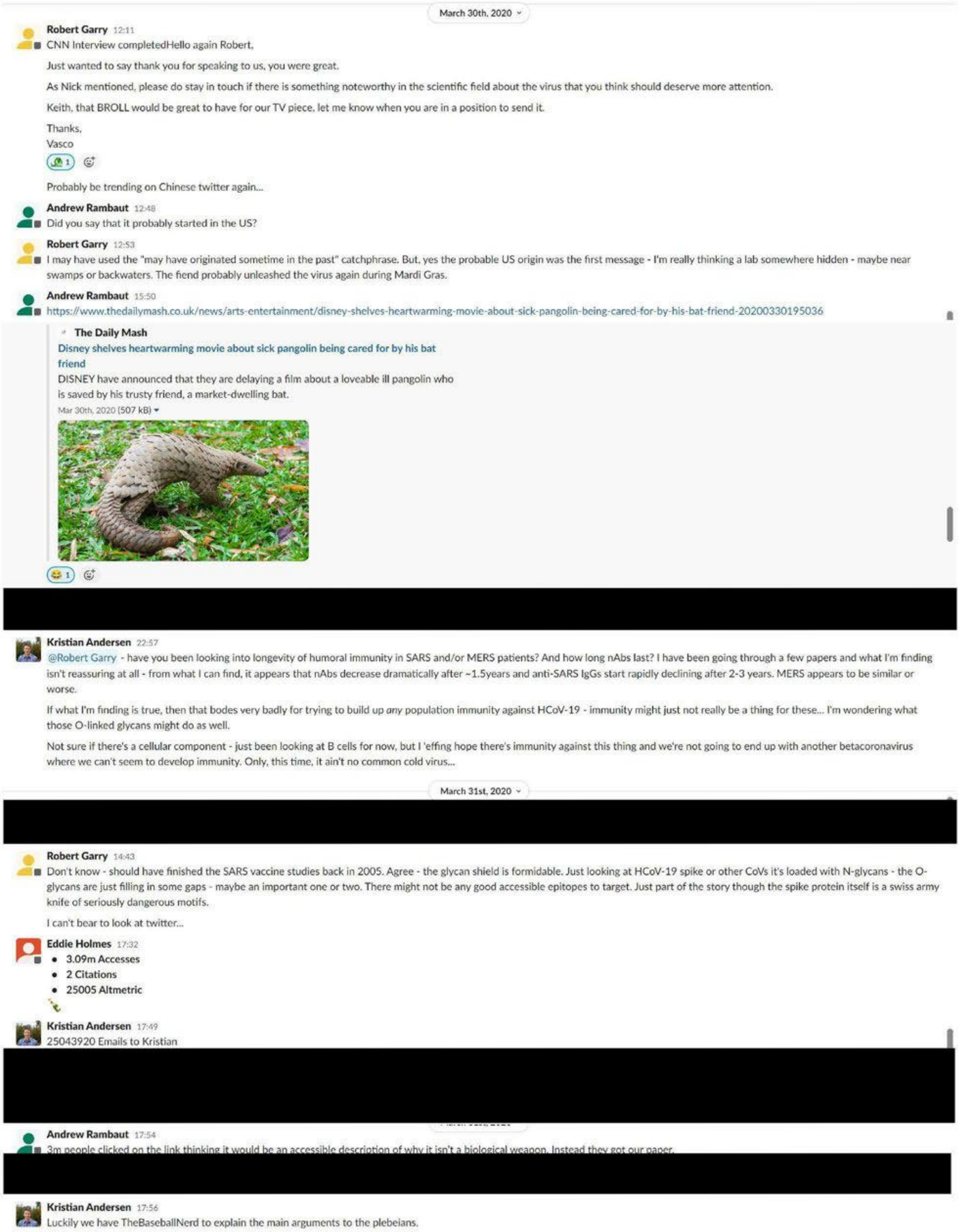
Kristian G. Andersen, Andrew Rambaut, W. Ian Lipkin, Edward C. Holmes & Robert F. Garry

Nature Medicine (2020) | Cite this article

2.90m Accesses | 1 Citations | 24415 Altmetric | Metrics

I think Andrew should go on CNN London since he is closest geographically.

[redacted]



March 30th, 2020

Robert Garry 12:11

CNN Interview completedHello again Robert,

Just wanted to say thank you for speaking to us, you were great.

As Nick mentioned, please do stay in touch if there is something noteworthy in the scientific field about the virus that you think should deserve more attention.

Keith, that BROLL would be great to have for our TV piece, let me know when you are in a position to send it.

Thanks,
Vasco

Probably be trending on Chinese twitter again...

Andrew Rambaut 12:48

Did you say that it probably started in the US?

Robert Garry 12:53

I may have used the "may have originated sometime in the past" catchphrase. But, yes the probable US origin was the first message - I'm really thinking a lab somewhere hidden - maybe near swamps or backwaters. The fiend probably unleashed the virus again during Mardi Gras.

Andrew Rambaut 15:50

https://www.thedailymash.co.uk/news/arts-entertainment/disney-shelves-heartwarming-movie-about-sick-pangolin-being-cared-for-by-his-bat-friend-20200330195036

> **The Daily Mash**
> Disney shelves heartwarming movie about sick pangolin being cared for by his bat friend
> DISNEY have announced that they are delaying a film about a loveable ill pangolin who is saved by his trusty friend, a market-dwelling bat.
> Mar 30th, 2020 (507 kB)

[redacted]

Kristian Andersen 22:57

@Robert Garry - have you been looking into longevity of humoral immunity in SARS and/or MERS patients? And how long nAbs last? I have been going through a few papers and what I'm finding isn't reassuring at all - from what I can find, it appears that nAbs decrease dramatically after ~1.5years and anti-SARS IgGs start rapidly declining after 2-3 years. MERS appears to be similar or worse.

If what I'm finding is true, then that bodes very badly for trying to build up *any* population immunity against HCoV-19 - immunity might just not really be a thing for these... I'm wondering what those O-linked glycans might do as well.

Not sure if there's a cellular component - just been looking at B cells for now, but I 'effing hope there's immunity against this thing and we're not going to end up with another betacoronavirus where we can't seem to develop immunity. Only, this time, it ain't no common cold virus...

March 31st, 2020

[redacted]

Robert Garry 14:43

Don't know - should have finished the SARS vaccine studies back in 2005. Agree - the glycan shield is formidable. Just looking at HCoV-19 spike or other CoVs it's loaded with N-glycans - the O-glycans are just filling in some gaps - maybe an important one or two. There might not be any good accessible epitopes to target. Just part of the story though the spike protein itself is a swiss army knife of seriously dangerous motifs.

I can't bear to look at twitter...

Eddie Holmes 17:32

- 3.09m Accesses
- 2 Citations
- 25005 Altmetric

Kristian Andersen 17:49

25043920 Emails to Kristian

[redacted]

Andrew Rambaut 17:54

3m people clicked on the link thinking it would be an accessible description of why it isn't a biological weapon. Instead they got our paper.

[redacted]

Kristian Andersen 17:56

Luckily we have TheBaseballNerd to explain the main arguments to the plebeians.

[redacted]

Kristian Andersen 20:57

@Andrew Rambaut - where you previously asked about the deletion, is this the study you were referring to? Pretty interesting: http://virological.org/t/identification-of-a-common-deletion-in-the-spike-protein-of-sars-cov-2/451

> **Virological**
>
> Identification of a common deletion in the spike protein of SARS-CoV-2
>
> Identification of a common deletion in the spike protein of SARS-CoV-2 Zhe Liu1,2, Huanying Zheng2, Runyu Yuan1,2, Mingyue Li3, Huifang Lin1,2, Jingju Peng1,2, Qianlin Xiong1,2, Jiufeng Sun1,2, Baisheng Li2, Jie Wu2, Ruben J.G. Hulswit4, Thomas A. Bowden4, Andrew Rambaut5, Nick Loman6, Oliver G Pybus4, Changwen Ke2, Jing Lu1,2 Affiliations: 1 Guangdong Provincial Institution of Public Health, Guangzhou, China; 2 Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, China...
>
> **Reading time**
> 4 mins
>
> **Likes**
> 2 ❤
>
> Mar 31st, 2020

April 1st, 2020

Andrew Rambaut 02:55
It was. Also this ... https://www.biorxiv.org/content/10.1101/2020.03.22.002204v1

bR **bioRxiv**
Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site
Direct RNA sequencing using an Oxford Nanopore MinION characterised the transcriptome of SARS-CoV-2 grown in Vero E6 cells. This cell line is being widely used to propagate the novel coronavirus. The viral transcriptome was analysed using a recently developed ORF-centric pipeline. This revealed the pattern of viral transcripts, (i.e. subgenomic mRNAs), generally fitted the predicted replication and transcription model for coronaviruses. A 24 nt in-frame deletion was detected in subgenomic mRNAs encoding the spike (S) glycoprotein. This feature was identified in over half of the mapped transcripts and was predicted to remove a proposed furin cleavage site from the S glycoprotein. This motif d... Show more
Mar 24th, 2020

Kristian Andersen 10:24
Very interesting. Honestly don't know what to make of it.

Robert Garry 15:15
https://www.snopes.com/news/2020/04/01/covid-19-bioweapon/

Snopes.com
Why You Shouldn't Fall for the COVID-19 'Bioweapon' Conspiracy Theory
The coronavirus responsible for COVID-19 has deadly adaptations that make it perfect for infecting humans. But this is a testament to natural selection, not bioengineering.
(195 kB) ▾

April 1st, 2020

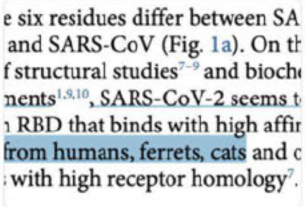
Kristian Andersen 15:50
Thanks Bob for answering his emails - I got several but had to blank them (together with a million others...). Request from BBC coming through too - I'll loop you in if anybody has time

Robert Garry 19:37
Snopes - actually pretty legit....

1 reply 3 years ago

Kristian Andersen 20:58
Our comparative genomics juju is unparalleled. Almost as if we created the virus ourselves...😮

2 files ▾



e six residues differ between SA
and SARS-CoV (Fig. 1a). On th
f structural studies7–9 and bioch
nents1,9,10, SARS-CoV-2 seems t
1 RBD that binds with high affir
from humans, ferrets, cats and c
; with high receptor homology7.

[redacted]

April 2nd, 2020

Kristian Andersen 10:10
I guess we didn't consider this possibility...

Email from Slack for Gmail ▾

Re: The proximal origin of SARS-CoV-2 Apr 2nd, 2020
From Thomas Busse (No content)

Andrew Rambaut 10:12
Is this coming from the whole HIV-denial thing?

Kristian Andersen 10:18
Oh interesting. I'm not much of a HIV denial'ologist, but that would make sense - he does mention retroviruses... Shame on them exosomes!

Andrew Rambaut 10:27
'widespread innumeracy in the Medical Field'

Kristian Andersen 11:00
I have to give him props for that part

Kristian Andersen 13:18
BBC - "Just to be clear, I read the research and other pieces about it" - proceeds to asking questions that are all answered in the paper... I don't have time for this kind of journalism!

4 replies Last reply 3 years ago

Kristian Andersen 13:49
This one is actually pretty interesting!

Email from Slack for Gmail ▾

(No Subject) Apr 2nd, 2020
From Wharton, Kristi (No content)

April 2nd, 2020 ▾

Here's the paper she mentions

PDF ▾

Anderson JBC 2017 complete.pdf
PDF

JBC Papers in Press. Published on September 18, 2017 as Manuscript M117.793513

Edward N. Anderson, Kristi A. Wharton

Eddie Holmes 18:47
Dear Prof Holmes, I have been reading a bit, in the context of the current crisis, on the subject of virology. I came upon a text written by some of your UK colleagues entitled: The antigenic evolution of Influenza: Drift or Thrift?
In it, they refer to the expression: **Antigenic Thrift** as an expression coined by Eddy Holmes: "*This model—which we*
*will henceforward refer to as the '**antigenic thrift**' model*
*(**as suggested by Eddie Holmes**)—departs from the conventional*
'antigenic drift' hypothesis in a number of important ways:" The problem, it is done in such a familiar way...I could not find the document in which you first used it.
It would be interesting in the current context of political insecurity if you culd somewhat find a way to put your expression at the forefront, which may help explain the variability in fatalities among some Caucasian dominant countries vs China. We know that standardized hygiene of the food chain, while increasing safety in an individual perspective as a consumer, lower the general immunity of a population, is epitope cross immunity protection stands as a model or theory.
Thompson et al. also covers the expression in a recent 2018 article: A naturally protective epitope of limited variability as an influenza vaccine target
Reclaim your expression and help diffuse political paranoia, in a setting where politics are already quite noisy.
Thank you for your work.
Denys Picard
US and Canadian citizen
and dilettante of course...

April 2nd, 2020 ▾

> **PubMed Central (PMC)**
> **The antigenic evolution of influenza: drift or thrift?**
> It is commonly assumed that antibody responses against the influenza virus are polarized in the following manner: strong antibody responses are directed at highly variable antigenic epitopes, which consequently undergo 'antigenic drift', ...

> **Nature Communications**
> **A naturally protective epitope of limited variability as an influ**
> Current influenza vaccine approaches largely focus on highly variable epitopes with high immunogenicity or epitopes of low variability that often have low immunogenicity. Here, Thompson et al. identify a highly immunogenic epitope of limited variability in the head domain of the H1 haemagglutinin and show protection from diverse H1N1 strains in mice.

I've literally no clue what the fuck that was about.

Kristian Andersen 18:52
Haha, this is completely nonsensical - but thank you for your work.

Are these types of emails sent by actual people? I get lots that don't make any sense at all - e.g.,

Screen Shot 2020-04-02 at 15.50.53.png ▾

Enquiry 03042020 Covid-19 17 March 2020

WHO THE ORGANIC CHEMIST ATTACHED TO YOUR CLAM OF COVID 19 A NATURAL ORIGIN?
REGARDS

Get Outlook for Android

[do read the one from Kristi above though - that one is really cool and could help explain that mysterious 'P' insertion, which is just such a cool evolutionary trick given that those O-linked residues already existed, but weren't O-linked until the insertion of P].

Eddie Holmes 19:21
There are a lot of actual very mad people. (edited)

April 3rd, 2020

Kristian Andersen 13:35

This whole furin site being messed with in T/C has me second-guessing myself. When we went through this whole process, remember we talked about "passage might make viruses acquire these sites"? We couldn't find a reference, but somebody just posted on Virological, which led me to this: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0052752#pone-0052752-t002

> **journals.plos.org**
> The Role of Viral Population Diversity in Adaptation of Bovine Coronavirus to New Host Environments
> The high mutation rate of RNA viruses enables a diverse genetic population of viral genotypes to exist within a single infected host. In-host genetic diversity could better position the virus population to respond and adapt to a diverse array of selective pressures such as host-switching events. Multiple new coronaviruses, including SARS, have been identified in human samples just within the last ten years, demonstrating the potential of coronaviruses as emergent human pathogens. Deep sequencing was used to characterize genomic changes in coronavirus quasispecies during simulated host-switching. Three bovine nasal samples infected with bovine coronavirus were used to infect human and bovine... Show more

Specifically "The consensus sequence of many of the passaged samples had a 12 nucleotide insert in the consensus sequence of the spike gene, and multiple point mutations were associated with the presence of the insert" - those insertions being Arg rich, which is exactly what HCoV has.

