**From:** Saif, Linda
**To:** Liu, Shan-Lu; lishan_su@med.unc.edu
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 1:28:39 PM
**Attachments:** EMI-2019-nCoV_Commentary_LJS_SLL_Refs-rsb.docx

---

Hi

Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!

Regards,

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>

**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

**Commented [BRS1]:** Not a dna virus

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

**Commented [BRS2]:** In Chinese social media

**Commented [BRS3]:** >5,000 nts

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of SARS CoV in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations

**Commented [BRS4]:** No, wildtype was passaged

**Commented [BRS5]:** wildtype

associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

**Commented [BRS6]:** these six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age.....leading to severe pathogenesis in aged, but not young animals [6].

**Commented [BRS7]:** SARS-CoV, as well as its closely related SHC014 bat strain and the chimera all differ by over 6,000 nts as compared with SARS-CoV 2.
Genome identities

Differences between Genomes.xlsx

**Commented [BRS8]:** This is not correct.

But was fully attenuated and displayed reduced virus infection in the airway epithelium as compared to SARS-CoV MA15 which is lethal.

Did not produce lethal disease like wildtype sars, so its attenuated!

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were subject to pause, reviewed and later approved under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

**Commented [BRS9]:** reduced

**Commented [BRS10]:** as written, suggests experiments were done before review. May want to reformulate

**Commented [BRS11]:** PMC6954302 PMC5567817

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.


1. Wang, D., *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang, *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N., *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N., *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D., *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y., *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W., *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F., *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

**From:** Saif, Linda
**To:** Liu, Shan-Lu; Su, Lishan
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 2:38:08 PM
**Attachments:** image001.png
EMI-2019-nCoV_Commentary_Final LJS 2020.docx

---

Thanks—a few minor last edits

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 2:05 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 2:00 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 1:34 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss.
Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

**SARVS-CoV-2: no evidence of a laboratory origin**

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between

the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS

**Formatted:** Font: (Default) Arial, Font color: Black

molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice. It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-~~

~~MA15 can replicate efficiently in the mouse lung, leading to severe pathogiogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 ~~nucleotides~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV

**Formatted:** Highlight

**Formatted:** Line spacing: Double

**Commented [LS1]:** Lishan: see Ralph's comments to revise, as I am confused!

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [BRS2]:** PMC6954302 PMC5567817

sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.

5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Saif, Linda
**To:** Liu, Shan-Lu; Su, Lishan
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 2:54:23 PM
**Attachments:** image001.png
EMI-2019-nCoV_Commentary_Final LJS 2020.docx

---

Sorry just caught the error in the title!

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:38 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks—a few minor last edits

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 2:05 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 2:00 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 1:34 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University

1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the

**Formatted:** Font: (Default) Arial, Font color: Black

severe disease phenotype in mice. It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathoglogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 nucleotide~~st~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is

**Formatted:** Highlight

**Formatted:** Line spacing: Double

**Commented [LS1]:** Lishan: see Ralph's comments to revise, as I am confused!

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [BRS2]:** PMC6954302 PMC5567817

not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb

15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Saif, Linda
**To:** Liu, Shan-Lu; Su, Lishan
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 2:56:06 PM
**Attachments:** image001.png
EMI-2019-nCoV_Commentary_Final LJS2x 2020.docx

---

Also sent prior draft—here is latest one LJS2x

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:54 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Sorry just caught the error in the title!

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:38 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks—a few minor last edits

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 2:05 PM

**To:** "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 2:00 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 1:34 PM

**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry~~have~~ CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the

**Formatted:** Font: (Default) Arial, Font color: Black

severe disease phenotype in mice. It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was ~~fully~~ attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathoglogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were only later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 nucleotide~~s~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical pre-clinical~~IND~~ data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is

**Formatted:** Highlight

**Formatted:** Line spacing: Double

**Formatted:** Highlight

**Commented [LS1]:** Lishan: see Ralph's comments to revise, as I am confused!

**Commented [J2]:** Ralph wants us to clarify that these exp were not restricted when he did them—only later!

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [BRS3]:** PMC6954302 PMC5567817

not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.

6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Cc:** Su, Lishan
**Subject:** FW: [External] Commentary for EMI
**Date:** Wednesday, February 12, 2020 4:09:18 PM
**Attachments:** EMI-2019-nCoV_Commentary_Final for submission .docx
image001.png
image002.png

---

Hi Linda,

Susan Weiss has decided to join the authorship – see the final version attached.

Shan-Lu


THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu


---

**From:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Date:** Wednesday, February 12, 2020 at 4:00 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Re: [External] Commentary for EMI

Shan-LU
I am still in Spain, going home on Saturday.
Yes please add my name as a co-author. This is important!!
Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>

**Date:** Wednesday, February 12, 2020 at 5:26 PM
**To:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me ). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu[3, 5,6.7]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[5] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[6] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[7] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

**References**


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

| | |
|---|---|
| **From:** | Emerging Microbes and Infections |
| **To:** | Liu, Shan-Lu |
| **Cc:** | Liu, Shan-Lu; Saif, Linda; weisssr@pennmedicine.upenn.edu; lishan_su@med.unc.edu |
| **Subject:** | Emerging Microbes & Infections - Manuscript ID TEMI-2020-0121 has been submitted online |
| **Date:** | Wednesday, February 12, 2020 9:55:21 PM |

12-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections

Your manuscript ID is TEMI-2020-0121

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__!!KGKeukY!iZuBSqFeEfVqrLHbkUeoF7XsNXtARiE1kDnvBvwaKd9rBosvuwrxy7vjNjs58A$ and edit your user information as appropriate

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__!!KGKeukY!iZuBSqFeEfVqrLHbkUeoF7XsNXtARiE1kDnvBvwaKd9rBosvuwrxy7vjNjs58A$

Thank you for submitting your manuscript to Emerging Microbes & Infections

Sincerely,
Emerging Microbes & Infections Editorial Office

**From:** Min Yang
**To:** Liu, Shan-Lu; Su, Lishan; Saif, Linda; Weiss, Susan
**Cc:** Lu, Shan
**Subject:** Re: EMI commentary
**Date:** Thursday, February 13, 2020 1:10:05 AM
**Attachments:** image001.png

---

Dear Dr Liu,

Thank you for your quick response.

This is to confirm that we have already received your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin". (TEMI-2020-0121)

Thanks and regards,

Min Yang

*Emerging Microbes & Infections* (EMI) Editorial Office
4F Fuxing Building
131 Dongan Road
Shanghai
China
Tel: 86-21-54237992
E-mail: min.yang@emi2012.org

---

发件人: "Liu, Shan-Lu" <liu.6244@osu.edu>
日期: 2020年2月13日 星期四 上午11:25
收件人: Min Yang <min.yang@emi2012.org>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
抄送: "Lu, Shan" <Shan.Lu@umassmed.edu>
主题: Re: EMI commentary

Min:

It should have been successfully submitted. See below email:

12-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections.

Your manuscript ID is TEMI-2020-0121.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to

ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!klcqOriA-xIzLyrzwwKWtghNAQgvfbCh7pqavzMYm77fJJsm_iShbXJWIKEtRML7ExI$ and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!klcqOriA-xIzLyrzwwKWtghNAQgvfbCh7pqavzMYm77fJJsm_iShbXJWIKEtRML7ExI$ .

Thank you for submitting your manuscript to Emerging Microbes & Infections.

Sincerely,
Emerging Microbes & Infections Editorial Office

---

**From:** Min Yang <min.yang@emi2012.org>
**Date:** Wednesday, February 12, 2020 at 10:17 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Re: EMI commentary

Dear Dr Liu,

Thank you for your support to EMI.

According to the attachment, it looks like your submission is a DRAFT still which has not been submitted successfully yet.

Could you please check and confirm?

Thanks and regards,

Min Yang

*Emerging Microbes & Infections* (EMI) Editorial Office
4F Fuxing Building
131 Dongan Road
Shanghai
China
Tel: 86-21-54237992
E-mail: min.yang@emi2012.org

---

发件人: "Liu, Shan-Lu" <liu.6244@osu.edu>
日期: 2020年2月13日 星期四 上午10:58
收件人: "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

抄送: Min Yang <min.yang@emi2012.org>, "Lu, Shan" <Shan.Lu@umassmed.edu>
主题: EMI commentary

Dear all,

I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

**From:** Liu, Shan-Lu
**To:** Su, Lishan; Saif, Linda
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 3:34:29 PM
**Attachments:** EMI-2019-nCoV_Commentary_Final for submission .docx
image001.png
image002.png

---

Hi Linda and Lishan,

I have finalized it, please take a look at it and let me know.

Not sure if abstract and acknowledgment are needed at this point. Will check with the editor.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 3:17 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>, "Saif, Linda" <saif.2@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu and Linda:
I have incorporated all comments and added the two references (in both text and endnote file).
Please do a final proof read, and finalize it.
Thanks,

-Lishan

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 at 3:03 PM

**To:** "Saif, Linda" <saif.2@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks Linda, all good!

Shan-Lu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 2:56 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Also sent prior draft—here is latest one LJS2x
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:54 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Sorry just caught the error in the title!
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:38 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks—a few minor last edits
Linda J. Saif, PhD
Distinguished University Professor

Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 2:05 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 2:00 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 1:34 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss.

Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

**References**


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Cc:** Su, Lishan
**Subject:** Re: [External] Commentary for EMI
**Date:** Wednesday, February 12, 2020 5:00:51 PM
**Attachments:** EMI-2019-nCoV_Commentary_Final for submission .docx
image001.png
image002.png
image003.png

---

Thank you, Linda. Susan is in Barcelona, with no comments.

I have made all your requested changes, but would like to check on your suggestion for Ralph's point. I thought the word "later" is sufficient. See attached updated version and let me know if there are still errors.

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research).

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 4:37 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: [External] Commentary for EMI

Hi All
There were a few minor edits on this prior draft. Did Susan provide any edits?
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 4:09 PM
**To:** Linda Saif <saif.2@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: [External] Commentary for EMI

Hi Linda,

Susan Weiss has decided to join the authorship – see the final version attached.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Date:** Wednesday, February 12, 2020 at 4:00 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Re: [External] Commentary for EMI

Shan-LU

I am still in Spain, going home on Saturday.

Yes please add my name as a co-author. This is important!!

Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>

**Date:** Wednesday, February 12, 2020 at 5:26 PM

**To:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>

**Subject:** [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me ). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473

Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# SARS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu[3, 5,6.7]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[5] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[6] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[7] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring

pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

**References**


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

**From:** Saif, Linda
**To:** Liu, Shan-Lu
**Cc:** Su, Lishan
**Subject:** Re: [External] Commentary for EMI
**Date:** Wednesday, February 12, 2020 4:37:32 PM
**Attachments:** image001.png
image002.png
EMI-2019-nCoV_Commentary_Final LJS2x 2020.docx

---

Hi All

There were a few minor edits on this prior draft. Did Susan provide any edits?

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 4:09 PM
**To:** Linda Saif <saif.2@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: [External] Commentary for EMI

Hi Linda,

Susan Weiss has decided to join the authorship – see the final version attached.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Date:** Wednesday, February 12, 2020 at 4:00 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Re: [External] Commentary for EMI

Shan-LU
I am still in Spain, going home on Saturday.
Yes please add my name as a co-author. This is important!!
Is the new virus now names SARS-2; maybe not a good name – should be different from SARS

I hope I am not too late

susan

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 at 5:26 PM
**To:** "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** [External] Commentary for EMI

Dear Susan,

Hope your trip back to Philly was safe and pleasant.

Dr. Lishan Su at UNC and I have just wrapped up a commentary, at invitation by the editor in chief of "Emerging Microbes and Infections", Dr. Shan Lu (don't get confused, it's not me ). We are wondering if you would be interested in joining us as a coauthor. We feel that this is an important issue, and as scientist, we should clear this thing up if we can.

Please let us know as soon as possible, as we will try to submit it today. If you feel someone else (other coronavirus experts), whom might be interested in becoming a coauthor, kindly let us know as well.

Best wishes.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program

Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry~~have~~ CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the

**Formatted:** Font: (Default) Arial, Font color: Black

severe disease phenotype in mice. It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was ~~fully~~ attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathoglogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were only later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 nucleotide~~s~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical pre-clinical~~IND~~ data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is

**Formatted:** Highlight

**Formatted:** Line spacing: Double

**Formatted:** Highlight

**Commented [LS1]:** Lishan: see Ralph's comments to revise, as I am confused!

**Commented [J2]:** Ralph wants us to clarify that these exp were not restricted when he did them—only later!

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [BRS3]:** PMC6954302 PMC5567817

not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.

6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Saif, Linda
**To:** Liu, Shan-Lu
**Subject:** FW: Lancet Statement Posted!
**Date:** Tuesday, February 18, 2020 3:00:45 PM
**Attachments:** Lancet Statement 2020.pdf

---

Hi Shan-lu,
Here is the statement with the opportunity for others to sign. Please distribute to colleagues!
Thanks
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Peter Daszak <daszak@ecohealthalliance.org>
**Date:** Tuesday, February 18, 2020 at 12:44 PM
**To:** Christian Drosten <drosten@virology-bonn.de>, John Mackenzie <J.Mackenzie@curtin.edu.au>, Jonna Mazet <jkmazet@ucdavis.edu>, "llmpoon@hku.hk" <llmpoon@hku.hk>, Larry Madoff <lmadoffpmm@gmail.com>, Prof Lam Sai Kit <sklam@nipahvirus.org>, "j.farrar@wellcome.ac.uk" <j.farrar@wellcome.ac.uk>, "peter.palese@mssm.edu" <peter.palese@mssm.edu>, Dennis Carroll <dcarroll008@gmail.com>, "bernard.roizman@bsd.uchicago.edu" <bernard.roizman@bsd.uchicago.edu>, "Stanley-Perlman@uiowa.edu" <Stanley-Perlman@uiowa.edu>, Charles H Calisher <calisher@cybersafe.net>, "a.e.gorbalenya@lumc.nl" <a.e.gorbalenya@lumc.nl>, "L.Enjuanes@cnb.csic.es" <L.Enjuanes@cnb.csic.es>, "b.haagmans@erasmusmc.nl" <b.haagmans@erasmusmc.nl>, "rcolwell@umiacs.umd.edu" <rcolwell@umiacs.umd.edu>, "JMHUGHE@emory.edu" <jmhughe@emory.edu>, Hume Field <hume.field@ecohealthalliance.org>, "jlubroth@gmail.com" <jlubroth@gmail.com>, Linda Saif <saif.2@osu.edu>, "William B. Karesh" <karesh@ecohealthalliance.org>, "rbcorley@bu.edu" <rbcorley@bu.edu>, "Keusch, Gerald T" <keusch@bu.edu>, "Subbarao, Kanta" <kanta.subbarao@influenzacentre.org>, "J.Golding@wellcome.ac.uk" <J.Golding@wellcome.ac.uk>, Mike Turner <M.Turner@wellcome.ac.uk>
**Cc:** Hongying Li <li@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>
**Subject:** Lancet Statement Posted!

Dear All,

Our statement is live as of just a few minutes ago!

https://www.thelancet.com/lancet/article/s0140-6736(20)30418-9

Please take time to send this out via twitter, email to your networks, post on your institution or other websites, and distribute as widely as possible to get the word out. Include the link too ( http://chng.it/SDpTB9Kf ), so other people can register their support of the statement.

I really want to thank all of you for rallying for this - especially with such a short timeline. This looks terrific and I know it will do a world of good towards buoying the spirits of our colleagues in China and gaining an ear from those in policy to support collaborative, open approaches to fighting this as well as future outbreaks.

Cheers,

Peter

**Peter Daszak**
*President*

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +[redacted]
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

*EcoHealth Alliance develops science-based solutions tp prevent pandemics and promote conservation.*

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Subject:** Re: Revised commentary for EMI - final!
**Date:** Tuesday, February 18, 2020 11:05:34 AM
**Attachments:** image001.png

---

Thanks. Would love to see it in Lancet, so please chare.

Shan-Lu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Tuesday, February 18, 2020 at 11:00 AM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: Revised commentary for EMI - final!

Thanks—Good seminar this AM and so glad we could access it.
I will send you a copy of joint correspondence on SARS-CoV-2 initiated by Peter Daszak that will be published today in Lancet!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Tuesday, February 18, 2020 at 10:55 AM
**To:** Linda Saif <saif.2@osu.edu>
**Subject:** Re: Revised commentary for EMI - final!

Hi Linda,

I will be out for an NIH virology B study section Feb 20-21 so will miss your webinar. I am sure it will go well!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Tuesday, February 18, 2020 at 9:37 AM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: Revised commentary for EMI - final!

Can you ask Speaker if he tried camel strains in his model and how do mice react since camel strains less pathogenic in camels?

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Monday, February 17, 2020 at 10:15 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

I agree too

Shan-Lu Liu sent from iPhone

On Feb 17, 2020, at 9:54 PM, Su, Lishan <lishan_su@med.unc.edu> wrote:

I agree. We should try to cite the link if possible.