Robert Garry 13:48

We're passaging HCov-19 on lung cell lines and Veros. But yes - totally missed that 2013 paper! I guess if we get the deletions we should pass those back on lung cells. The 12 base insertion is freaky though.

Kristian Andersen 13:50

Yeah, I'd be very interesting in knowing whether an HCoV-19 *without* the furin site could acquire it again. I haven't fully read that PLOS paper yet, but the similarity is very interesting.

I also thought this one was interesting - some talk about lab too: https://www.scientificamerican.com/article/how-chinas-bat-woman-hunted-down-viruses-from-sars-to-the-new-coronavirus1/

> **SA Scientific American**
> How China’s “Bat Woman” Hunted Down Viruses from SARS to the New Coronavirus
> Wuhan-based virologist Shi Zhengli has identified dozens of deadly SARS-like viruses in bat caves, and she warns there are more out there (376 kB) ▾

The 2013 paper is summarized nicely here: http://virological.org/t/identification-of-a-common-deletion-in-the-spike-protein-of-sars-cov-2/451/6

> **Virological**
> Identification of a common deletion in the spike protein of SARS-CoV-2
> The presence of inserts or deletions in consensus sequences or as variants of SARS-like coronaviruses is also observed in bovine coronavirus, also a member of betacoronavirus (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0052752#pone-0052752-t002) . For example, after passing 3 different naturally infected bovine nasal samples in different cell lines we observed the consensus sequences of many viral samples acquired a 12-nucleotide insert encoding 4 amino acids (Ser, Arg, Ar...
> Apr 3rd, 2020

Especially: "For example, after passing 3 different naturally infected bovine nasal samples in different cell lines we observed the consensus sequences of many viral samples acquired a 12-nucleotide insert encoding 4 amino acids (Ser, Arg, Arg, Arg) located at nt 2737 of the spike gene (S2 subunit), whereas none of the unpassaged samples contained this insert at the consensus level"

It's not just a single experiment - three different strains all exactly acquired a 12bp furin cleavage site. That's definitely peculiar.

This too very interesting as a potential mechanism "Deep sequencing revealed that the insert genotype was present but very rare in the unpassaged samples but quickly became consensus after passage in cell culture." - so it's there in their input (presumably directly from cow).

Robert Garry 14:09

Mutations,
including point mutations, insertions and deletions, can occur near the S1/S2 junction of
coronaviruses 34,40–43 suggesting that the polybasic site could arise by a natural evolutionary process.

I think this covers us pretty well - yes - there is natural variation adding and subtracting the furin site in several CoVs - also note that Bovine Cov is really a very broad host range virus

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2395124/

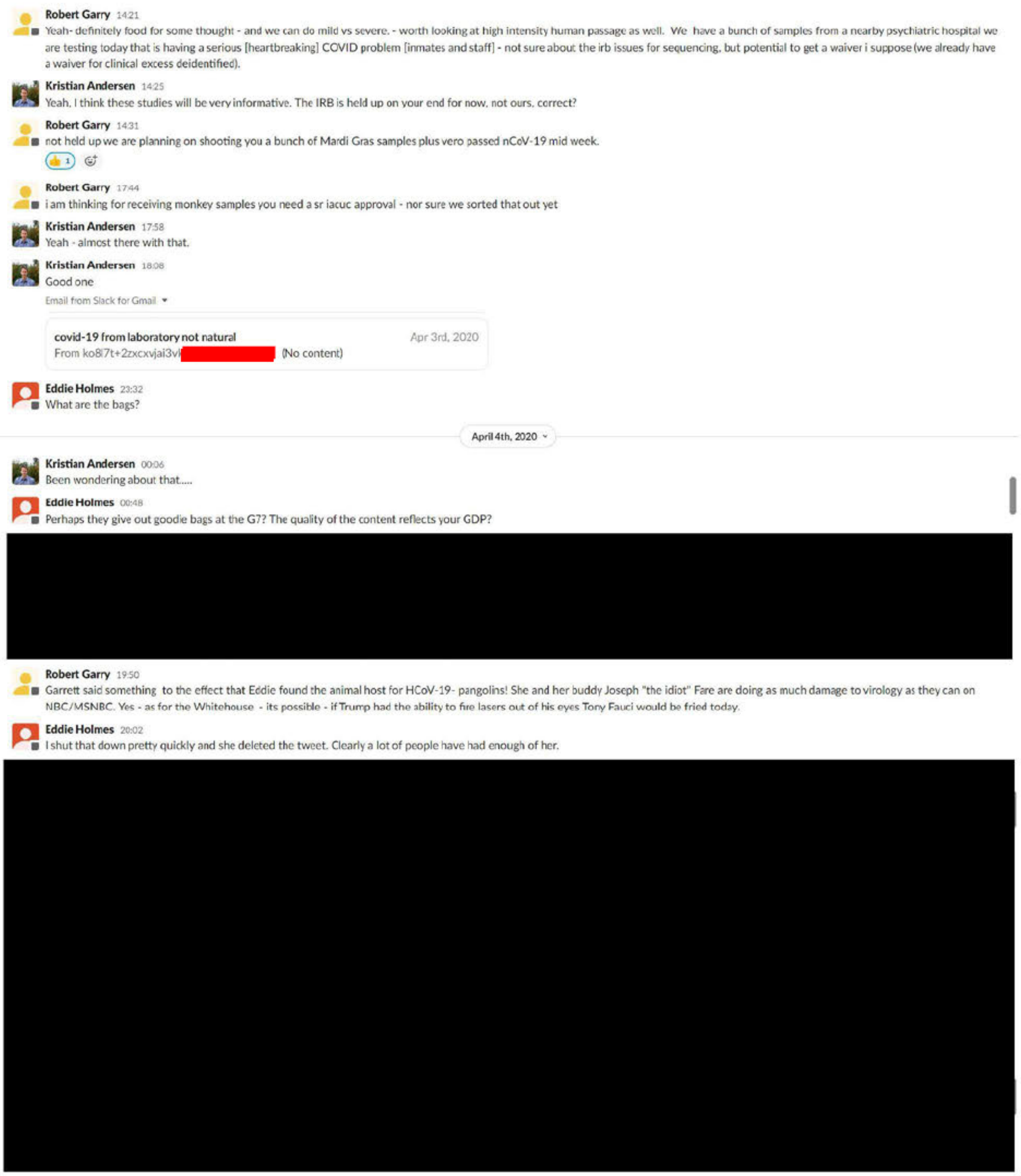
> **PubMed Central (PMC)**
> Cleavage of Group 1 Coronavirus Spike Proteins: How Furin Cleavage Is Traded Off against Heparan Sulfate Binding upon Cell Culture Adaptation
> A longstanding enigmatic feature of the group 1 coronaviruses is the uncleaved phenotype of their spike protein, an exceptional property among class I fusion proteins. Here, however, we show that some group 1 coronavirus spike proteins carry a furin enzyme ...

Kristian Andersen 14:13

Yeah, clearly this part of the genome is very 'active' - which is super freaky, because are we just waiting for other SARS-like CoVs popping up that have pandemic potential too.

I don't think any of this new knowledge goes against what we said in the paper, but it does make our "definitely not passage" argument weaker.

I would be very interested in seeing some very in-depth studies of high coverage longitudinal viral sequencing of mild vs severe cases. I wouldn't be surprised if we might observe loss of the furin site in more severe cases.



Robert Garry 14:21
Yeah- definitely food for some thought - and we can do mild vs severe. - worth looking at high intensity human passage as well. We have a bunch of samples from a nearby psychiatric hospital we are testing today that is having a serious [heartbreaking] COVID problem [inmates and staff] - not sure about the irb issues for sequencing, but potential to get a waiver i suppose (we already have a waiver for clinical excess deidentified).

Kristian Andersen 14:25
Yeah, I think these studies will be very informative. The IRB is held up on your end for now, not ours, correct?

Robert Garry 14:31
not held up we are planning on shooting you a bunch of Mardi Gras samples plus vero passed nCoV-19 mid week.
👍 1

Robert Garry 17:44
i am thinking for receiving monkey samples you need a sr iacuc approval - nor sure we sorted that out yet

Kristian Andersen 17:58
Yeah - almost there with that.

Kristian Andersen 18:08
Good one
Email from Slack for Gmail ▾

covid-19 from laboratory not natural Apr 3rd, 2020
From ko8l7t+2zxcxvjai3v[redacted] (No content)

Eddie Holmes 23:32
What are the bags?

April 4th, 2020

Kristian Andersen 00:06
Been wondering about that.....

Eddie Holmes 00:48
Perhaps they give out goodie bags at the G7? The quality of the content reflects your GDP?

[redacted]

Robert Garry 19:50
Garrett said something to the effect that Eddie found the animal host for HCoV-19- pangolins! She and her buddy Joseph "the idiot" Fare are doing as much damage to virology as they can on NBC/MSNBC. Yes - as for the Whitehouse - its possible - if Trump had the ability to fire lasers out of his eyes Tony Fauci would be fried today.

Eddie Holmes 20:02
I shut that down pretty quickly and she deleted the tweet. Clearly a lot of people have had enough of her.

[redacted]

April 5th, 2020

Andrew Rambaut 14:02

@channel Been helping out a colleague of Oli's with a little paper about deletions that take out the furin cleavage site.
https://www.biorxiv.org/content/10.1101/2020.03.31.015941v1.full.pdf+html

> bR **bioRxiv**
> Identification of a common deletion in the spike protein of SARS-CoV-2
> Abstract Two notable features have been identified in the SARS-CoV-2 genome: (1) the receptor binding domain of SARS-CoV-2; (2) a unique insertion of twelve nucleotide or four amino acids (PRRA) at the S1 and S2 boundary. For the first feature, the similar RBD identified in SARs-like virus from pangolin suggests the RBD in SARS-CoV-2 may already exist in animal host(s) before it transmitted into human. The left puzzle is the history and function of the insertion at S1/S2 boundary, which is uniquely identified in SARS-CoV-2. In this study, we identified two variants from the first Guangdong SARS-CoV-2 cell strain, with deletion mutations on polybasic cleavage site (PRRAR) and its flank sites... Show more
> Apr 2nd, 2020

I just wanted to run by an idea by you all... What do think about the hypothesis that knocking out the furin site is being selected in cells and in some patients but basically it needs it to successfully shed in the lungs and/or infect the next lungs?

Thus without it it is more SARS like in its transmissibility.

April 5th, 2020

Robert Garry 15:24

This is massively important. I very much agree with the hypothesis - needs to be tested in animal models ASAP.

Kristian Andersen 17:32

@Andrew Rambaut - yeah, reasonable hypotheses and you can see a posed something similar above. It's possible that a lack of the furin cleavage site might 'drive' the virus deeper into the lungs hence leading to more severe disease - the opposite would then also be true, but could then lead to more spread.

I'm not convinced passage *per se* in tissue culture will lead to the deletion of the site. I think this is likely going to be highly dependent on what cell line it's being passaged in - e.g., Vero cells are (monkey) kidney epithelial cells, so likely pretty different than the main cells HCoV would typically infect - unlike, e.g., passage on lung cells. Some of the experiments Bob and I discussed above could be very illuminating here and it'd **definitely** be interesting to do a clinical outcome association study with absence/presence of furin site.

Kristian Andersen 20:25

@Andrew Rambaut one question that just occurred to me - did they grow the viruses in the presence or absence of trypsin? (SARS needs trypsin, HCoV does not, but if this was done similar to SARS then they might have added trypsin to the culture - which could drive the deletion of the furin site).

Andrew Rambaut 20:56

Yes - I think we discussed this earlier up the thread somewhere. I believe they did use trypsin in the cell medium (this is normal I think to stop the cells bunching?).

Kristian Andersen 21:02

Interesting - I think this might drive it. Yes, trypsin is often used to dislodge the cells when you split them - but then it's typically washed off pretty thoroughly, so shouldn't really be present at a high level in the culture itself - but it might be sufficient here. Veros can be split without adding trypsin though - just by scraping the cells off. If possible, it'd be **very** interested in seeing an experiment with or without trypsin to get a sense of whether that might drive the phenotype.

Eddie Holmes 22:11

And on it goes: https://www.nationalreview.com/2020/04/coronavirus-china-trail-leading-back-to-wuhan-labs/

> NR **National Review**
> The Trail Leading Back to the Wuhan Labs | National Review
> There's no proof the coronavirus originated in a laboratory, but we can't take the Chinese government's denials at face value.
> Apr 3rd, 2020 (144 kB)

Robert Garry 22:32

yes - good idea K - passaging with and without trypsin.