-Lishan

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Monday, February 17, 2020 at 9:25 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

Hi all
Since this is so relevant to our commentary, is it possible to cite it in our commentary?
Thanks
Linda

Sent from my iPhone

> On Feb 17, 2020, at 6:12 PM, Liu, Shan-Lu <liu.6244@osu.edu> wrote:
>
> See a very relevant online posting:
>
> # The Proximal Origin of SARS-CoV-2
>
> http://virological.org/t/the-proximal-origin-of-sars-cov-2/398
>
> Shan-Lu
>
> ---
>
> **From:** "Saif, Linda" <saif.2@osu.edu>
> **Date:** Sunday, February 16, 2020 at 7:20 PM

**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

Attached
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Sunday, February 16, 2020 3:14 PM
**To:** "Lu, Shan" <Shan.Lu@umassmed.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

See a typo in the title, and the last sentence as we had discussed.
Thanks,

-Lishan

---

**From:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Date:** Sunday, February 16, 2020 at 1:55 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** RE: Revised commentary for EMI - final!

Good to me.

---

**From:** Liu, Shan-Lu <liu.6244@osu.edu>
**Sent:** Sunday, February 16, 2020 1:45 PM
**To:** Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
**Cc:** Lu, Shan <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu

<image001.png>
Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>
**Date:** Sunday, February 16, 2020 at 1:38 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

<image001.png>
Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and

Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

**From:** Weiss, Susan
**To:** Liu, Shan-Lu; Saif, Linda; Su, Lishan; Lu, Shan
**Subject:** Re: [External] Re: Revised commentary for EMI - final!
**Date:** Monday, February 17, 2020 6:58:27 PM
**Attachments:** image001.png

---

Thanks, this is good

susan

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Monday, February 17, 2020 at 6:13 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** [External] Re: Revised commentary for EMI - final!

See a very relevant online posting:

# The Proximal Origin of SARS-CoV-2

http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

Shan-Lu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Sunday, February 16, 2020 at 7:20 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

Attached
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Sunday, February 16, 2020 3:14 PM

**To:** "Lu, Shan" <Shan.Lu@umassmed.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

See a typo in the title, and the last sentence as we had discussed.
Thanks,

-Lishan

---

**From:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Date:** Sunday, February 16, 2020 at 1:55 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** RE: Revised commentary for EMI - final!

Good to me.

---

**From:** Liu, Shan-Lu <liu.6244@osu.edu>
**Sent:** Sunday, February 16, 2020 1:45 PM
**To:** Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
**Cc:** Lu, Shan <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>

**Date:** Sunday, February 16, 2020 at 1:38 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Subject:** Re: Revised commentary for EMI - final!
**Date:** Tuesday, February 18, 2020 9:44:01 AM

---

Yes

Shan-Lu Liu sent from iPhone

> On Feb 18, 2020, at 9:37 AM, Saif, Linda <saif.2@osu.edu> wrote:
>
> Can you ask Speaker if he tried camel strains in his model and how do mice react since camel strains less pathogenic in camels?
>
> Linda J. Saif, PhD
> Distinguished University Professor
> Food Animal Health Research Program
> OARDC/The Ohio State University
> 1680 Madison Ave
> Wooster, Oh 44691
>
> ---
>
> **From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
> **Date:** Monday, February 17, 2020 at 10:15 PM
> **To:** "Su, Lishan" <lishan_su@med.unc.edu>
> **Cc:** Linda Saif <saif.2@osu.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
> **Subject:** Re: Revised commentary for EMI - final!
>
> I agree too
>
> Shan-Lu Liu sent from iPhone
>
> > On Feb 17, 2020, at 9:54 PM, Su, Lishan <lishan_su@med.unc.edu> wrote:
> >
> > I agree. We should try to cite the link if possible.
> >
> > -Lishan
> >
> > ---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Monday, February 17, 2020 at 9:25 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Cc:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

Hi all
Since this is so relevant to our commentary, is it possible to cite it in our commentary?
Thanks
Linda

Sent from my iPhone

> On Feb 17, 2020, at 6:12 PM, Liu, Shan-Lu <liu.6244@osu.edu> wrote:
>
> See a very relevant online posting:
>
> **The Proximal Origin of SARS-CoV-2**
>
> http://virological.org/t/the-proximal-origin-of-sars-cov-2/398
>
> Shan-Lu
>
> ---
>
> **From:** "Saif, Linda" <saif.2@osu.edu>
> **Date:** Sunday, February 16, 2020 at 7:20 PM
> **To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Lu, Shan" <Shan.Lu@umassmed.edu>, Shan-Lu Liu <liu.6244@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
> **Subject:** Re: Revised commentary for EMI - final!
>
> Attached

Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Sunday, February 16, 2020 3:14 PM
**To:** "Lu, Shan" <Shan.Lu@umassmed.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

See a typo in the title, and the last sentence as we had discussed.
Thanks,

-Lishan

---

**From:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Date:** Sunday, February 16, 2020 at 1:55 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** RE: Revised commentary for EMI - final!

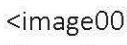
Good to me.

---

**From:** Liu, Shan-Lu <liu.6244@osu.edu>
**Sent:** Sunday, February 16, 2020 1:45 PM
**To:** Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
**Cc:** Lu, Shan <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu


Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>

**Date:** Sunday, February 16, 2020 at 1:38 PM

**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>

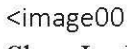
**Subject:** Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu


Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210

Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

**From:** Liu, Shan-Lu
**To:** Su, Lishan; Saif, Linda; Weiss, Susan
**Cc:** Lu, Shan
**Subject:** Revised commentary for EMI - final!
**Date:** Sunday, February 16, 2020 1:44:54 PM
**Attachments:** Liu et al_EMI Commentary_Revision_Final.docx
image001.png
image002.png

---

Please look at this new version, sorry!

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>
**Date:** Sunday, February 16, 2020 at 1:38 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.

Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# No Credible evidence supporting claims of the laborary engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,
Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,
Ohio Agricultural Research and Development Center, CFAES
Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural

evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed

to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already

exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a

virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

| | |
|---|---|
| **From:** | Liu, Shan-Lu |
| **To:** | Su, Lishan; Saif, Linda; Weiss, Susan |
| **Cc:** | Lu, Shan |
| **Subject:** | Revised commentary for EMI |
| **Date:** | Sunday, February 16, 2020 1:38:44 PM |
| **Attachments:** | Liu et al_EMI Commentary_revision_Feb 16"2020.docx<br>image001.png |

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu


Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# No credible evidence supporting claims of the laborary engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,
Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,
Ohio Agricultural Research and Development Center, CFAES
Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a

wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to

use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that

could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should

emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerging Microbes & Infections. 2020 Feb 14. 9 (1): 378-381. DOI: 10.1080/22221751.2020.1727299.

**From:** Saif, Linda
**To:** Su, Lishan; Lu, Shan; Liu, Shan-Lu; Weiss, Susan
**Subject:** Re: Revised commentary for EMI - final!
**Date:** Sunday, February 16, 2020 7:20:04 PM
**Attachments:** image001.png
Liu et al_EMI Commentary_Revision_Final-sls.docx

---

Attached

Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Sunday, February 16, 2020 3:14 PM
**To:** "Lu, Shan" <Shan.Lu@umassmed.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** Re: Revised commentary for EMI - final!

See a typo in the title, and the last sentence as we had discussed.
Thanks,

-Lishan

---

**From:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Date:** Sunday, February 16, 2020 at 1:55 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** RE: Revised commentary for EMI - final!

Good to me.

---

**From:** Liu, Shan-Lu <liu.6244@osu.edu>
**Sent:** Sunday, February 16, 2020 1:45 PM
**To:** Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
**Cc:** Lu, Shan <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>

**Date:** Sunday, February 16, 2020 at 1:38 PM

**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>

**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>

**Subject:** Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# No ~~Credible~~ credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,
Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,
Ohio Agricultural Research and Development Center, CFAES
Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a

close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable

to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that

could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More

studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.~~We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.~~

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

**From:** Su, Lishan
**To:** Lu, Shan; Liu, Shan-Lu; Saif, Linda; Weiss, Susan
**Subject:** Re: Revised commentary for EMI - final!
**Date:** Sunday, February 16, 2020 3:17:09 PM
**Attachments:** image001.png
Liu et al_EMI Commentary_Revision_Final-sls.docx

---

See a typo in the title, and the last sentence as we had discussed.
Thanks,

-Lishan

---

**From:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Date:** Sunday, February 16, 2020 at 1:55 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Subject:** RE: Revised commentary for EMI - final!