Kristian Andersen 23:32

@Eddie Holmes we almost have a 30k Altmetric score so I welcome any crazy theory ;)

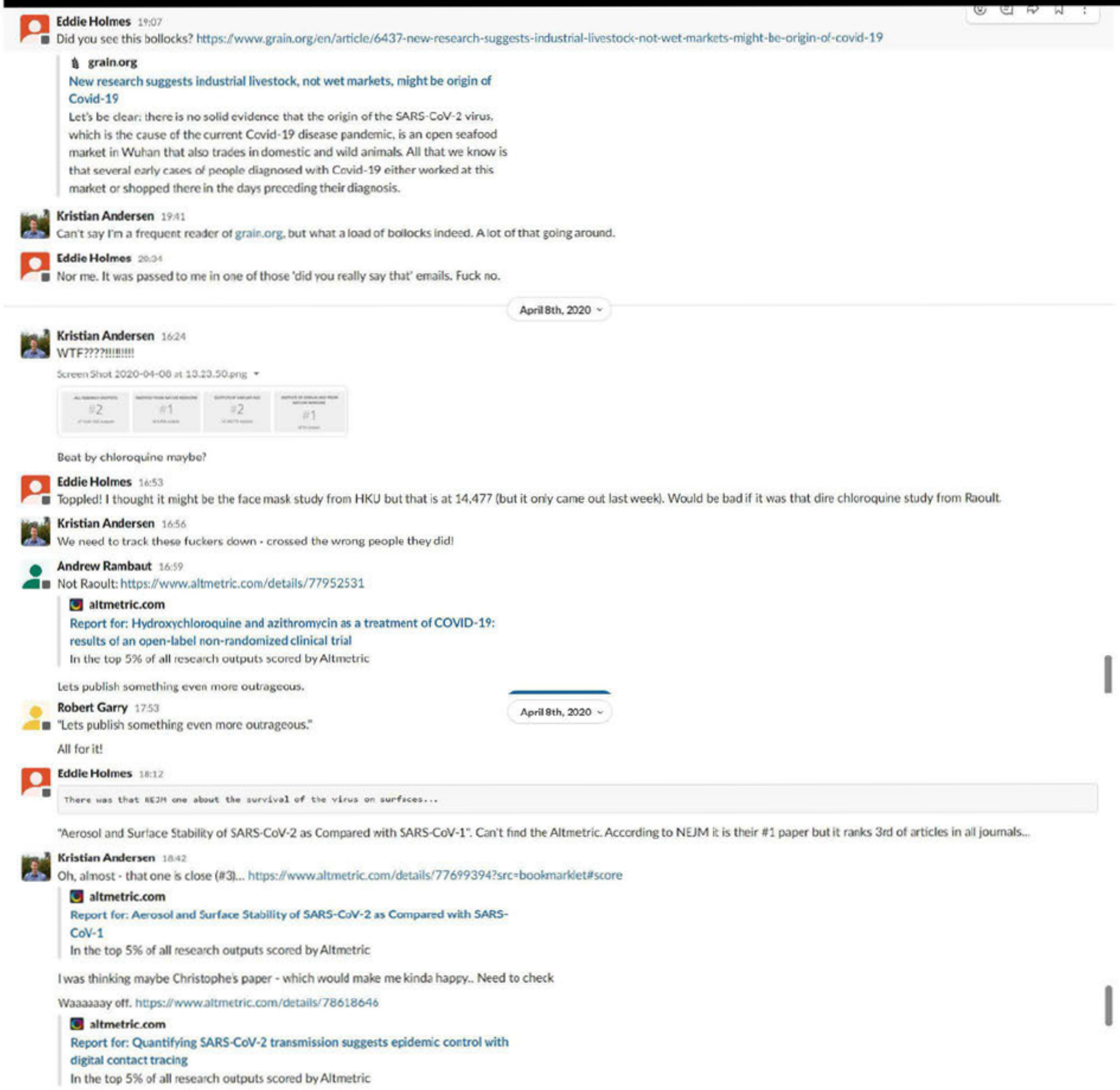
Eddie Holmes 23:47

Good point. Let's keep pushing for 30k.

April 6th, 2020

[redacted]

[redacted]



Eddie Holmes 19:07
Did you see this bollocks? https://www.grain.org/en/article/6437-new-research-suggests-industrial-livestock-not-wet-markets-might-be-origin-of-covid-19

> **grain.org**
> New research suggests industrial livestock, not wet markets, might be origin of Covid-19
> Let's be clear: there is no solid evidence that the origin of the SARS-CoV-2 virus, which is the cause of the current Covid-19 disease pandemic, is an open seafood market in Wuhan that also trades in domestic and wild animals. All that we know is that several early cases of people diagnosed with Covid-19 either worked at this market or shopped there in the days preceding their diagnosis.

Kristian Andersen 19:41
Can't say I'm a frequent reader of grain.org, but what a load of bollocks indeed. A lot of that going around.

Eddie Holmes 20:34
Nor me. It was passed to me in one of those 'did you really say that' emails. Fuck no.

April 8th, 2020

Kristian Andersen 16:24
WTF????!!!!!!!!!

Screen Shot 2020-04-08 at 13.23.50.png

Beat by chloroquine maybe?

Eddie Holmes 16:53
Toppled! I thought it might be the face mask study from HKU but that is at 14,477 (but it only came out last week). Would be bad if it was that dire chloroquine study from Raoult.

Kristian Andersen 16:56
We need to track these fuckers down - crossed the wrong people they did!

Andrew Rambaut 16:59
Not Raoult: https://www.altmetric.com/details/77952531

> **altmetric.com**
> Report for: Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial
> In the top 5% of all research outputs scored by Altmetric

Lets publish something even more outrageous.

April 8th, 2020

Robert Garry 17:53
"Lets publish something even more outrageous."

All for it!

Eddie Holmes 18:12

`There was that NEJM one about the survival of the virus on surfaces...`

"Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1". Can't find the Altmetric. According to NEJM it is their #1 paper but it ranks 3rd of articles in all journals...

Kristian Andersen 18:42
Oh, almost - that one is close (#3)... https://www.altmetric.com/details/77699394?src=bookmarklet#score

> **altmetric.com**
> Report for: Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1
> In the top 5% of all research outputs scored by Altmetric

I was thinking maybe Christophe's paper - which would make me kinda happy.. Need to check

Waaaaaay off. https://www.altmetric.com/details/78618646

> **altmetric.com**
> Report for: Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing
> In the top 5% of all research outputs scored by Altmetric

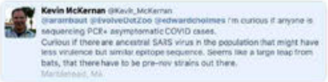
Eddie Holmes 19:08
Let's hope it's some bat shit crazy wankfest so we can still claim the moral high ground. I'm keen to find out...without asking Twitter thereby admitting that I am keen to find out.

April 9th, 2020

[redacted]

Andrew Rambaut 12:55
This question...

image.png



Kevin McKernan @Kevin_McKernan
@arambaut @EvolveDotZoe @edwardcholmes I'm curious if anyone is sequencing PCR+ asymptomatic COVID cases.
Curious if there are ancestral SARS virus in the population that might have less virulence but similar epitope sequence. Seems like a large leap from bats, that there have to be pre-nov strains out there.
Marblehead, MA

makes sense now...

image.png

Kevin M:Kernan
@Kevin_McKernan
FOLLOWS YOU
Cannabis Genome Project,2011. Developed the SOLID sequencer. R&D lead Human Genome Project at MIT/WIBR
Marblehead
http://medicinalgenomics.com

Kristian Andersen 13:00
Haha, I think he might have done more than just sequence the genome of that 2011 project.

Andrew Rambaut 13:37
Yup.

'sequenced it' if you know what I mean, man.

April 10th, 2020

Robert Garry 07:47
sequence evidence for SARS-Cov-2 existed five years ago.

SECRET email -

```
link£ºhttps://pan.baidu.com/s/1QnUdYJ3mmBy0-MkIm7PB4A
passwd£ºtlwm
```

Dear ALL professors,
I have found out that the SARS-Cov-2 is existed in Wuhan in the year 2015, 2017, 2018.
The sequence evidence detected for patients with infectious disease is in the attached folders. I think you can do more similar work to the sequence data submitted by guys in Hubei province, China.
I think you are right, SARS-Cov-2 is existed in Hubei for a long time, maybe the common corona virus have some communication with other viruses such as novel Bunya virus on genetic materials. Maybe the environment in Hubei trigger some switch to speed up the evolution of SARS-Cov-2, since high temperature environment in Wuhan, make the ecosystem there chaos, some food chains was destroyed by people there and make the virus jump into human being and begin the long journey to finish revolution to kill more old people to balance the ecosystem there, so that the food chain can be restored.
Please keep the data secret for me, since the data is from our company, and the data are actually from CDC in the country. And I have emailed to Kristian G. Andersen.
Yours,
Shaofei Liu

Robert Garry 07:54
phish?

Andrew Rambaut 07:55
Strange link in an email from China? Sure to be legit.

Andrew Rambaut 08:01
Mind you, I so want to see this. Perhaps I will break into another office and use a student's computer...

Robert Garry 08:48
Let us know what you find down the rabbit hole...

Kristian Andersen 10:33
The link is legit enough and there are fastq files in there...

https://pan.baidu.com/s/1QnUdYJ3mmBy0-MWim7PB4A
Pass: tlwm

I find it kinda interesting that he emailed y'all separately - could be a Chinese whistleblower... I'll download some of these and run a Kraken screen, because why the heck not. (edited)

Andrew Rambaut 10:35
Glad you were willing to take the bullet for us.

Look forward to hearing about what you find.

Kristian Andersen 10:50
Always count on me to do the dumbest things. 🤪

Kristian Andersen 12:27
I swear there are fastq files in there - and all named logically. Issue is, I can't bloody figure out how to download stuff since it's all in Mandarin.

Andrew Rambaut 12:45
Get the google translate app on your phone - it can do live translating through the camera.

Kristian Andersen 12:46
Brilliant!

Andrew Rambaut 12:46
No. It offers you a software download - presumably what you need to install so the Chinese government can take control of your computer

Kristian Andersen 12:47
Exactly - need to download the Baidu app. I trust my Mac won't be taken over... (I created a protected account just for this)

I'm sufficiently intrigued here because these are clearly sequencing files and this guy could be from BGI

Kristian Andersen 13:37
Still trying to work through this... Here's the readme
image (2).png ▾

13:37 I think we *do* have a whistleblower here - just not sure what the data is actually going to show...

Kristian Andersen 15:40
Very slow going, but at least now we know that it's legit (but could very well be misclassification)
Screen Shot 2020-04-10 at 12.40.00.png ▾

Robert Garry 15:47
Wow- keep after this and keep us posted - BTW - I think that this individual provided a female name....did they send the message thru an encrypted site?

Kristian Andersen 16:54
Yeah, this was a very strange email so while the message itself wasn't encrypted, I think this person went to some length to hide their tracks. The data download is very slow so it'll take me a while to take a look at the actual data - I suspect these are just misclassifications, but I'll definitely take a look.

Eddie Holmes 17:20
I can easily get a Mandarin speaker to look at these Kristian. Just let me know.

↓ Latest messages

Do you want to try to find out who this person is? I can ask around.

Eddie Holmes 17:27
The Chinese govt have control of my computer anyway so no worries there. Whistleblower, hoax, or set-up? Remember, we looked at 600 metatranscriptomic samples from Wuhan in 2018 and saw no know SARS-CoV-2.

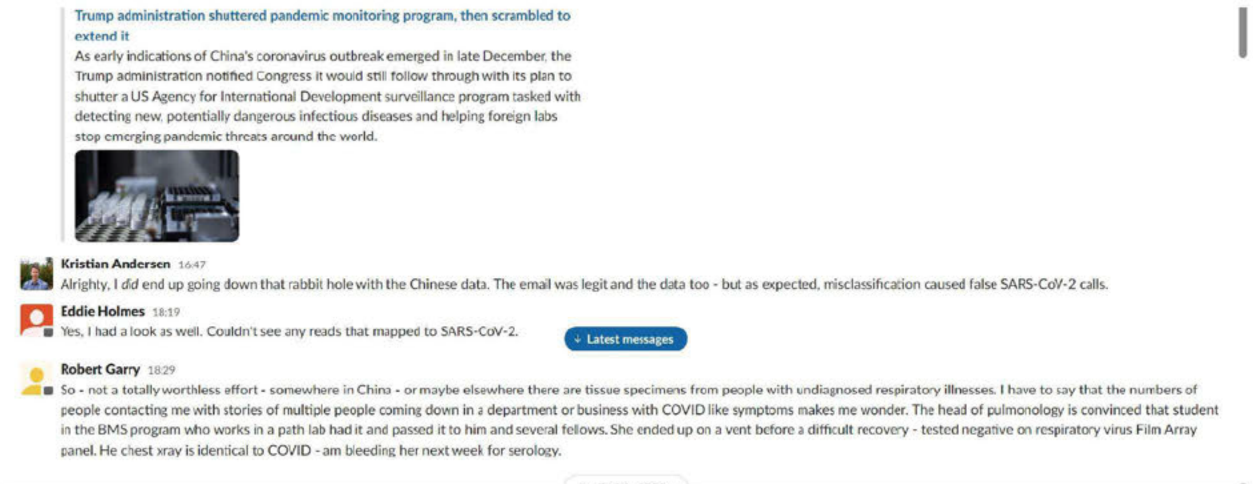
Kristian Andersen 17:41
We have two guys from China here at our institute and they managed to start the downloads. They're downloading as we speak, albeit slowly.

It looks to me that these are single reads aligning, so most likely misclassification - but let's see once I have the fastqs

Eddie Holmes 19:01
Makes sense. Cock-up is always the most likely explanation.

April 11th, 2020 ▾

Kristian Andersen 00:08
PREDICT resurrected... https://www.cnn.com/2020/04/10/politics/trump-usaid-prevent-program-coronavirus/index.html
CNN



Trump administration shuttered pandemic monitoring program, then scrambled to extend it

As early indications of China's coronavirus outbreak emerged in late December, the Trump administration notified Congress it would still follow through with its plan to shutter a US Agency for International Development surveillance program tasked with detecting new, potentially dangerous infectious diseases and helping foreign labs stop emerging pandemic threats around the world.

Kristian Andersen 16:47
Alrighty, I *did* end up going down that rabbit hole with the Chinese data. The email was legit and the data too - but as expected, misclassification caused false SARS-CoV-2 calls.

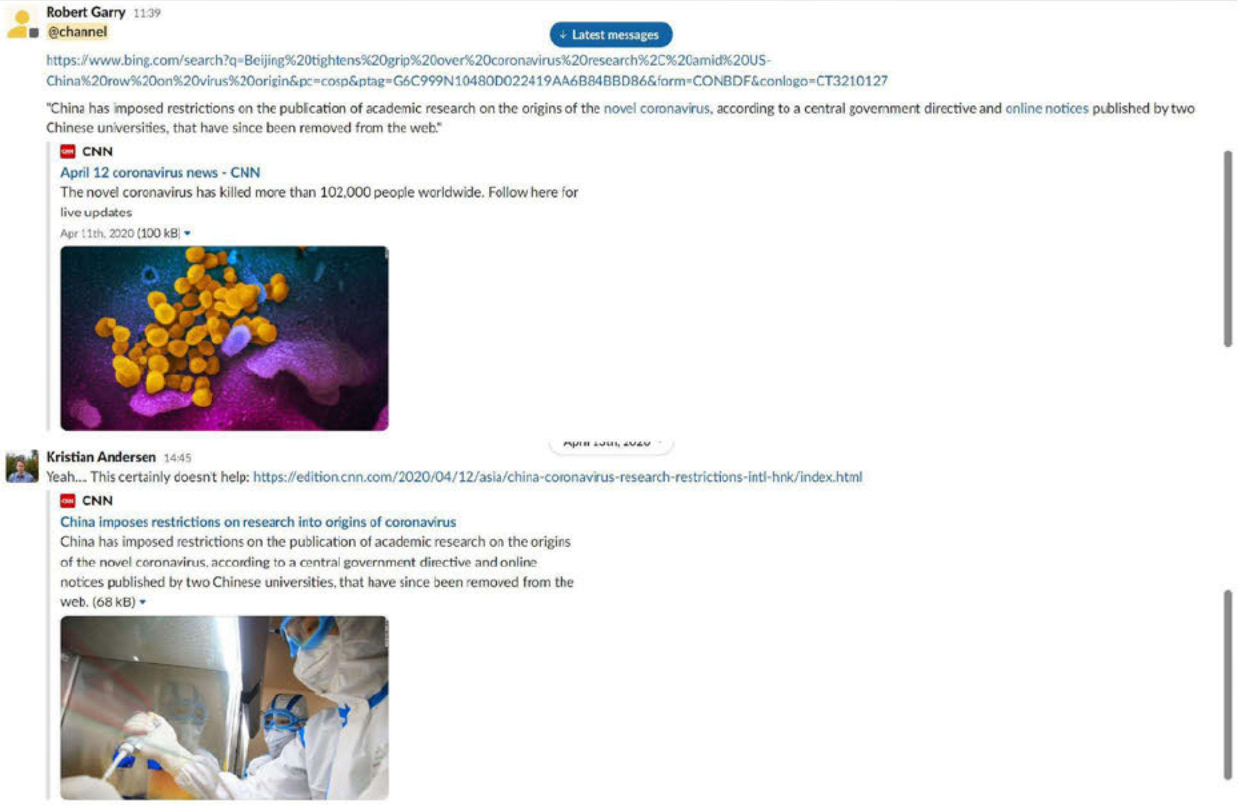
Eddie Holmes 18:19
Yes, I had a look as well. Couldn't see any reads that mapped to SARS-CoV-2.