Good to me.

---

**From:** Liu, Shan-Lu <liu.6244@osu.edu>
**Sent:** Sunday, February 16, 2020 1:45 PM
**To:** Su, Lishan <lishan_su@med.unc.edu>; Saif, Linda <saif.2@osu.edu>; Weiss, Susan <weisssr@pennmedicine.upenn.edu>
**Cc:** Lu, Shan <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI - final!

Please look at this new version, sorry!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>
**Date:** Sunday, February 16, 2020 at 1:38 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Weiss, Susan" <weisssr@pennmedicine.upenn.edu>
**Cc:** "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** Revised commentary for EMI

Dear All,

Following some discussions in the weekend, I had made a change in the title, and also added a sentence to the end of commentary – the latter is based on the concerns of lab safety for this new virus and also other viruses previously.

Let me know what you think.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# No ~~Credible~~ credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,
Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,
OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,
Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,
The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,
Ohio Agricultural Research and Development Center, CFAES
Department of Veterinary Preventive Medicine,
The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural

evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed

to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already

exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses

with such great public health threats must be handled properly in the laboratory and also properly regulated by scientific community and governments. ~~We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, such a virus, and closely related, do pose great public health threats and must be handled properly in the laboratory and also properly regulated by governments and scientific community.~~

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.
15. Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378-381.

**From:** Emerging Microbes and Infections
**To:** Liu, Shan-Lu; Saif, Linda; weisssr@pennmedicine.upenn.edu; lishan_su@med.unc.edu
**Subject:** Emerging Microbes & Infections - Decision on Manuscript ID TEMI-2020-0121
**Date:** Thursday, February 13, 2020 9:49:29 AM

13-Feb-2020

Dear Professor Liu:

Ref: SARS-CoV-2: no evidence of a laboratory origin

Our reviewers have now considered your paper and have recommended publication in Emerging Microbes & Infections. We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter, along with those of the editor who coordinated the review of your paper.

You will receive proofs for checking, and instructions for transfer of copyright in due course.

The publisher also requests that proofs are checked through the publisher's tracking system and returned within 48 hours of receipt.

Taylor & Francis strives to be compliant with the open access (OA) policies of the major national and international funding bodies. Information about funder compliance and OA policies can be found on our Author Services website. Details of our Green OA policy, including deposit in institutional and subject repositories, can be found on the Sharing your work (https://urldefense.com/v3/__http://authorservices.taylorandfrancis.com/sharing-your-work/__;!!KGKeukY!gLRVkVTBooblmp3n3FBf74RjT1VCSRXFKKIf47wWHj0jB5i5PHNQJrpQtwG4AQ$ ) page. We recommend that you retain your Accepted Manuscript for this purpose.

For more information regarding the versions of your article that can be used to meet Green OA requirements, and the embargo period that applies, please see the open access journal finder (https://urldefense.com/v3/__http://authorservices.taylorandfrancis.com/journal-list/__;!!KGKeukY!gLRVkVTBooblmp3n3FBf74RjT1VCSRXFKKIf47wWHj0jB5i5PHNQJrqR9F4xLg$ ) page.

Thank you for your contribution to Emerging Microbes & Infections and we look forward to receiving further submissions from you.

Sincerely,

Shan Lu
Editor-in-Chief
Emerging Microbes & Infections

Review Editor Comments to the Author:
EMI would like to thank the authors for providing a timely piece. It will have major impact to clear many people's confusion.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
This is a timely commentary. It is perfectly written. All four authors are well established virologists. I suggest to publish it right away.

| | |
|---|---|
| **From:** | Emerging Microbes and Infections |
| **To:** | Liu, Shan-Lu |
| **Cc:** | Liu, Shan-Lu; Saif, Linda; weisssr@pennmedicine.upenn.edu; lishan_su@med.unc.edu |
| **Subject:** | Emerging Microbes & Infections - Manuscript ID TEMI-2020-0121 has been submitted online |
| **Date:** | Thursday, February 13, 2020 9:11:20 AM |

13-Feb-2020

Dear Professor Liu:

Your manuscript entitled "SARS-CoV-2: no evidence of a laboratory origin" has been successfully submitted online and is presently being given full consideration for publication in Emerging Microbes & Infections.

Your manuscript ID is TEMI-2020-0121.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!g1on9C-9Z6nI-dSjtB5Ub-UfJstd8c_r64qQXOqSWkMHVLgs8YcAW0auDGDzEQ$ and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://urldefense.com/v3/__https://mc.manuscriptcentral.com/temi__;!!KGKeukY!g1on9C-9Z6nI-dSjtB5Ub-UfJstd8c_r64qQXOqSWkMHVLgs8YcAW0auDGDzEQ$ .

Thank you for submitting your manuscript to Emerging Microbes & Infections.

Sincerely,
Emerging Microbes & Infections Editorial Office

| | |
|---|---|
| **From:** | Liu, Shan-Lu |
| **To:** | Su, Lishan; Saif, Linda; Weiss, Susan |
| **Cc:** | min.yang@emi2012.org; Lu, Shan |
| **Subject:** | EMI commentary |
| **Date:** | Wednesday, February 12, 2020 9:58:16 PM |
| **Attachments:** | EMI_Commentary_submitted.pdf<br>image001.png |

Dear all,

I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu


Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu


# SARS-CoV-2: no evidence of a laboratory origin

| | |
|---|---|
| Journal: | *Emerging Microbes & Infections* |
| Manuscript ID | Draft |
| Manuscript Type: | Commentary |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Liu, Shan-Lu; The Ohio State University, Infectious Diseases Institute<br>Saif, Linda J.; The Ohio State University<br>Weiss, Susan; University of Pennsylvania<br>Su, Lishan; University of North Carolina at Chapel Hill |
| Keywords: | SARS-CoV-2, COIVD-2019, origin, evolution |
| Abstract: | |
| | |

# SARS-CoV-2: no evidence of a laboratory origin

Shan-Lu Liu [1, 2,3,4], Linda J. Saif [4,5], Susan Weiss [6], and Lishan Su [7]

[1] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[2] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[3] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

[4] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[6] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[7] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.

16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

**From:** Liu, Shan-Lu
**To:** Lu, Shan
**Cc:** Su, Lishan; Saif, Linda; Weiss, Susan
**Subject:** EMI commentary
**Date:** Wednesday, February 12, 2020 5:12:43 PM
**Attachments:** EMI-2019-nCoV_Commentary_Final for submission .docx

---

Hi Shan,

Attached please find the final version of the commentary for your consideration to be published at EMI.

Kindly advise.

Regards.

Shan-Lu

# SARS-CoV-2: no evidence of a laboratory origin

Lishan Su [1], and Linda J. Saif [2,3], Susan Weiss [4], and Shan-Lu Liu [3, 5,6.7]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbiology, Perelman School of Medicine,

University of Pennsylvania, Philadelphia, Pennsylvania, USA

[5] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[6] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[7] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact: Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding sequences, and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that

RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-

director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo [15, 16], providing critical pre-clinical data that has led to the ongoing clinical trials in China and is critical for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

**References**


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015

Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.

8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.
15. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017 Jun 28;9(396). doi: 10.1126/scitranslmed.aal3653. PubMed PMID: 28659436; PubMed Central PMCID: PMCPMC5567817.
16. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020 Jan 10;11(1):222. doi: 10.1038/s41467-019-13940-6. PubMed PMID: 31924756; PubMed Central PMCID: PMCPMC6954302.

**From:** Su, Lishan
**To:** Liu, Shan-Lu; Saif, Linda
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 3:17:49 PM
**Attachments:** image001.png
EMI-2019-nCoV_Commentary_Final LJS 2020ls.docx
2019 CoV Copy.enl

---

Shan-Lu and Linda:
I have incorporated all comments and added the two references (in both text and endnote file).
Please do a final proof read, and finalize it.
Thanks,

-Lishan

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 at 3:03 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks Linda, all good!

Shan-Lu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 2:56 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Also sent prior draft—here is latest one LJS2x
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:54 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Sorry just caught the error in the title!
Linda J. Saif, PhD

Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** Linda Saif <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 2:38 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Thanks—a few minor last edits
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 2:05 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute

Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 2:00 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 1:34 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM

**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between

the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. ~~These six mutations were reintroduced into a SARS~~

**Formatted:** Font: (Default) Arial, Font color: Black, Strikethrough

~~molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice~~ (Not necessary here, I had deleted these details from previous versions to shorten the description of the study). It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes ~~regardless of~~in aged mice ~~Importantly,~~

**Formatted:** Font: (Default) Arial, Font color: Black
**Formatted:** Not Strikethrough
**Formatted:** Not Strikethrough
**Formatted:** Not Strikethrough
**Formatted:** Not Strikethrough

~~SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogiogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (~~from Oct. 2014 to Dec. 2017;~~ https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >~~5~~5,000 nucleotide~~nt~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad ~~-based~~spectrum inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo (Sheahan, T.P. et al. Sci Transl Med 9 (2017), Sheahan, T.P. et al. Nat Commun 11, 222 (2020) ), providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a

**Formatted:** Highlight

**Formatted:** Line spacing: Double

**Commented [LS1]:** Lishan: see Ralph's comments to revise, as I am confused!

**Formatted:** Highlight

manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In aA rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.

4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Liu, Shan-Lu
**To:** Su, Lishan; Saif, Linda
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 2:05:52 PM
**Attachments:** EMI-2019-nCoV_Commentary_LJS_SLL_Refs-rsb.docx
EMI-2019-nCoV_Commentary_Final LJS 2020.docx
image001.png

---

Hi Lishan and Linda,

I have just tried to incorporate Ralph's comments into the version from Linda to make a new "final" version, please see attached.

Lishan: you will need to add two new references for Ralph's new sentences. Send me the updated new Endote, along with your final version.

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Su, Lishan" <lishan_su@med.unc.edu>
**Date:** Wednesday, February 12, 2020 at 2:00 PM
**To:** "Saif, Linda" <saif.2@osu.edu>, Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Shan-Lu:

I will incorporate his comments, if needed, in the final version from you, and send to you for a real final version.
Best,

-Lishan

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 1:34 PM
**To:** "Liu, Shan-Lu" <liu.6244@osu.edu>, "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** FW: A commentary on 2019 nCoV vs lab engineered viruses

Hi
Please note that Ralph made these changes on an earlier copy sent to him so hopefully the 2 of you can incorporate them into the updated draft I sent this AM!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 12:32 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Cc:** Linda Saif <saif.2@osu.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss.
Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>

**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

**Commented [BRS1]:** Not a dna virus

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

**Commented [BRS2]:** In Chinese social media

**Commented [BRS3]:** >5,000 nts

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of SARS CoV in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations

**Commented [BRS4]:** No, wildtype was passaged

**Commented [BRS5]:** wildtype

associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

**Commented [BRS6]:** these six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age.....leading to severe pathogenesis in aged, but not young animals [6].

**Commented [BRS7]:** SARS-CoV, as well as its closely related SHC014 bat strain and the chimera all differ by over 6,000 nts as compared with SARS-CoV 2.
Genome identities

Differences between Genomes.xlsx

**Commented [BRS8]:** This is not correct.

But was fully attenuated and displayed reduced virus infection in the airway epithelium as compared to SARS-CoV MA15 which is lethal.

Did not produce lethal disease like wildtype sars, so its attenuated!

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were subject to pause, reviewed and later approved under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

**Commented [BRS9]:** reduced

**Commented [BRS10]:** as written, suggests experiments were done before review. May want to reformulate

**Commented [BRS11]:** PMC6954302 PMC5567817

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.


1. Wang, D., *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang, *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N., *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N., *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D., *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y., *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W., *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F., *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

**SARVS-CoV-2: no evidence of a laboratory origin**

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5,6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new ~~novel~~ human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding ~~DNA~~ sequences ~~(CDSs)~~, and among the 128 nonsynonymous mutations, 89 led to ~~a~~ predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between

the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern ~~and~~ following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The ~~recombinant~~ mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. These six mutations were reintroduced into a SARS

**Formatted:** Font: (Default) Arial, Font color: Black

molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice. It is ~~also~~ likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6, 12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age ~~Importantly, SHC014-~~

~~MA15 can replicate efficiently in the mouse lung, leading to severe pathogiogyenesis~~ [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 14], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5,000 ~~nucleotides~~ differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad-based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV

**Formatted:** Highlight

**Formatted:** Line spacing: Double

**Commented [LS1]:** Lishan: see Ralph's comments to revise, as I am confused!

**Formatted:** Highlight

**Formatted:** Highlight

**Commented [BRS2]:** PMC6954302 PMC5567817

sequence in it and was thus likely generated in the laboratory. In a~~A~~ rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they ~~has~~ used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 ~~was~~ originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.

5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8. doi: 10.1126/science.1087139. PubMed PMID: 12958366.
13. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.
14. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Baric, Ralph S
**To:** Su, Lishan
**Cc:** Saif, Linda
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses
**Date:** Wednesday, February 12, 2020 12:34:00 PM
**Attachments:** EMI-2019-nCoV_Commentary_LJS_SLL_Refs-rsb.docx

---

My comments. I've included an excel file comparing the differences in the genome length sequences of the parental and chimeric viruses. Also made some text changes. I think the community needs to write these editorials and I thank you for your efforts. ralph

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 10:11 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** Re: A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:
We are trying to finish it and had no plan to get you too involved, but I do value your input. It is almost final and we are also getting comments from Perlman and Weiss. Thanks,

-Lishan

---

**From:** "Baric, Ralph S" <rbaric@email.unc.edu>
**Date:** Wednesday, February 12, 2020 at 10:02 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>
**Subject:** RE: A commentary on 2019 nCoV vs lab engineered viruses

sure, but don't want to be cited in as having commented prior to submission.

---

**From:** Su, Lishan <lishan_su@med.unc.edu>
**Sent:** Wednesday, February 12, 2020 1:12 AM
**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Subject:** A commentary on 2019 nCoV vs lab engineered viruses

Hi Ralph:

In response to the EMI journal editor's request, Drs. Shan-Lu Liu, Lin Saif and myself are writing a commentary (1-2 pages) to dispute the rumors of 2019 nCoV origin. Will you be interested, and have time, to have a quick read/comment? Please let me know if you have time.

Tentative Title: Is 2019-nCoV laboratory origin?

Thanks!

-Lishan

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], XXX, XXX, and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr Linda J. Saif, Saif.2@osu.edu

XXX, XXX

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (WHO website link ref).

According to what has been reported [1-3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes (Song, H.D. et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A 102, 2430-2435 (2005)). Given that there are greater than 1000 nt differences between the human

**Commented [BRS1]:** Not a dna virus

SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (website link ref).

Another claim points to a Nature Medicine paper published in 2015 [6], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [7]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

**Commented [BRS2]:** In Chinese social media

**Commented [BRS3]:** >5,000 nts

The recombinant mouse-adapted SARS virus (MA15) (Roberts, A. et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog 3, e5 (2007)) was generated by serial passage of SARS CoV in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations

**Commented [BRS4]:** No, wildtype was passaged

**Commented [BRS5]:** wildtype

associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

**Commented [BRS6]:** these six mutations were reintroduced into a SARS molecular clone to isolate a SARS MA15 recombinant virus, which recapitulated the severe disease phenotype in mice.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [8,9]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans (need to find refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [7]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [10], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. While SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was fully attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes regardless of age.....leading to severe pathogenesis in aged, but not young animals [6].

**Commented [BRS7]:** SARS-CoV, as well as its closely related SHC014 bat strain and the chimera all differ by over 6,000 nts as compared with SARS-CoV 2.
Genome identities

Differences between Genomes.xlsx

**Commented [BRS8]:** This is not correct.

But was fully attenuated and displayed reduced virus infection in the airway epithelium as compared to SARS-CoV MA15 which is lethal.

Did not produce lethal disease like wildtype sars, so its attenuated!

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were subject to pause, reviewed and later approved under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,11], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus. Finally, we note that the synthetic and chimeric panels of bat and SARS-like CoV led to the identification of remdesivir as a broad based inhibitor of all group 2b SARS-like coronaviruses tested in vitro or in vivo, providing critical preIND data that led to the ongoing clinical trials in China and for the future development of universal vaccines for all the SARS-like coronaviruses.

**Commented [BRS9]:** reduced

**Commented [BRS10]:** as written, suggests experiments were done before review. May want to reformulate

**Commented [BRS11]:** PMC6954302 PMC5567817

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (a manuscript sharing site prior to any peer review ~~and not yet peer reviewed for accuracy~~) claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.---And should not be present? in naturally isolated viruses such as RaTG13. Currently, there is no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.