Robert Garry 18:29
So - not a totally worthless effort - somewhere in China - or maybe elsewhere there are tissue specimens from people with undiagnosed respiratory illnesses. I have to say that the numbers of people contacting me with stories of multiple people coming down in a department or business with COVID like symptoms makes me wonder. The head of pulmonology is convinced that student in the BMS program who works in a path lab had it and passed it to him and several fellows. She ended up on a vent before a difficult recovery - tested negative on respiratory virus Film Array panel. He chest xray is identical to COVID - am bleeding her next week for serology.

April 12th, 2020

[redacted]

[redacted]



Robert Garry 11:39

@channel

https://www.bing.com/search?q=Beijing%20tightens%20grip%20over%20coronavirus%20research%2C%20amid%20US-China%20row%20on%20virus%20origin&pc=cosp&ptag=G6C999N10480D022419AA6B84BBD86&form=CONBDF&conlogo=CT3210127

"China has imposed restrictions on the publication of academic research on the origins of the novel coronavirus, according to a central government directive and online notices published by two Chinese universities, that have since been removed from the web."

> **CNN**
>
> April 12 coronavirus news - CNN
>
> The novel coronavirus has killed more than 102,000 people worldwide. Follow here for live updates
>
> Apr 11th, 2020 (100 kB)

Kristian Andersen 14:45

Yeah.... This certainly doesn't help: https://edition.cnn.com/2020/04/12/asia/china-coronavirus-research-restrictions-intl-hnk/index.html

> **CNN**
>
> China imposes restrictions on research into origins of coronavirus
>
> China has imposed restrictions on the publication of academic research on the origins of the novel coronavirus, according to a central government directive and online notices published by two Chinese universities, that have since been removed from the web. (68 kB)

April 13th, 2020

Andrew Rambaut 16:54

On the other hand this is an interesting read: https://www.theguardian.com/world/2020/apr/10/birth-of-a-pandemic-inside-the-first-weeks-of-the-coronavirus-outbreak-in-wuhan?CMP=Share_iOSApp_Other

the Guardian

Birth of a pandemic: inside the first weeks of the coronavirus outbreak in Wuhan

Interviews with patients, medical workers and residents reveal delays with far-reaching consequences for the city, the world and China's leadership

Apr 10th, 2020 (65 kB)

Eddie Holmes 21:41

Once this is over the shit will hit the fan. Lots of stories will need to be told.

April 14th, 2020

[redacted]

Robert Garry 15:01

Hi Dr. Garry,

Our episode on virus hunting and bat virology for Short Wave, NPR's daily science podcast, will publish tomorrow at 4 a.m. EST.

You'll find it at the top of this web page here: https://www.npr.org/podcasts/510351/short-wave or wherever you get your podcasts. It includes quotes from yourself, Dr. Linfa Wang in Singapore, and Dr. Peter Daszak at EcoHealth Alliance. Thank you so much for taking the time to speak with me, and I hope you're taking care in New Orleans.

-Emily

NPR.org

Short Wave

New discoveries, everyday mysteries, and the science behind the headlines — all in about 10 minutes, every weekday. It's science for everyone, using a lot of creativity and a little humor. Join host Maddie Sofia for science on a different wavelength.

April 14th, 2020

Kristian - I hope you are proud of what you got me into here - LOL.

Kristian Andersen 15:06

I hope so too Bob, I hope so too....

Eddie Holmes 18:16

Did you lot get this?

Screen Shot 2020-04-15 at 8.16.01 am.png

Dear Professor Holmes,

I am a medical correspondent for the Daily Mail national newspaper here in the UK.

I have been asked to examine allegations made by in a new report by the *Epoch Times* that the coronavirus Covid-19 pandemic was actually caused by Chinese government scientists weaponizing a SARS virus, and that the outbreak therefore did not originate in the Wuhan wet food market.

The Epoch Times report quotes selected scientists as claiming that the virus actually appears to be man-made.

By way of evidence, the report states that Covid-19 has 100 per cent similarity to SARS-type viruses found in bats, and that: "It is hard to see proteins being 100 per cent identical when a virus jumps species. This means that the virus could have been generated by a reverse-engineered process."

Can I ask please whether you have seen any evidence that might support this theory?

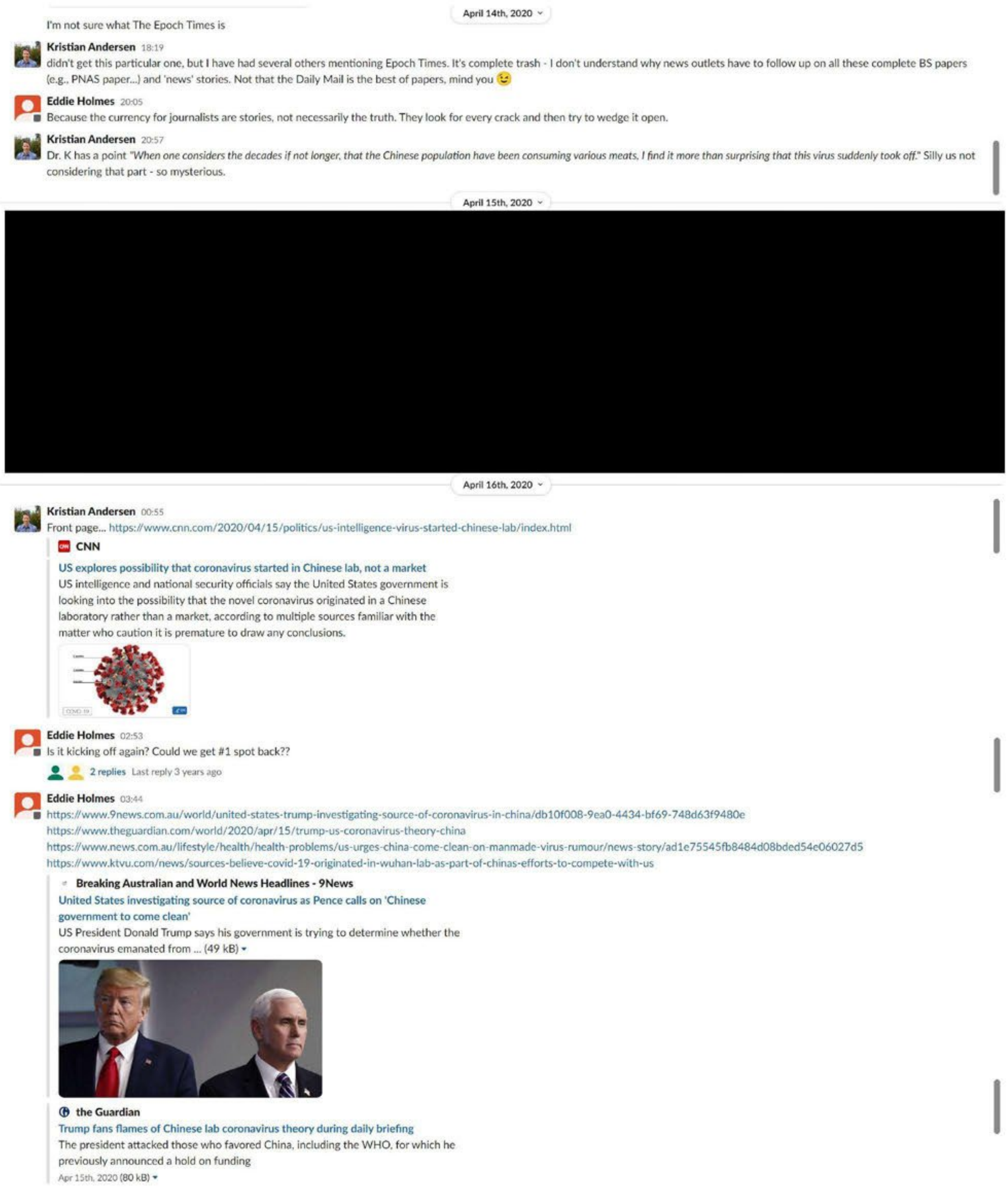
Also, would it be unlikely in nature for a zoonotic SARS virus to mutate in the way that it has?

I am endeavouring to establish as much reliable, authoritative science as possible to critique claims that are getting attached to this subject amid all the speculation and conspiracy hysteria that's flying around the internet.

Therefore any assistance you could give me would be most gratefully received.

With thanks for your time,

John Naish



April 14th, 2020

I'm not sure what The Epoch Times is

Kristian Andersen 18:19
didn't get this particular one, but I have had several others mentioning Epoch Times. It's complete trash - I don't understand why news outlets have to follow up on all these complete BS papers (e.g., PNAS paper...) and 'news' stories. Not that the Daily Mail is the best of papers, mind you 😉

Eddie Holmes 20:05
Because the currency for journalists are stories, not necessarily the truth. They look for every crack and then try to wedge it open.

Kristian Andersen 20:57
Dr. K has a point *"When one considers the decades if not longer, that the Chinese population have been consuming various meats, I find it more than surprising that this virus suddenly took off."* Silly us not considering that part - so mysterious.

April 15th, 2020

[redacted]

April 16th, 2020

Kristian Andersen 00:55
Front page... https://www.cnn.com/2020/04/15/politics/us-intelligence-virus-started-chinese-lab/index.html

CNN
US explores possibility that coronavirus started in Chinese lab, not a market
US intelligence and national security officials say the United States government is looking into the possibility that the novel coronavirus originated in a Chinese laboratory rather than a market, according to multiple sources familiar with the matter who caution it is premature to draw any conclusions.

Eddie Holmes 02:53
Is it kicking off again? Could we get #1 spot back??
2 replies Last reply 3 years ago

Eddie Holmes 03:44
https://www.9news.com.au/world/united-states-trump-investigating-source-of-coronavirus-in-china/db10f008-9ea0-4434-bf69-748d63f9480e
https://www.theguardian.com/world/2020/apr/15/trump-us-coronavirus-theory-china
https://www.news.com.au/lifestyle/health/health-problems/us-urges-china-come-clean-on-manmade-virus-rumour/news-story/ad1e75545fb8484d08bded54e06027d5
https://www.ktvu.com/news/sources-believe-covid-19-originated-in-wuhan-lab-as-part-of-chinas-efforts-to-compete-with-us

Breaking Australian and World News Headlines - 9News
United States investigating source of coronavirus as Pence calls on 'Chinese government to come clean'
US President Donald Trump says his government is trying to determine whether the coronavirus emanated from ... (49 kB)

the Guardian
Trump fans flames of Chinese lab coronavirus theory during daily briefing
The president attacked those who favored China, including the WHO, for which he previously announced a hold on funding
Apr 15th, 2020 (80 kB)

Apr 15th, 2020 (80 kB) ▾

April 16th, 2020 ⌄

NewsComAu

US urges China: 'Come clean' on virus

The US is urging China to 'come clean' about the origin of COVID-19 as claims circulate that it was manufactured in a Wuhan laboratory.

Apr 16th, 2020 (22 kB) ▾

April 16th, 2020 ⌄

KTVU FOX 2

Sources believe COVID-19 originated in Wuhan lab as part of China's efforts to compete with US

This may be the "costliest government coverup of all time," one of the sources said.

(30 kB) ▾

April 16th, 2020 ⌄

Robert Garry 08:57

Trump/Faux really need to settle on one cospiracy theory or another rather than somehow conflating the two into one grand conspiratorial mash-up.

Either NCoV-19 1) came from the market or 2) it was created or escaped from WIV or 3) it can from natural processes.

FIne - push 1 or 2 I suppose, but what Trump/Flox is pushing is a mash-up conspiracy theory where someone from WIV released NCoV-19 into the fish market.

Andrew Rambaut 09:09

Project restore #1 Altmetric is under way -

👍 1 🦜 1

Kristian Andersen 10:04

It's disgusting what's going on here. Once again he will manage to blame others and come out stronger with his base. Put it all on China and WHO - he obviously did his job perfectly along the way.

Andrew Rambaut 10:23

And the way it is made to look like his own rambling thoughts. This is done by design by the people who run him.

Kristian Andersen 10:25

It's not exactly elegant, but it's (unfortunately) effective. I want out. Anybody has contacts in Norway?

Andrew Rambaut 10:31

A colleague is from Norway. But he is a bit concerned about the rise of the right-wing there too.

Robert Garry 10:33

ABC - national news - so a start. - Hi Dr. Garry!

I hope you're doing well!

As conspiracy theories continue to posit that SARS-CoV-2 is anthropogenic, I thought it could be an apt time to revisit your team's findings and hear how your thoughts may have evolved over the past few weeks.