1. Wang, D., *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* (2020).
2. Chang, *et al.* Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* (2020).
3. Chen, N., *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020).
4. Zhou, P., *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
5. Zhu, N., *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020).
6. Menachery, V.D., *et al.* A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med* **21**, 1508-1513 (2015).
7. Ge, X.Y., *et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).
8. Li, F., Li, W., Farzan, M. & Harrison, S.C. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864-1868 (2005).
9. Li, W., *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454 (2003).
10. Demogines, A., Farzan, M. & Sawyer, S.L. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. *J Virol* **86**, 6350-6353 (2012).
11. Wu, F., *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* (2020).

**From:** Saif, Linda
**To:** rbaric@email.unc.edu
**Subject:** EIM commentary
**Date:** Wednesday, February 12, 2020 11:49:11 AM
**Attachments:** EMI-2019-nCoV_Commentary_Final LJS 2020.docx

---

Hi Ralph,
My 2 Chinese colleagues and I have prepared this commentary to try to scientifically address some of the rumors and conspiracy theories on the internet about the origin of the 2019-nCoV, now designated SARS-2. Since we have tried to address concerns about some of your chimeric SARS constructs, it would be extremely helpful if you could review this and edit or add anything that might be useful. I realize from what Peter said you may not want to add your name but certainly your unacknowledged input and insights would be helpful to be certain we have provided the key evidence against such rumors and a false claims.
I recognize that it is essential for scientists to do whatever they can to counter fake news and false information and to support our esteemed colleagues and scientists like yourself which is what prompted this commentary!

In another matter Dr Wang and I want to try to get the SARS-2 CoV from BEI and attempt to infect pigs in our BSL 3 Ag facility. Do you know of any funds we could apply for to do these pilot studies, just to see if pigs are susceptible based on similar ACE2?

Hope you are well in spite of all the turmoil!
Regards,
Linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathology [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding

that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 expert Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Cc:** Su, Lishan; Lu, Shan
**Subject:** Re: Commentary for Emerging Microbes & Infections
**Date:** Wednesday, February 12, 2020 11:01:25 AM
**Attachments:** EMI-2019-nCoV_Commentary_Final.docx
image001.png

---

Dear Linda;

Attached please find almost the final version of the commentary for EMI, so please feel free to share it with Ralph. Let me know if you have additional suggestions – all your points are incorporated into the new version, please check.

Note that I was trying to find official website links for the new names of the virus (ICTV) and diseases (WHO), but failed; I therefore decided to use the following website, which contains both.

https://globalbiodefense.com/novel-coronavirus-covid-19-portal/

We will try to submit it today, but are considering to add a few more coronavirus experts – anyone that you would like to suggest? We will contact Stanley Perlman right now.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Wednesday, February 12, 2020 at 9:37 AM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: Commentary for Emerging Microbes & Infections

Can you please send me the updated version first and then I will try to share with Ralph!

Thanks
Linda
Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 12:47 AM
**To:** Linda Saif <saif.2@osu.edu>
**Subject:** Re: Commentary for Emerging Microbes & Infections

Hi Linda.
Thanks so much, and your comments are extremely helpful. Please feel free to share with Ralph to get his feedback if possible. We would like to publish this in the next few days.
I will work on reference tomorrow and send you a updated version.

Shan-Lu Liu sent from iPhone

> On Feb 11, 2020, at 11:54 PM, Saif, Linda <saif.2@osu.edu> wrote:
>
> Hi Shan-Lu,
> I edited this version and added my name as I too feel strongly about denouncing this.
> Here are more comments and some refs that I have made in replies to some reporters about this issue if you think any are useful to include. I also wonder if we might share this with Ralph Baric since he is a conspiracy target and maybe he could add additional points, but I know he would not want to be a co-author—not sure if he has time to answer.
>
> The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that 2019-nCoV evolved by natural evolution. Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.

The closest virus relative to 2019-nCoV is bat CoV RaTG13. There are 4% nt differences between 2019-nCoV and RaTG13, corresponding to >1000 nt based on a genome size of 29k. These changes (SNP) are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region.
(Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature doi:10.1038/s41586-020-2012-7.

Regarding differences between civet cat SARSr-CoV and SARS-CoV, here is the accurate data: . A total of 202 SNVs with multiple occurrences were identified, among which 200 were in the CDSs. Among the 128 nonsynonymous mutations, 89 led to a predicted radical amino acid changes
Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4.
Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human.
Song HD1, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L, Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP.

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Tuesday, February 11, 2020 10:32 PM
**To:** Linda Saif <saif.2@osu.edu>
**Subject:** Commentary for Emerging Microbes & Infections

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu

<image001.png>
Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

<image001.png>
<EMI-2019-nCoV_Commentary LJS.docx>

# SARVS-CoV-2: no evidence of a laboratory origin

Lishan Su[1], and Linda J. Saif [2,3], and Shan-Lu Liu[3, 4,5.6]

[1] Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology,

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Food Animal Health Research Program,

Ohio Agricultural Research and Development Center, CFAES

Department of Veterinary Preventive Medicine,

The Ohio State University, Wooster, Ohio 44691, USA

[3] Viruses and Emerging Pathogens Program, Infectious Diseases Institute,

The Ohio State University, Columbus, OH 43210, USA

[4] Center for Retrovirus Research, The Ohio State University,

Columbus, OH 43210, USA

[5] Department of Veterinary Biosciences, The Ohio State University, Columbus,

OH 43210, USA

[6] Department of Microbial Infection and Immunity, The Ohio State University,

Columbus, OH 43210, USA

Contact:

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1, 2, 3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4, 5].

Currently, there are speculations, rumors and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide variations (SNVs) identified across the genome; among these SNVs, 200 were in the coding DNA sequences (CDSs), and among the 128 nonsynonymous mutations, 89 led to a predicted radical amino-acid changes [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout

the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might have CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2.

The recombinant mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10, 11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [12], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus were restricted as gain of function (GOF) studies under the US government-mandated pause policy (from Oct. 2014 to Dec. 2017: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding these bat CoVs

already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5, 13], the SARS-CoV-2 is undoubtedly distinct from SHC014-MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (Gao et al., EMI paper 2/12/2020 in press). Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 was originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2.

## References


1. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570.
2. Chang, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1623. PubMed PMID: 32031568.
3. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. doi: 10.1016/S0140-6736(20)30211-7. PubMed PMID: 32007143.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.
5. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945.
6. Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. doi: 10.1073/pnas.0409608102. PubMed PMID: 15695582; PubMed Central PMCID: PMCPMC548959.
7. Menachery VD, Yount BL, Jr., Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. PubMed PMID: 26552008; PubMed Central PMCID: PMCPMC4797993.
8. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8. doi: 10.1038/nature12711. PubMed PMID: 24172901; PubMed Central PMCID: PMCPMC5389864.
9. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5. doi: 10.1371/journal.ppat.0030005. PubMed PMID: 17222058; PubMed Central PMCID: PMCPMC1769406.
10. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864-8. doi: 10.1126/science.1116480. PubMed PMID: 16166518.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4. doi: 10.1038/nature02145. PubMed PMID: 14647384.
12. Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350-3. doi: 10.1128/JVI.00311-12. PubMed PMID: 22438550; PubMed Central PMCID: PMCPMC3372174.

13. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508.

**From:** Saif, Linda
**To:** Liu, Shan-Lu
**Subject:** Re: Commentary for Emerging Microbes & Infections
**Date:** Tuesday, February 11, 2020 11:54:35 PM
**Attachments:** image001.png
EMI-2019-nCoV_Commentary LJS.docx

---

Hi Shan-Lu,
I edited this version and added my name as I too feel strongly about denouncing this.
Here are more comments and some refs that I have made in replies to some reporters about this issue if you think any are useful to include. I also wonder if we might share this with Ralph Baric since he is a conspiracy target and maybe he could add additional points, but I know he would not want to be a co-author—not sure if he has time to answer.

The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that 2019-nCoV evolved by natural evolution. Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of randomly occurring mutations.

The closest virus relative to 2019-nCoV is bat CoV RaTG13. There are 4% nt differences between 2019-nCoV and RaTG13, corresponding to >1000 nt based on a genome size of 29k. These changes (SNP) are distributed throughout the genome in a naturally occurring pattern and follow the evolution characteristics typical of CoVs, including the S gene as the most variable region.
(Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature doi:10.1038/s41586-020-2012-7.

Regarding differences between civet cat SARSr-CoV and SARS-CoV, here is the accurate data: .
A total of 202 SNVs with multiple occurrences were identified, among which 200 were in the CDSs. Among the 128 nonsynonymous mutations, 89 led to a predicted radical amino acid changes
Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2430-5. Epub 2005 Feb 4.
Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human.
Song HD1, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L,

Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP.