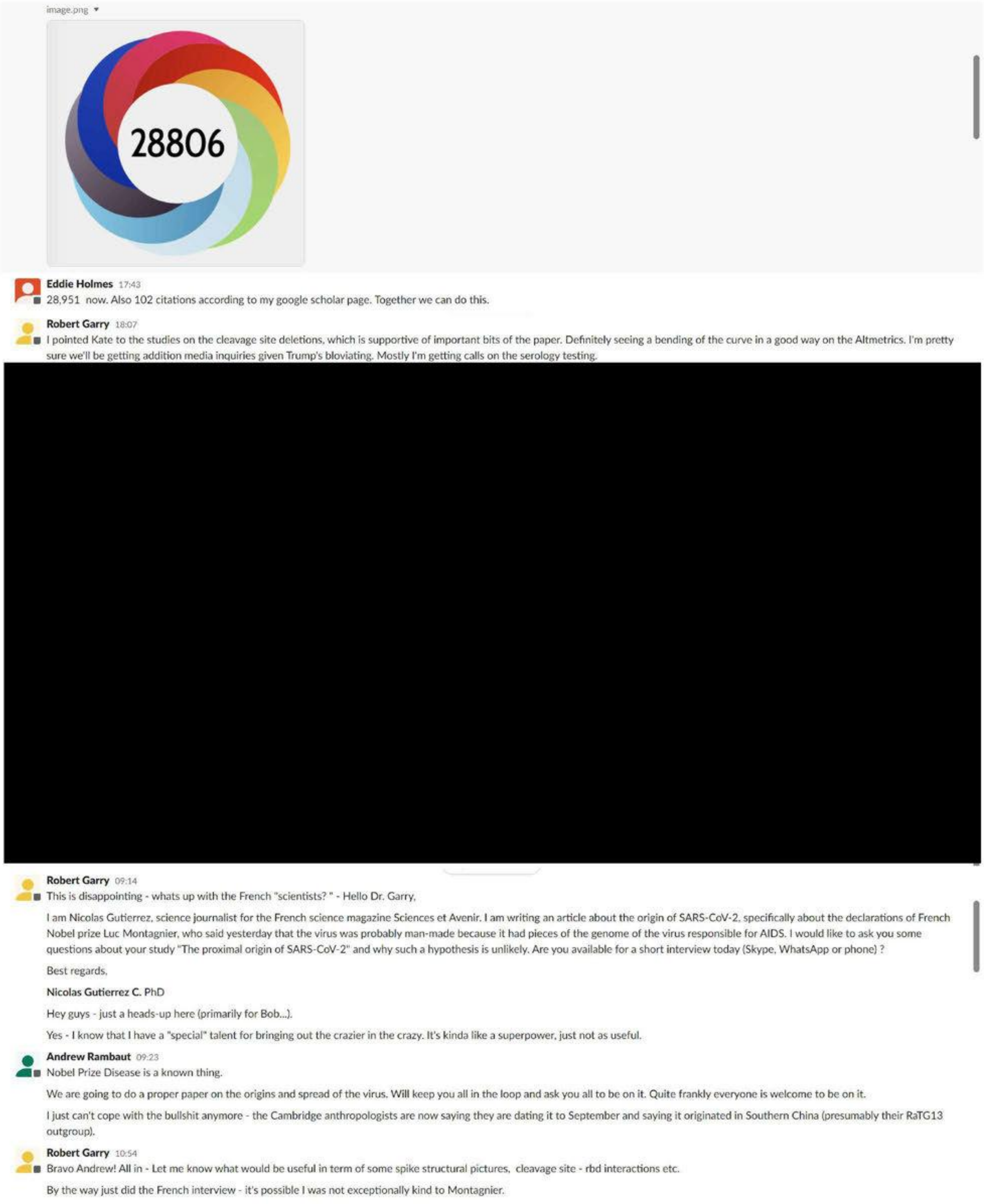
What are you and your colleagues thinking and hearing? Has new evidence surfaced to further support your research?

Please let me know when you might be available to speak again! I would love to do some kind of follow-up.

Thank you!
Kate
[redacted]

Andrew Rambaut 12:32

Up another 120. Keep it up



Eddie Holmes 17:43
28,951 now. Also 102 citations according to my google scholar page. Together we can do this.

Robert Garry 18:07
I pointed Kate to the studies on the cleavage site deletions, which is supportive of important bits of the paper. Definitely seeing a bending of the curve in a good way on the Altmetrics. I'm pretty sure we'll be getting addition media inquiries given Trump's bloviating. Mostly I'm getting calls on the serology testing.

[redacted]

Robert Garry 09:14
This is disappointing - whats up with the French "scientists? " - Hello Dr. Garry,

I am Nicolas Gutierrez, science journalist for the French science magazine Sciences et Avenir. I am writing an article about the origin of SARS-CoV-2, specifically about the declarations of French Nobel prize Luc Montagnier, who said yesterday that the virus was probably man-made because it had pieces of the genome of the virus responsible for AIDS. I would like to ask you some questions about your study "The proximal origin of SARS-CoV-2" and why such a hypothesis is unlikely. Are you available for a short interview today (Skype, WhatsApp or phone) ?

Best regards,

Nicolas Gutierrez C. PhD

Hey guys - just a heads-up here (primarily for Bob...).

Yes - I know that I have a "special" talent for bringing out the crazier in the crazy. It's kinda like a superpower, just not as useful.

Andrew Rambaut 09:23
Nobel Prize Disease is a known thing.

We are going to do a proper paper on the origins and spread of the virus. Will keep you all in the loop and ask you all to be on it. Quite frankly everyone is welcome to be on it.

I just can't cope with the bullshit anymore - the Cambridge anthropologists are now saying they are dating it to September and saying it originated in Southern China (presumably their RaTG13 outgroup).

Robert Garry 10:54
Bravo Andrew! All in - Let me know what would be useful in term of some spike structural pictures, cleavage site - rbd interactions etc.

By the way just did the French interview - it's possible I was not exceptionally kind to Montagnier.

https://www.researchgate.net/publication/340100582_WUHAN_COVID-19_SYNTHETIC_ORIGINS_AND_EVOLUTION

Here's the link to the new paper that Montagnier thinks is wonderful - my head started to explode about a page or so in (but go figure I had the same response when I started to read Harry Potter).

Andrew Rambaut 11:33

I think this may be French post-modernism. "Curiously, these digital waves characterizing the 9 SARS genomes studied here are characteristic whole numbers: the "Fibonacci numbers","

Robert Garry 11:52

https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.foxnews.com%2Fpolitics%2Fcoronavirus-wuhan-lab-china-compete-us-sources&data=02%7C01%7Crfgarry%40tulane.edu%7C8e15fc5745344661c8c808d7e2e31306%7C9de9818325d94b139fc34de5489c1f3b%7C0%7C0%7C637227337228352836&sdata=TJUNUjpxjZygqeolaFMx56KzNkT5HfDF95iuL93941E%3D&reserved=0

> **Fox News**
>
> Sources believe coronavirus outbreak originated in Wuhan lab as part of China's efforts to compete with US
>
> There is increasing confidence that COVID-19 likely originated in a Wuhan laboratory not as a bioweapon, but as part of China's effort to demonstrate that its efforts to identify and combat viruses are equal to or greater than the capabilities of the United States, multiple sources who have been briefed on the details of early actions by China's government and seen relevant materials tell Fox News.

Coronavirus: Is there any evidence for lab release theory?

BBC News examines allegations that the coronavirus was accidentally released from a lab.

> **BBC News**
>
> Is there any evidence for coronavirus lab release idea?
>
> BBC News examines allegations that the coronavirus was accidentally released from a lab. (67 kB) ▾

Fox - BBC it's really hard to tell the diff

Kristian Andersen 16:57

> We are going to do a proper paper on the origins and spread of the virus

@Andrew Rambaut - please keep us posted - I'd love to be part of this if I can be helpful (or even if I can't... 😉.

Okay, so about the current news. Is there *any* reason to believe that they might be onto something, or is it all smoke and mirrors? @Eddie Holmes - any insights on the China side? The main things from my perspective:

1. Bioweapon and engineered totally off the table
2. If there is **no** engineering and **no** culturing, then it means that somebody magically found a pre-formed pandemic virus, put it in the lab, and then infected themselves. The prior on that vs somebody coming into contact with an animal source infected with the virus is as close to zero as you can get. Humans come into contact *all the time* with SARS-like CoVs, but the likelihood of somebody finding exactly that pandemic virus and infecting themselves is very very low (make no mistake - if they *did* find that pandemic virus, then they *would* get infected if they grew it in the lab - but the likelihood of them finding it in the first place is exceedingly small (or so one would hope - otherwise, good luck World avoiding future pandemic).
3. But here's the issue - I'm still not fully convinced that **no** culture was involved. If culture *was* involved, then the prior completely changes - because this could have happened with any random SARS-like CoV, of which there are very many. So are we **absolutely certain** that no culture could have been involved? What concerns me here are some of the comments by Shi in the SciAm article ("I had to check the lab", etc.) and the fact that the furin site is being messed with *in vitro*. Yes, it loses it, but that could be context dependent. Finally, the paper that was shared with us showing a very similar phenomenon (exactly 12bp insertion) in other CoVs has me concerned: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0052752 - best summarized here: http://virological.org/t/identification-of-a-common-deletion-in-the-spike-protein-of-sars-cov-2/451/6

I really really want to go out there guns swinging saying "don't be such an idiot believing these dumb theories - the president is deflecting from the **real** problems", but I'm worried that we can't fully disprove culture (our argument was mostly based on the presence of the O-linked glycans - but they could likely play a different role: https://www.ncbi.nlm.nih.gov/pubmed/28924042). We also can't fully rule out engineering (for basic research) - yes, no obvious signs of engineering anywhere, but that furin site could still have been inserted via gibson assembly (and clearly creating the reverse genetic system isn't hard - the Germans managed to do exactly that for SARS-CoV-2 in less than a month).

April 17th, 2020 ⌄

> **journals.plos.org**
>
> The Role of Viral Population Diversity in Adaptation of Bovine Coronavirus to New Host Environments
>
> The high mutation rate of RNA viruses enables a diverse genetic population of viral genotypes to exist within a single infected host. In-host genetic diversity could better position the virus population to respond and adapt to a diverse array of selective pressures such as host-switching events. Multiple new coronaviruses, including SARS, have been identified in human samples just within the last ten years, demonstrating the potential of coronaviruses as emergent human pathogens. Deep sequencing was used to characterize genomic changes in coronavirus quasispecies during simulated host-switching. Three bovine nasal samples infected with bovine coronavirus were used to infect human and bovine... Show more

> **Virological**
>
> Identification of a common deletion in the spike protein of SARS-CoV-2
>
> The presence of inserts or deletions in consensus sequences or as variants of SARS-like coronaviruses is also observed in bovine coronavirus, also a member of betacoronavirus (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0052752#pone-0052752-t002) . For example, after passing 3 different naturally infected bovine nasal samples in different cell lines we observed the consensus sequences of many viral samples acquired a 12-nucleotide insert encoding 4 amino acids (Ser, Arg, Ar...

Apr 3rd, 2020

April 17th, 2020

ncbi.nlm.nih.gov

Alternative cleavage of the bone morphogenetic protein (BMP), Gbb, produces ligands with distinct developmental functions and receptor preferences. - PubMed - NCBI

J Biol Chem. 2017 Nov 24;292(47):19160-19178. doi: 10.1074/jbc.M117.793513. Epub 2017 Sep 18. Research Support, N.I.H., Extramural (13 kB)

Eddie Holmes 18:23

Yes, Andrew, I'm in. Very happy to help. Have the Cambridge anthropologists published anything else?

Eddie Holmes 18:38

This is what I know. 1. China are definitely trying to rewrite what happened, but I'm pretty certain that's because they don't what anyone to think about the origin in any context rather than trying to suppress the lab escape theory. They've been trying to suppress this from day 1 in December because the word 'SARS' is just so toxic to the regime. 2. There are lots more Chinese genome sequences available but the ones that I have seen don't provide any new insights. I am meant to be on a paper about the genetic diversity of the virus in Wuhan that they keep changing to say the virus might have emerged somewhere else and I keep changing back. 3. I've not heard of any cover-ups etc. George Gao has led most of the sampling and genomic work and he's too dumb to set up a sophisticated theory. 4. Was Dr. Shi from the WIV even doing GOF work in that lab? I thought all the relevant experiments were done in Baric's lab? I thought Shi just did sequencing/ecological work. 5. I think the simplest explanation is very likely the correct one: that the virus originated in bats, jumped to an as yet unknown intermediate host (I don't think it came straight from bats), and then jumped to humans in that market shortly before we detected it. The market is just too coincidental to ignore. All the component bits of this virus are found in nature and I see no reason to invoke lab escape whatsoever.

I'm very concerned that Ebright/Lipsitch/Bergstrom are going to try to use this to end GOF research when I think this is going to be time we need it most.

Kristian Andersen 18:51

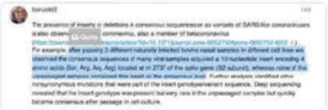
Shi didn't do any GOF work that I'm aware of - but GOF work isn't the concern here. She did A LOT of work that involved isolating and culturing SARS-like viruses from bats (in BSL-2) and that's my main concerning scenario (we cite several of those in the paper - if you have a look at those original publications, it's definitely concerning work, no question about it - and is the main reason I have been so concerned about the 'culture' scenario).

Eddie Holmes 19:00

Culturing in what? Why would culturing make it more human adapted? The WIV group sequence so many of their viruses I just be amazed if they were doing experiments on one for which they had no published the sequence, and all their viruses are from Yunnan. The closest bat virus to SARS-CoV-2 from that lab is RaTG13 which ain't that close. RmYN02 - which is not from WIV or any lab in Wuhan - is a bit closer to SARS-CoV-2 in most of the genome. We have a miniscule sample of bat virus in nature and almost none from Hubei. We know that people do get naturally spill-over infected by bat coronaviruses. Surely this route is far, far more likely than the lab escape scenario?

Kristian Andersen 19:02

Screen Shot 2020-04-17 at 16.02.10.png



Eddie Holmes 19:03

And RmYN02, a bat from nature, also includes insertions at that site.

Kristian Andersen 19:03

Here are just four examples of some of the culturing work that's concerning:

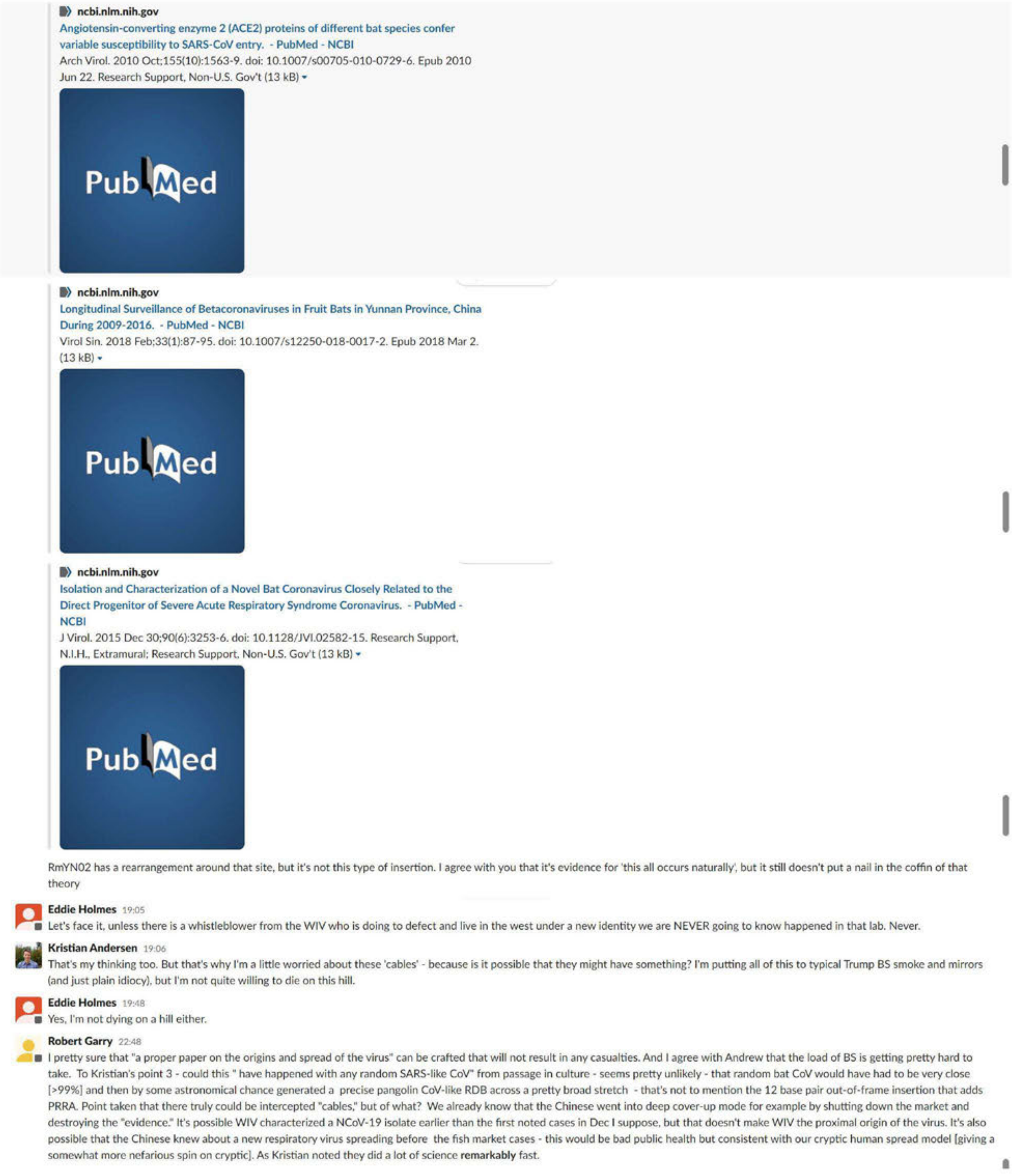
https://www.ncbi.nlm.nih.gov/pubmed/24172901
https://www.ncbi.nlm.nih.gov/pubmed/20567988
https://www.ncbi.nlm.nih.gov/pubmed/29500692
https://www.ncbi.nlm.nih.gov/pubmed/26719272

ncbi.nlm.nih.gov

Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. - PubMed - NCBI

Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. Epub 2013 Oct 30. Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S. (13 kB)

↓ Latest messages



ncbi.nlm.nih.gov
[Angiotensin-converting enzyme 2 (ACE2) proteins of different bat species confer variable susceptibility to SARS-CoV entry. - PubMed - NCBI]
Arch Virol. 2010 Oct;155(10):1563-9. doi: 10.1007/s00705-010-0729-6. Epub 2010 Jun 22. Research Support, Non-U.S. Gov't (13 kB) ▾

ncbi.nlm.nih.gov
[Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China During 2009-2016. - PubMed - NCBI]
Virol Sin. 2018 Feb;33(1):87-95. doi: 10.1007/s12250-018-0017-2. Epub 2018 Mar 2. (13 kB) ▾

ncbi.nlm.nih.gov
[Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. - PubMed - NCBI]
J Virol. 2015 Dec 30;90(6):3253-6. doi: 10.1128/JVI.02582-15. Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't (13 kB) ▾

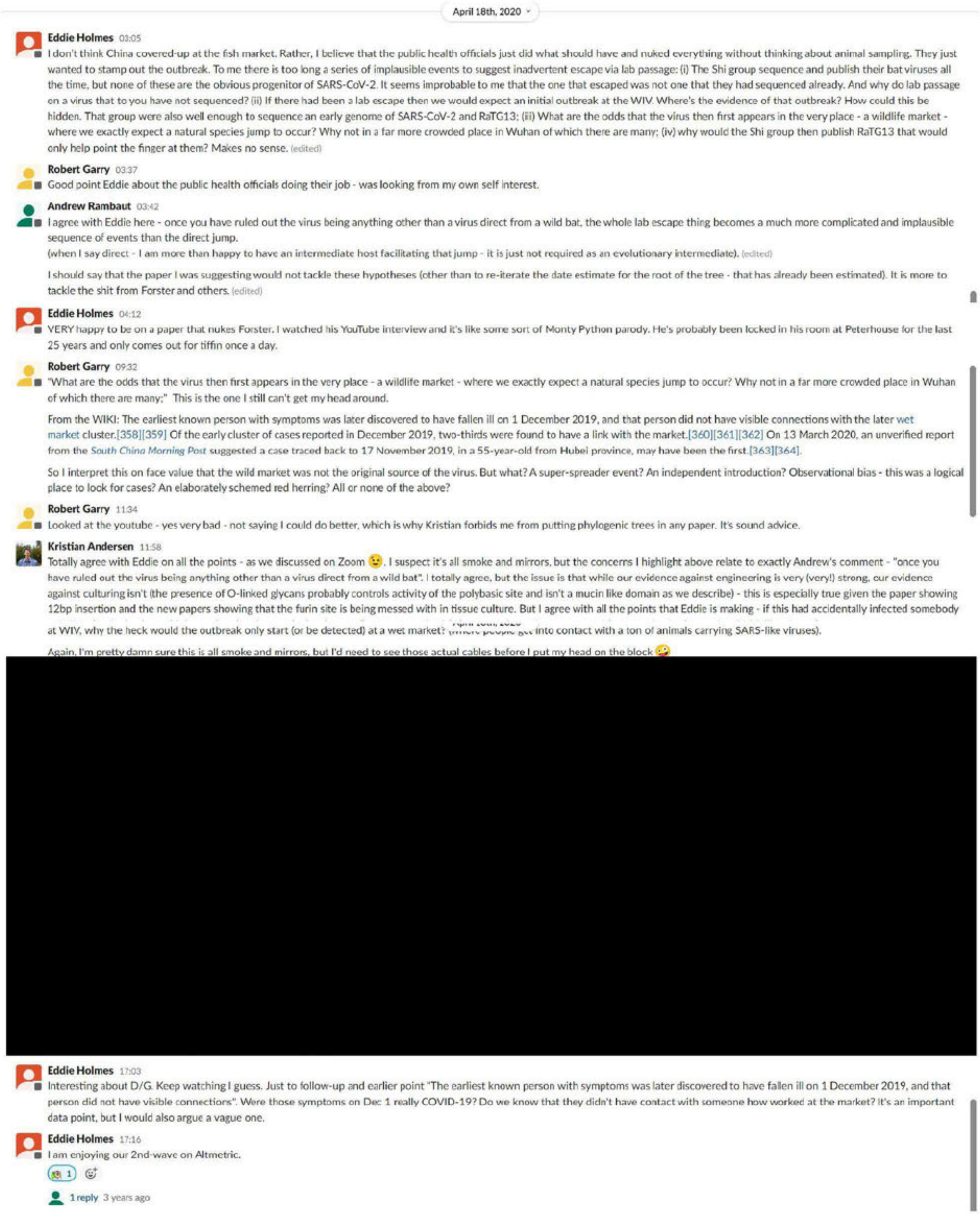
RmYN02 has a rearrangement around that site, but it's not this type of insertion. I agree with you that it's evidence for 'this all occurs naturally', but it still doesn't put a nail in the coffin of that theory

Eddie Holmes 19:05
Let's face it, unless there is a whistleblower from the WIV who is doing to defect and live in the west under a new identity we are NEVER going to know happened in that lab. Never.

Kristian Andersen 19:06
That's my thinking too. But that's why I'm a little worried about these 'cables' - because is it possible that they might have something? I'm putting all of this to typical Trump BS smoke and mirrors (and just plain idiocy), but I'm not quite willing to die on this hill.

Eddie Holmes 19:48
Yes, I'm not dying on a hill either.

Robert Garry 22:48
I pretty sure that "a proper paper on the origins and spread of the virus" can be crafted that will not result in any casualties. And I agree with Andrew that the load of BS is getting pretty hard to take. To Kristian's point 3 - could this " have happened with any random SARS-like CoV" from passage in culture - seems pretty unlikely - that random bat CoV would have had to be very close [>99%] and then by some astronomical chance generated a precise pangolin CoV-like RDB across a pretty broad stretch - that's not to mention the 12 base pair out-of-frame insertion that adds PRRA. Point taken that there truly could be intercepted "cables," but of what? We already know that the Chinese went into deep cover-up mode for example by shutting down the market and destroying the "evidence." It's possible WIV characterized a NCoV-19 isolate earlier than the first noted cases in Dec I suppose, but that doesn't make WIV the proximal origin of the virus. It's also possible that the Chinese knew about a new respiratory virus spreading before the fish market cases - this would be bad public health but consistent with our cryptic human spread model [giving a somewhat more nefarious spin on cryptic]. As Kristian noted they did a lot of science **remarkably** fast.



April 18th, 2020

Eddie Holmes 03:05
I don't think China covered-up at the fish market. Rather, I believe that the public health officials just did what should have and nuked everything without thinking about animal sampling. They just wanted to stamp out the outbreak. To me there is too long a series of implausible events to suggest inadvertent escape via lab passage: (i) The Shi group sequence and publish their bat viruses all the time, but none of these are the obvious progenitor of SARS-CoV-2. It seems improbable to me that the one that escaped was not one that they had sequenced already. And why do lab passage on a virus that to you have not sequenced? (ii) If there had been a lab escape then we would expect an initial outbreak at the WIV. Where's the evidence of that outbreak? How could this be hidden. That group were also well enough to sequence an early genome of SARS-CoV-2 and RaTG13; (iii) What are the odds that the virus then first appears in the very place - a wildlife market - where we exactly expect a natural species jump to occur? Why not in a far more crowded place in Wuhan of which there are many; (iv) why would the Shi group then publish RaTG13 that would only help point the finger at them? Makes no sense. (edited)

Robert Garry 03:37
Good point Eddie about the public health officials doing their job - was looking from my own self interest.

Andrew Rambaut 03:42
I agree with Eddie here - once you have ruled out the virus being anything other than a virus direct from a wild bat, the whole lab escape thing becomes a much more complicated and implausible sequence of events than the direct jump.
(when I say direct - I am more than happy to have an intermediate host facilitating that jump - it is just not required as an evolutionary intermediate). (edited)

I should say that the paper I was suggesting would not tackle these hypotheses (other than to re-iterate the date estimate for the root of the tree - that has already been estimated). It is more to tackle the shit from Forster and others. (edited)

Eddie Holmes 04:12
VERY happy to be on a paper that nukes Forster. I watched his YouTube interview and it's like some sort of Monty Python parody. He's probably been locked in his room at Peterhouse for the last 25 years and only comes out for tiffin once a day.

Robert Garry 09:32
"What are the odds that the virus then first appears in the very place - a wildlife market - where we exactly expect a natural species jump to occur? Why not in a far more crowded place in Wuhan of which there are many;" This is the one I still can't get my head around.

From the WIKI: The earliest known person with symptoms was later discovered to have fallen ill on 1 December 2019, and that person did not have visible connections with the later wet market cluster.[358][359] Of the early cluster of cases reported in December 2019, two-thirds were found to have a link with the market.[360][361][362] On 13 March 2020, an unverified report from the *South China Morning Post* suggested a case traced back to 17 November 2019, in a 55-year-old from Hubei province, may have been the first.[363][364].

So I interpret this on face value that the wild market was not the original source of the virus. But what? A super-spreader event? An independent introduction? Observational bias - this was a logical place to look for cases? An elaborately schemed red herring? All or none of the above?

Robert Garry 11:34
Looked at the youtube - yes very bad - not saying I could do better, which is why Kristian forbids me from putting phylogenic trees in any paper. It's sound advice.

Kristian Andersen 11:58
Totally agree with Eddie on all the points - as we discussed on Zoom 😉. I suspect it's all smoke and mirrors, but the concerns I highlight above relate to exactly Andrew's comment - "once you have ruled out the virus being anything other than a virus direct from a wild bat". I totally agree, but the issue is that while our evidence against engineering is very (very!) strong, our evidence against culturing isn't (the presence of O-linked glycans probably controls activity of the polybasic site and isn't a mucin like domain as we describe) - this is especially true given the paper showing 12bp insertion and the new papers showing that the furin site is being messed with in tissue culture. But I agree with all the points that Eddie is making - if this had accidentally infected somebody at WIV, why the heck would the outbreak only start (or be detected) at a wet market? (where people get into contact with a ton of animals carrying SARS-like viruses).

Again, I'm pretty damn sure this is all smoke and mirrors, but I'd need to see those actual cables before I put my head on the block 🤪

[redacted]

Eddie Holmes 17:03
Interesting about D/G. Keep watching I guess. Just to follow-up and earlier point "The earliest known person with symptoms was later discovered to have fallen ill on 1 December 2019, and that person did not have visible connections". Were those symptoms on Dec 1 really COVID-19? Do we know that they didn't have contact with someone how worked at the market? It's an important data point, but I would also argue a vague one.

Eddie Holmes 17:16
I am enjoying our 2nd-wave on Altmetric.

Robert Garry 17:18
True enough - as is the possible case from mid Nov. If I had a nickel for every person that said they thing they had COVID-19 in January or earlier --- well I would have a couple of dollars. But still it will be interesting to test some of these for antibodies. Yes - well over 30K now - can't see how #1 could be all that far ahead at this junction. (edited)

Robert Garry 18:18
I'm a little disappointed my smackdown of Montagnier, who was pushing the HIV recombinant engineering meme, got so watered down. Maybe it was just the translation to ~~cheese-eating surrender monkey language~~ French.

Eddie Holmes 18:59
It is so like HIV though. A bunch of conspiracy theories over its origin that were resolved through more sampling of widlife.

April 19th, 2020

Andrew Rambaut 04:10
Also like HIV there will be those that just continue to spout nonsense but they will be increasingly irrelevant.

Robert Garry 09:02
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4265931/ (edited)

Very insightful - HIV conspiracies used politically to major effect and very damaging.

https://mbio.asm.org/content/6/4/e01013-15 This paper making the rounds on the conservative underbelly of the Internet - cited as proof of intentional/accidental release of NCoV-19.

> **mBio**
> The Reemergent 1977 H1N1 Strain and the Gain-of-Function Debate
> The 1977-1978 influenza epidemic was probably not a natural event, as the genetic sequence of the virus was nearly identical to the sequences of decades-old strains. While there are several hypotheses that could explain its origin, the possibility that the 1977 epidemic resulted from a laboratory accident has recently gained popularity in discussions about the biosafety risks of gain-of-function (GOF) influenza virus research, as an argument for why this research should not be performed. There is now a moratorium in the United States on funding GOF research while the benefits and risks, including the potential for accident, are analyzed. Given the importance of this historical epidemic to on... Show more
> Sep 1st, 2015

Andrew Rambaut 11:38
Found number 3: https://dimensions.altmetric.com/details/77699394#score

> **dimensions.altmetric.com**
> Report for: Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1
> In the top 5% of all research outputs scored by Altmetric

https://app.dimensions.ai/discover/publication?order=altmetric

April 19th, 2020

> **app.dimensions.ai**
> Dimensions
> Re-imagining discovery and access to research: grants, datasets, publications, citations, clinical trials, patents and policy documents in one place. With more than 100 million publications and 1 billion citations freely available for personal use, Dimensions provides students and researchers access to the data and information they need - with the lowest barriers possible.