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Tuesday, February 11, 2020 10:32 PM
**To:** Linda Saif <saif.2@osu.edu>
**Subject:** Commentary for Emerging Microbes & Infections

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# Evidence refuting~~Is SARS-CoV-2 a~~ laboratory origin of COVID-19 (2019-nCoV)~~?~~

Lishan Su[1], ~~and~~ Shan-Lu Liu[2, 3,4,5] and Linda J. Saif [5,6]

[1] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA

[3] Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH 43210, USA

[5] Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, Ohio 44691, USA

[5,6] Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

Formatted: Left, Indent: Left: 0

Dr Linda J. Saif, Saif.2@osu.edu

Formatted: Font: (Default) Arial, 12 pt

Formatted: Font: (Default) Arial, 12 pt

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A novel human coronavirus, ~~SARS-CoV-2~~COVID-19, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease discovered in 2019 (COVID-19 cite WHO ref here).

**Commented [J1]:** Not sure how widely used or accepted this is—please check or to avoid confusion use COVID-19?

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The ~~SARS-CoV-2~~COVID-19 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity (Nature 2020 refs).

Currently, there are speculations, ~~or~~ rumors and conspiracy theories that COVID-19 ~~the 2019-CoV~~ is of ~~a~~ laboratory origin. ~~Some people have Certain people suspected~~ alleged that the human ~~SARS-CoV-2~~COVID-19 was~~is directly~~ leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the ~~SARS-CoV-2~~COVID-19 (Nature, 2020). However, as we know, the human SARS-CoV and intermediate host palm civet~~s~~ SARS-like CoV shared 99.8% homology, which is only about 60 nt differences in the whole 29Kb (ck) genome sequence (refs). Given that there are greater than 1000 nt differences between the human ~~SARS-CoV-2~~COVID-19 and the bat RaTG13-CoV (refs), it is highly unlikely that RaTG13 CoV is the immediate source of ~~SARS-CoV-2~~COVID-19 ~~; this is particularly true in light of a low mutation rate of the coronaviruses (refs)~~. ~~A s~~Searching for an

**Commented [J2]:** CoVs have a high mutation rate like other RNA viruses!

intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human COVID-19. There is speculation that pangolins might have CoVs closely related to COVID-19, but the data to substantiate this is not yet published (ref).

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells (refs). However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new COVID-19.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passage~~s~~ of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 ~~rounds of~~ passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is also likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was un~~not~~ able to use human ACE2 as a receptor for entry into human cells (refs). Civets were proposed to be an intermediate host of the bat-CoVs, capable of ~~before they~~ spreading SARS CoV

to humans (refs). However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats ~~in 2013~~ and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV (JVI 2012), it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts (refs). To directly address this possibility, the S gene from bat coronavirus SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could ~~can~~ indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat. Med. 2015).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are now restricted ~~considered~~ as gain of function (GOF) studies under the US government-mandated pause policy (refs). The current NCP epidemic has restarted the debate over the risks of constructing such viruses that could have~~with~~ pandemic potential, irrespective of the finding these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the ~~SARS-CoV-2~~COVID-19 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, once again there is no credible

evidence to support the claim that the ~~SARS-CoV-2~~COVID-19 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the ~~SARS-CoV-2~~COVID-19 was~~is~~ artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, (and not yet peer reviewed for accuracy) claiming that ~~SARS-CoV-2~~COVID-19 has HIV sequence in it and was~~is~~ thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the ~~SARS-CoV-2~~COVID-19 is not HIV-1 specific but random (EMI paper 2/12/2020). Because of the many concerns raised by the international community, the authors who made the initial claim have recently ~~decided to~~ withdrawn this report.

In summary, ~~we believe that~~ there is no credible evidence to support the claim that the ~~SARS-CoV-2~~COVID-19 ~~was~~ originated from a laboratory-engineered CoV. It is much more likely~~However, we cannot rule out the possibility~~ that ~~SARS-CoV-2~~COVID-19 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of ~~SARS-CoV-2~~COVID-19.

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Subject:** Commentary for Emerging Microbes & Infections
**Date:** Tuesday, February 11, 2020 10:32:03 PM
**Attachments:** EMI-2019-nCoV_Commentary.docx
image001.png

---

Hi Linda,

Invited by the editor in chief of EMI, Lushan Su from UNC and I have written a commentary on the possible origin of the 2019-nCoV or SARS-CoV-2 in order to dispute some rumors, and we would like to invite you as a coauthor. Attached please find an almost complete draft (references needed) of the commentary, so kindly let me know what you think. Your comments and suggestions are very much appreciated.

Thanks.

Shan-Lu


Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# Is SARS-CoV-2 a laboratory origin?

Lishan Su[1], and Shan-Lu Liu[2, 3,4.5]

[1] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA

[3] Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

[4] Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH 43210, USA

[5] Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH 43210, USA

Dr. Lishan Su, lsu@med.unc.edu

Dr. Shan-Lu Liu, Liu.6244@osu.edu

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1000 as of Feb. 10, 2020. A novel human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as novel coronavirus pneumonia (NCP) or coronavirus disease discovered in 2019 (COVID-19).

According to what has been reported (Lancet, NEJM 2020), NCP seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but is most similar to some bat beta-coronaviruses, with the highest being >96% identity (refs).

Currently, there are speculations or rumors that the 2019-CoV is of a laboratory origin. Certain people suspected that the SARS-CoV-2 is directly leaked from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 (Nature, 2020). However, as we know, the SARS-CoV and palm civets CoV shared 99.8% homology, which is only about 60 nt differences in the whole genome sequence (refs). Given that there are greater than 1000 nt differences between the SARS-CoV-2 and the RaTG13-CoV (refs), it is highly unlikely RaTG13 is the immediate source of SARS-CoV-2; this is particularly true in light of a low mutation rate of the coronaviruses (refs). Searching for an intermediate host between bat and humans is needed.

Another claim points to a Nature Medicine paper published in 2015, which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells (refs). However, this claim lacks any scientific basis and must be discounted.

The recombinant mouse-adapted SARS virus (MA15) (PLoS Pathog. 2007 Jan;3(1):e5) was generated by serial passages of an infectious SARS CoV clone in the respiratory tract of BALB/c mice. After 15 rounds of passage in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was not able to use human ACE2 as a receptor for entry (refs). Civets were proposed to be an intermediate host of the bat-CoVs before they spread to humans (refs). However, several novel bat coronaviruses were isolated from Chinese horseshoe bats in 2013 and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry (Nature 2013). Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as human ACE2 gene for interacting with SARS CoV (JVI 2012), it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts (refs). To directly address this possibility, the S gene from bat coronavirus

SL-SHC014 was used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus can indeed efficiently use human ACE2 and replicate efficiently in primary human airway cells to similar titers as epidemic strains of SARS-CoV. Importantly, SHC014-MA15 can replicate efficiently in the mouse lung, leading to severe pathogenesis (Nat. Med. 2015).

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SHC014- MA15 chimeric virus are considered as gain of function (GOF) studies under the US government-mandated pause policy (refs). The current NCP epidemic has restarted the debate over the risks constructing such viruses with pandemic potential. Regardless, upon careful phylogenetic analyses by multiple international groups (EMI, Nature…2020), the SARS-CoV-2 is undoubtedly distinct from SHC014- MA15, with >5000 nt differences across the whole genome. Therefore, there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SHC014-MA15 virus.

There are also rumors that the SARS-CoV-2 is artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv, claiming that SARS-CoV-2 has HIV sequence in it and is thus likely generated in the laboratory. A rebuttal paper led by an HIV-1 expert Dr. Feng Gao has used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random (EMI paper 2/12/2020). Because

of the many concerns raised by the international community, the authors who made the initial claim have recently decided to withdraw this report.

In summary, we believe that there is no credible evidence to support the claim that the SARS-CoV-2 was originated from a laboratory-engineered CoV. However, we cannot rule out the possibility that SARS-CoV-2 is a recombinant generated in nature between a bat CoV and another coronavirus in an intermediate host. More studies are needed to explore this possibility and resolve the origin of SARS-CoV-2.

**From:** Liu, Shan-Lu
**To:** Saif, Linda
**Subject:** Re: EMI commentary
**Date:** Saturday, April 4, 2020 1:24:35 PM
**Attachments:** EMI_Published.pdf
image001.png

---

Thought I sent, but here it is again.

Be safe!