Robert Garry 12:54
I find myself rooting for POTUS to say more dumb stuff about the origins of the China virus, possibly poisoning Sino-American relationships for decades. Does this make me a bad person?

Eddie Holmes 17:23
Keep rooting Bob because it is working: now at 31,175. What is #1 though? It's clearly something over which Twitter has gone mad.

April 20th, 2020

Kristian Andersen 13:15
I really want to know who's #1 too... Gotta be quite a wacky paper!!

Separately - this is from Ed Yong - any idea? *"Do you recall a paper or figure recently showing that bats don't actually harbor more viruses than expected for a group of their speciosity?"*

Robert Garry 13:53
Not sure that's the right word - maybe sometime about the numbers of bat species?

April 20th, 2020

image.png

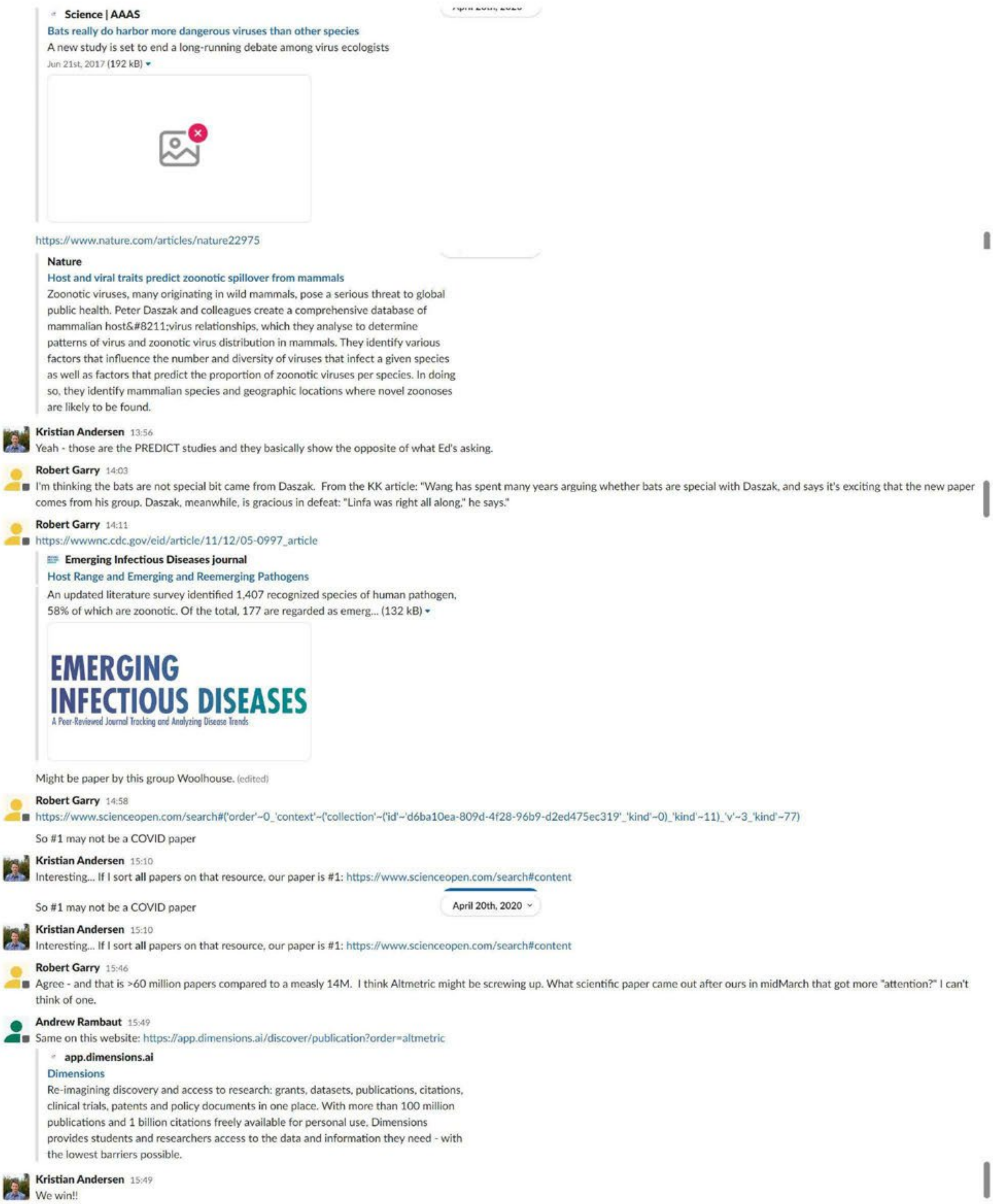
speciosity

spe·cious

speciosity

speciosity

https://www.sciencemag.org/news/2017/06/bats-really-do-harbor-more-dangerous-viruses-other-species



Science | AAAS
Bats really do harbor more dangerous viruses than other species
A new study is set to end a long-running debate among virus ecologists
Jun 21st, 2017 (192 kB)

https://www.nature.com/articles/nature22975

Nature
Host and viral traits predict zoonotic spillover from mammals
Zoonotic viruses, many originating in wild mammals, pose a serious threat to global public health. Peter Daszak and colleagues create a comprehensive database of mammalian host–virus relationships, which they analyse to determine patterns of virus and zoonotic virus distribution in mammals. They identify various factors that influence the number and diversity of viruses that infect a given species as well as factors that predict the proportion of zoonotic viruses per species. In doing so, they identify mammalian species and geographic locations where novel zoonoses are likely to be found.

Kristian Andersen 13:56
Yeah - those are the PREDICT studies and they basically show the opposite of what Ed's asking.

Robert Garry 14:03
I'm thinking the bats are not special bit came from Daszak. From the KK article: "Wang has spent many years arguing whether bats are special with Daszak, and says it's exciting that the new paper comes from his group. Daszak, meanwhile, is gracious in defeat: "Linfa was right all along," he says."

Robert Garry 14:11
https://wwwnc.cdc.gov/eid/article/11/12/05-0997_article

Emerging Infectious Diseases journal
Host Range and Emerging and Reemerging Pathogens
An updated literature survey identified 1,407 recognized species of human pathogen, 58% of which are zoonotic. Of the total, 177 are regarded as emerg... (132 kB)

Might be paper by this group Woolhouse. (edited)

Robert Garry 14:58
https://www.scienceopen.com/search#('order'~0_'context'~('collection'~('id'~'d6ba10ea-809d-4f28-96b9-d2ed475ec319'_'kind'~0)_'kind'~11)_'v'~3_'kind'~77)

So #1 may not be a COVID paper

Kristian Andersen 15:10
Interesting... If I sort **all** papers on that resource, our paper is #1: https://www.scienceopen.com/search#content

So #1 may not be a COVID paper

April 20th, 2020

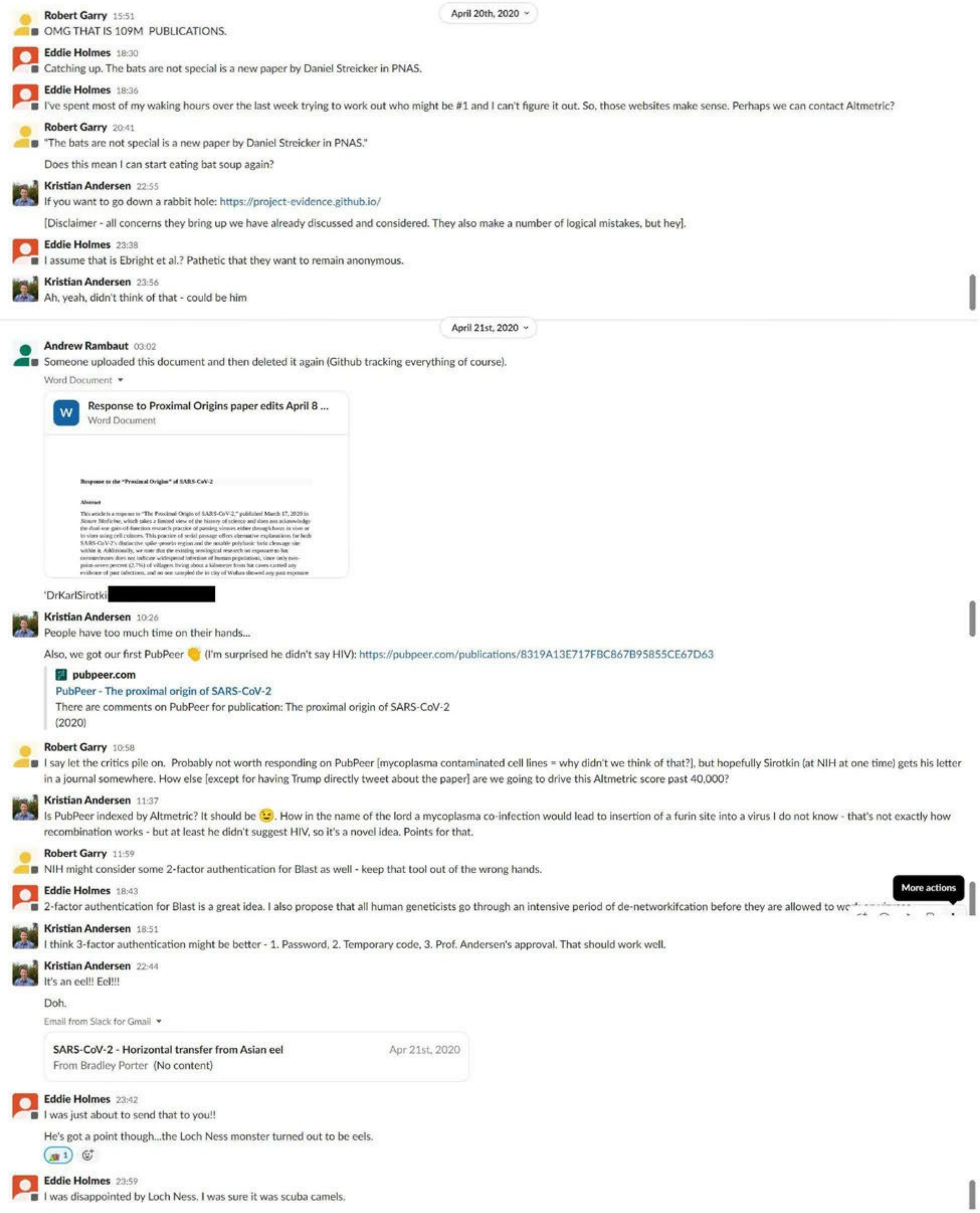
Kristian Andersen 15:10
Interesting... If I sort **all** papers on that resource, our paper is #1: https://www.scienceopen.com/search#content

Robert Garry 15:46
Agree - and that is >60 million papers compared to a measly 14M. I think Altmetric might be screwing up. What scientific paper came out after ours in midMarch that got more "attention?" I can't think of one.

Andrew Rambaut 15:49
Same on this website: https://app.dimensions.ai/discover/publication?order=altmetric

app.dimensions.ai
Dimensions
Re-imagining discovery and access to research: grants, datasets, publications, citations, clinical trials, patents and policy documents in one place. With more than 100 million publications and 1 billion citations freely available for personal use. Dimensions provides students and researchers access to the data and information they need - with the lowest barriers possible.

Kristian Andersen 15:49
We win!!



April 20th, 2020

Robert Garry 15:51
OMG THAT IS 109M PUBLICATIONS.

Eddie Holmes 18:30
Catching up. The bats are not special is a new paper by Daniel Streicker in PNAS.

Eddie Holmes 18:36
I've spent most of my waking hours over the last week trying to work out who might be #1 and I can't figure it out. So, those websites make sense. Perhaps we can contact Altmetric?

Robert Garry 20:41
"The bats are not special is a new paper by Daniel Streicker in PNAS."

Does this mean I can start eating bat soup again?

Kristian Andersen 22:55
If you want to go down a rabbit hole: https://project-evidence.github.io/

[Disclaimer - all concerns they bring up we have already discussed and considered. They also make a number of logical mistakes, but hey].

Eddie Holmes 23:38
I assume that is Ebright et al.? Pathetic that they want to remain anonymous.

Kristian Andersen 23:56
Ah, yeah, didn't think of that - could be him

April 21st, 2020

Andrew Rambaut 03:02
Someone uploaded this document and then deleted it again (Github tracking everything of course).

'DrKarlSirotki[redacted]

Kristian Andersen 10:26
People have too much time on their hands...

Also, we got our first PubPeer 👏 (I'm surprised he didn't say HIV): https://pubpeer.com/publications/8319A13E717FBC867B95855CE67D63

pubpeer.com
PubPeer - The proximal origin of SARS-CoV-2
There are comments on PubPeer for publication: The proximal origin of SARS-CoV-2 (2020)

Robert Garry 10:58
I say let the critics pile on. Probably not worth responding on PubPeer [mycoplasma contaminated cell lines = why didn't we think of that?], but hopefully Sirotkin (at NIH at one time) gets his letter in a journal somewhere. How else [except for having Trump directly tweet about the paper] are we going to drive this Altmetric score past 40,000?

Kristian Andersen 11:37
Is PubPeer indexed by Altmetric? It should be 😉. How in the name of the lord a mycoplasma co-infection would lead to insertion of a furin site into a virus I do not know - that's not exactly how recombination works - but at least he didn't suggest HIV, so it's a novel idea. Points for that.

Robert Garry 11:59
NIH might consider some 2-factor authentication for Blast as well - keep that tool out of the wrong hands.

Eddie Holmes 18:43
2-factor authentication for Blast is a great idea. I also propose that all human geneticists go through an intensive period of de-networkifcation before they are allowed to wc

Kristian Andersen 18:51
I think 3-factor authentication might be better - 1. Password, 2. Temporary code, 3. Prof. Andersen's approval. That should work well.

Kristian Andersen 22:44
It's an eel!! Eel!!!

Doh.

SARS-CoV-2 - Horizontal transfer from Asian eel — Apr 21st, 2020
From Bradley Porter (No content)

Eddie Holmes 23:42
I was just about to send that to you!!

He's got a point though...the Loch Ness monster turned out to be eels.

Eddie Holmes 23:59
I was disappointed by Loch Ness. I was sure it was scuba camels.

Kristian Andersen 00:05
I believe that theory is still being explored.

Robert Garry 07:47
Scuba camels is definitely a thing. It's in Egypt, where they have fruit bats. IIRC camels do have a little betacoronavirus. Like Fox news said about WIV the dots are falling in place. (edited)

image.png ▾

[redacted]

April 27th, 2020 ⌄

Eddie Holmes 18:29
Charming.

Screen Shot 2020-04-28 at 8.28.47 am.png ▾

Not a man made virus you say?
To: Edward Holmes

I am wondering how much the Chinese pay you to publish lies?
You work for the Chinese CDC right, doesn't take an investigative journalist to see your affiliations, you even publish it on your university profile.

You are a traitor.

Kristian Andersen 18:33
Okay, traitor, so how much *are* they actually paying you? I think they got me kinda cheap, so maybe I could have made a better deal.

Eddie Holmes 18:41
Have never paid me a cent, although I did get that presidential plate and a wooden elephant from Yunnan. In many ways I found the following email even more disturbing:

Screen Shot 2020-04-28 at 8.38.30 am.png ▾

animal gene in coronavirus from coral
To: Edward Holmes

Dear Professor Holmes,
The animal gene in the coronavirus is from a coral. This would make sense since the pandemic started at a seafood market? I have attached top result of BLAST of polyprotein against human genome and top result of BLAST of polyprotein against Animalia.

Best regards
John Gardiner

Kristian Andersen 18:48
Well, I can't really blame these people - I mean, I live in a country where the president suggested we treat this by drinking bleach. And blasting it with UV "inside the body, or maybe outside with very strong light". So compared to that, John's a fucking genius - I mean, BLAST = advanced stuff.

April 27th, 2020 ⌄

Eddie Holmes 18:50
Honestly, about 80% of daily inbox is composed of press (e.g. Vanity Fair today), threats and accusations, amazing treatments based on things like bathing in the natural essence of rhubarb and goat's piss, nutters who think they have found something profound, and conspiracy theory loons.

Kristian Andersen 18:55
Sounds remarkably like my inbox... The good thing about that is that I can pretty much just ignore everything coming in and go drink beer instead.

Eddie Holmes 19:00
I drink to that.

April 28th, 2020 ⌄

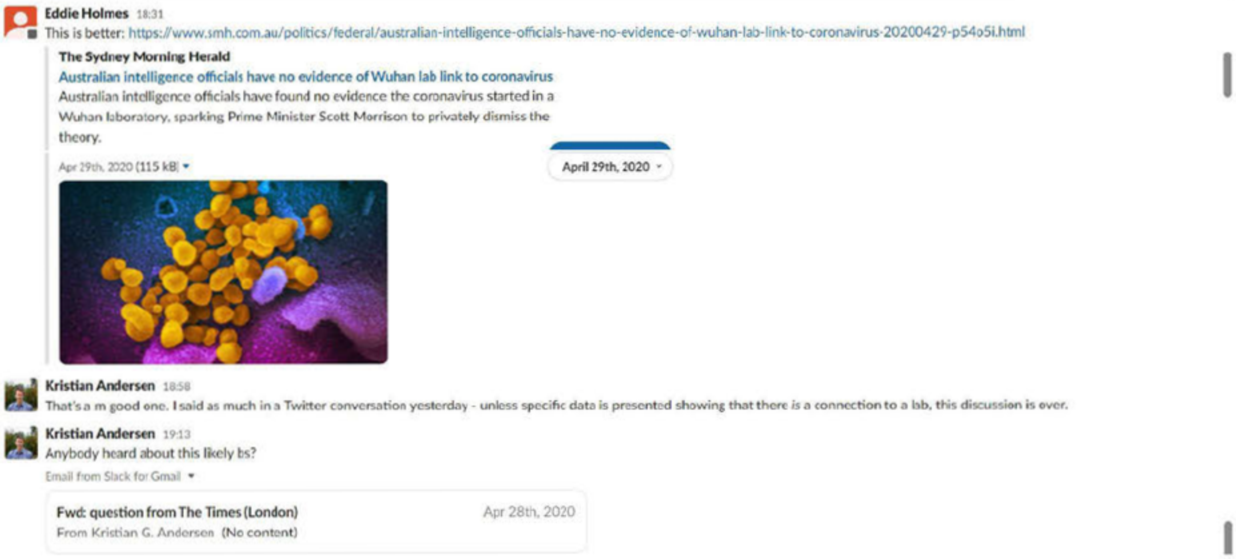
Robert Garry 09:29
https://mercola.fileburst.com/PDF/ExpertInterviewTranscripts/Interview-FrancisBoyle-SARS-COV-2.pdf

I get shit like this - same old same old - email started out calling me a traitor.

https://naijagists.com/zaire-ebola-virus-originated-from-us-bio-warfare-labs-in-west-africa-american-professor-francis-boyle-blows-whistle/

[redacted]

[redacted]



Eddie Holmes 18:31

This is better: https://www.smh.com.au/politics/federal/australian-intelligence-officials-have-no-evidence-of-wuhan-lab-link-to-coronavirus-20200429-p54o5i.html

The Sydney Morning Herald

Australian intelligence officials have no evidence of Wuhan lab link to coronavirus

Australian intelligence officials have found no evidence the coronavirus started in a Wuhan laboratory, sparking Prime Minister Scott Morrison to privately dismiss the theory.

Apr 29th, 2020 (115 kB)

Kristian Andersen 18:58

That's a m good one. I said as much in a Twitter conversation yesterday - unless specific data is presented showing that there *is* a connection to a lab, this discussion is over.

Kristian Andersen 19:13

Anybody heard about this likely bs?

Email from Slack for Gmail

Fwd: question from The Times (London) Apr 28th, 2020

From Kristian G. Andersen (No content)

Eddie Holmes 20:21
I've not heard this. They can't have any more data than we've looked at. I wonder where it will be 'published'. A large prior on this being complete bollocks.

[redacted]

Eddie Holmes 23:21
PDF ▾

Tele 28 April 2020.pdf
PDF

NSW

Coronavirus Australia: Chinese scientists linked to virus probe studied live bats in Australia

Two Chinese scientists — who Western intelligence agencies are looking into as part of their probe into the origins of the global coronavirus contagion — studied live bats in Australia in research jointly funded by the Australian and Chinese governments.

April 29th, 2020 ⌄

Sorry, the cover is the best bit:

April 29th, 2020 ⌄

Such shit. This guy did a bit of his PhD in Australia then went back to WIV.

Kristian Andersen 23:25
Haha. Former student of yours? I thought for a second you'd be the one on the frontpage - Eddie 'Bat Man' Holmes. It's got a nice ring to it.

And this is fucking unbelievable - the stupidity of people and journalists these days...

Eddie Holmes 23:25
I'd be the 'Twat Man'.
🤣 1

April 29th, 2020 ⌄

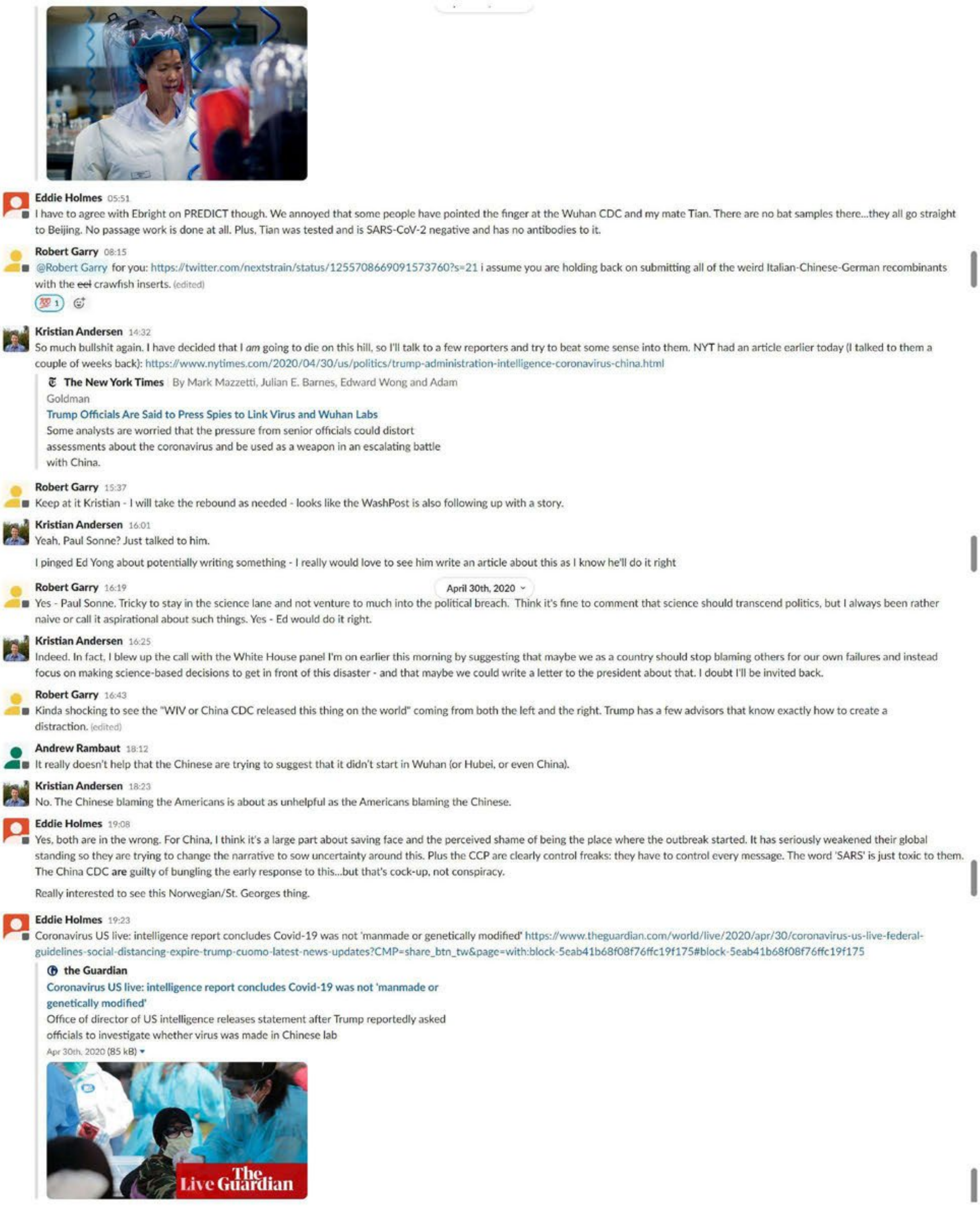
April 30th, 2020 ⌄

Kristian Andersen 01:29
@Robert Garry for you: https://twitter.com/nextstrain/status/1255708669091573760?s=21

Andrew Rambaut 03:49
This is just going on and on.

This article just flips back and forth:
https://www.newsweek.com/controversial-wuhan-lab-experiments-that-may-have-started-coronavirus-pandemic-1500503

> **Newsweek**
> The controversial experiments and Wuhan lab suspected of starting the coronavirus pandemic
> After reporting that Covid-19 occurred naturally, U.S. intelligence modified its stance to say it might have leaked from a lab.
> Apr 27th, 2020 (829 kB) ▾



Eddie Holmes 05:51
I have to agree with Ebright on PREDICT though. We annoyed that some people have pointed the finger at the Wuhan CDC and my mate Tian. There are no bat samples there...they all go straight to Beijing. No passage work is done at all. Plus, Tian was tested and is SARS-CoV-2 negative and has no antibodies to it.

Robert Garry 08:15
@Robert Garry for you: https://twitter.com/nextstrain/status/1255708669091573760?s=21 i assume you are holding back on submitting all of the weird Italian-Chinese-German recombinants with the ~~eel~~ crawfish inserts. (edited)

💯 1

Kristian Andersen 14:32
So much bullshit again. I have decided that I *am* going to die on this hill, so I'll talk to a few reporters and try to beat some sense into them. NYT had an article earlier today (I talked to them a couple of weeks back): https://www.nytimes.com/2020/04/30/us/politics/trump-administration-intelligence-coronavirus-china.html

> **The New York Times** By Mark Mazzetti, Julian E. Barnes, Edward Wong and Adam Goldman
> Trump Officials Are Said to Press Spies to Link Virus and Wuhan Labs
> Some analysts are worried that the pressure from senior officials could distort assessments about the coronavirus and be used as a weapon in an escalating battle with China.

Robert Garry 15:37
Keep at it Kristian - I will take the rebound as needed - looks like the WashPost is also following up with a story.

Kristian Andersen 16:01
Yeah, Paul Sonne? Just talked to him.

I pinged Ed Yong about potentially writing something - I really would love to see him write an article about this as I know he'll do it right

April 30th, 2020

Robert Garry 16:19
Yes - Paul Sonne. Tricky to stay in the science lane and not venture to much into the political breach. Think it's fine to comment that science should transcend politics, but I always been rather naive or call it aspirational about such things. Yes - Ed would do it right.

Kristian Andersen 16:25
Indeed. In fact, I blew up the call with the White House panel I'm on earlier this morning by suggesting that maybe we as a country should stop blaming others for our own failures and instead focus on making science-based decisions to get in front of this disaster - and that maybe we could write a letter to the president about that. I doubt I'll be invited back.

Robert Garry 16:43
Kinda shocking to see the "WIV or China CDC released this thing on the world" coming from both the left and the right. Trump has a few advisors that know exactly how to create a distraction. (edited)

Andrew Rambaut 18:12
It really doesn't help that the Chinese are trying to suggest that it didn't start in Wuhan (or Hubei, or even China).

Kristian Andersen 18:23
No. The Chinese blaming the Americans is about as unhelpful as the Americans blaming the Chinese.

Eddie Holmes 19:08
Yes, both are in the wrong. For China, I think it's a large part about saving face and the perceived shame of being the place where the outbreak started. It has seriously weakened their global standing so they are trying to change the narrative to sow uncertainty around this. Plus the CCP are clearly control freaks: they have to control every message. The word 'SARS' is just toxic to them. The China CDC are guilty of bungling the early response to this...but that's cock-up, not conspiracy.

Really interested to see this Norwegian/St. Georges thing.

Eddie Holmes 19:23
Coronavirus US live: intelligence report concludes Covid-19 was not 'manmade or genetically modified' https://www.theguardian.com/world/live/2020/apr/30/coronavirus-us-live-federal-guidelines-social-distancing-expire-trump-cuomo-latest-news-updates?CMP=share_btn_tw&page=with:block-5eab41b68f08f76ffc19f175#block-5eab41b68f08f76ffc19f175

> **the Guardian**
> Coronavirus US live: intelligence report concludes Covid-19 was not 'manmade or genetically modified'
> Office of director of US intelligence releases statement after Trump reportedly asked officials to investigate whether virus was made in Chinese lab
> Apr 30th, 2020 (85 kB)

Eddie Holmes 19:36
https://www.bbc.com/news/world-us-canada-52496098

BBC News
US intelligence debunks manmade coronavirus theory
US spies say they are still investigating the virus origins, as Mr Trump suggests it came from a lab. (74 kB)

Kristian Andersen 19:43
Yes yes, but our Great Leader sets the record straight with some clear language.

Screen Shot 2020-04-30 at 4.41.45 PM.png

What did President Trump say?

At the White House on Thursday, Mr Trump was asked by a reporter: "Have you seen anything at this point that gives you a high degree of confidence that the Wuhan Institute of Virology was the origin of this virus?"

The president replied: "Yes, I have. Yes, I have. And I think the World Health Organization should be ashamed of themselves because they're like the public relations agency for China."

He added: "Whether they [China] made a mistake, or whether it started off as a mistake and then they made another one, or did somebody do something on purpose?

"I don't understand how traffic, how people weren't allowed into the rest of China, but they were allowed into the rest of the world. That's a bad, that's a hard question for them to answer."

[redacted]