Shan-Lu

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** "Saif, Linda" <saif.2@osu.edu>
**Date:** Saturday, April 4, 2020 at 1:21 PM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Re: EMI commentary

Please email me the final published pdf of this commentary
Thanks,
linda

Linda J. Saif, PhD
Distinguished University Professor
Food Animal Health Research Program
OARDC/The Ohio State University
1680 Madison Ave
Wooster, Oh 44691

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Wednesday, February 12, 2020 10:58 PM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, Linda Saif <saif.2@osu.edu>, "Weiss, Susan"

<weisssr@pennmedicine.upenn.edu>
**Cc:** "min.yang@emi2012.org" <min.yang@emi2012.org>, "Lu, Shan" <Shan.Lu@umassmed.edu>
**Subject:** EMI commentary

Dear all,

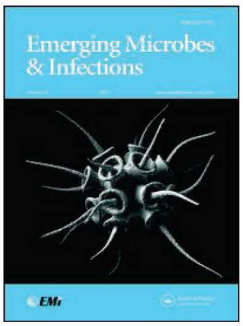
I have just submitted a commentary to EMI. See attached the submitted version.

Thank you.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu



Emerging Microbes & Infections

ISSN: (Print) 2222-1751 (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su

**To cite this article:** Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su (2020) No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2, Emerging Microbes & Infections, 9:1, 505-507, DOI: 10.1080/22221751.2020.1733440

**To link to this article:** https://doi.org/10.1080/22221751.2020.1733440



Published online: 26 Feb 2020.

Emerging Microbes & Infections
2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

OPEN ACCESS Check for updates

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan R. Weiss [f] and Lishan Su[g]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [b]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [c]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA; [d]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA; [e]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [f]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [g]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

**ARTICLE HISTORY** Received 13 February 2020; Accepted 13 February 2020

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1,100 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It was proposed that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary

**CONTACT** Shan Lu Liu Liu.6244@osu.edu; Lishan Su lsu@med.unc.edu

evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to MA15 chimeric virus with the original human SARS S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## ORCID

*Susan R. Weiss* http://orcid.org/0000 0002 8155 4528

## References

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1585

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426(6965): 450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

**From:** Liu, Shan-Lu
**To:** Zihai.Li@OSUMC.edu; Weller, Kevin; Vilgelm, Anna; Funderburg, Nicholas; Mitton-Fry, Mark J.; Gourapura, Renukaradhya; Larue, Ross; Kim, Sanggu; Garabed, Rebecca; Pomeroy, Laura; Bowman, Andrew; Li, Jianrong; Octavio.Ramilo@nationwidechildrens.org; Mejias, Asuncion; Peeples, Mark; Liyanage, Namal; Davis, Ian; Ghoneim, Hazem; Sullivan, Matthew; Tridandapani, Susheela; Artsimovitch, Irina; Moroi-Fetters, Sayoko; Kenney, Scott P.; Vlasova, Anastasia; Yount, Jacob; Behbehani, Gregory; Saif, Linda; Sullivan, Matthew; Boyaka, Prosper; Lee, Dean; Robins, Elizabeth; Riesenberg, Brian; Song, No joon; Wu, Xingjun; Bucci, Donna; Velegraki, Maria; Yang, Yiping - Division Director Hematology; Mardis, Elaine; Carson, William; Carbone, David; Li, Lang; Oglesbee, Michael; Hale, Vanessa; Lee, Jiyoung; Popovich, Phillip; Wang, Qiuhong; Reed, Erica; Ibba, Michael; Oltz, Eugene; Benson, Don; Baiocchi, Robert; Bednash, Joseph; El Boghdadly, Zeinab; Stiff, Andrew; Ahmer, Brian; Sharma, Amit; Kudryashov, Dmitri S.; Faith, Seth A.; Scrape, Scott; Gentry, James; Mohler, Peter; Shaffer, Rick; McAfee, Katherine A.; Koletar, Susan; Guerau, Mireia; Ranganathan, Parvathi; Chung, Dongjun; raj.muthusamy@osumc.edu; Lozanski, Gerard; Wen, Haitao; Ghoneim, Hazem; Dubey, Purnima; Rajaram, Murugesan; Robinson, Richard; Schelhorn, Jean; Shaffer, Rick; Polina.Shindiapina@osumc.edu
**Subject:** Re: OSU COVID-19 working groups
**Date:** Friday, March 27, 2020 8:13:06 AM
**Attachments:** image001.png

---

The genome release time was Jan 11 in Chinese time but Jan 10 in the US. The email was based on my note.
Just a clarification.

Shan-Lu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>
**Date:** Friday, March 27, 2020 at 8:02 AM
**To:** "Zihai.Li@OSUMC.edu" <Zihai.Li@OSUMC.edu>, Shan-Lu Liu <liu.6244@osu.edu>, "Weller, Kevin" <Kevin.Weller@osumc.edu>, "Vilgelm, Anna" <Anna.Vilgelm@osumc.edu>, "Funderburg, Nicholas" <Nicholas.Funderburg@osumc.edu>, "Mitton-Fry, Mark J." <mitton-fry.1@osu.edu>, "Gourapura, Renukaradhya" <gourapura.1@osu.edu>, "Larue, Ross" <larue.22@osu.edu>, "Kim, Sanggu" <kim.6477@osu.edu>, "Garabed, Rebecca" <garabed.1@osu.edu>, "Pomeroy, Laura" <pomeroy.26@osu.edu>, "Bowman, Andrew" <bowman.214@osu.edu>, "Li, Jianrong" <li.926@osu.edu>, "Octavio.Ramilo@nationwidechildrens.org" <Octavio.Ramilo@nationwidechildrens.org>, "Mejias, Asuncion" <Asuncion.Mejias@nationwidechildrens.org>, "Peeples, Mark" <Mark.peeples@nationwidechildrens.org>, "Liyanage, Namal" <Namal.MalimbadaLiyanage@osumc.edu>, "Davis, Ian" <davis.2448@osu.edu>, "Ghoneim, Hazem" <Hazem.Ghoneim@osumc.edu>, "Sullivan, Matthew" <sullivan.948@osu.edu>, "Tridandapani, Susheela" <Susheela.Tridandapani@osumc.edu>, "Artsimovitch, Irina" <artsimovitch.1@osu.edu>, "Moroi-Fetters, Sayoko" <Sayoko.Moroi@osumc.edu>, "Kenney, Scott P." <kenney.157@osu.edu>, "Vlasova, Anastasia" <vlasova.1@osu.edu>, "Yount, Jacob" <Jacob.Yount@osumc.edu>, "Behbehani, Gregory" <Gregory.Behbehani@osumc.edu>, "Saif, Linda" <saif.2@osu.edu>, "Sullivan, Matthew" <sullivan.948@osu.edu>, "Boyaka, Prosper" <boyaka.1@osu.edu>, "Lee, Dean" <Dean.Lee@nationwidechildrens.org>, "Robins, Elizabeth" <Elizabeth.Robins@osumc.edu>, "Riesenberg, Brian" <Brian.Riesenberg@osumc.edu>, "Song, No joon" <Nojoon.Song@osumc.edu>, "Wu, Xingjun" <Xingjun.Wu@osumc.edu>, "Bucci, Donna" <Donna.Bucci@osumc.edu>, "Velegraki, Maria" <Maria.Velegraki@osumc.edu>, "Yang, Yiping - Division Director Hematology" <Yiping.Yang2@osumc.edu>, "Mardis, Elaine" <Elaine.Mardis@nationwidechildrens.org>, "Carson, William" <William.Carson@osumc.edu>,

"Carbone, David" <David.Carbone@osumc.edu>, "Li, Lang" <Lang.Li@osumc.edu>, "Oglesbee, Michael" <oglesbee.1@osu.edu>, "Hale, Vanessa" <hale.502@osu.edu>, "Lee, Jiyoung" <lee.3598@osu.edu>, "Popovich, Phillip" <Phillip.Popovich@osumc.edu>, "Wang, Qiuhong" <wang.655@osu.edu>, "Reed, Erica" <reed.983@osu.edu>, "Ibba, Michael" <ibba.1@osu.edu>, "Oltz, Eugene" <Eugene.Oltz@osumc.edu>, "Benson, Don" <Don.Benson@osumc.edu>, "Baiocchi, Robert" <Robert.Baiocchi@osumc.edu>, "Bednash, Joseph" <Joseph.Bednash@osumc.edu>, "El Boghdadly, Zeinab" <Zeinab.ElBoghdadly@osumc.edu>, "Stiff, Andrew" <Andrew.Stiff@osumc.edu>, "Ahmer, Brian" <ahmer.1@osu.edu>, "Sharma, Amit" <sharma.157@osu.edu>, "Kudryashov, Dmitri S." <kudryashov.1@osu.edu>, "Faith, Seth A." <faith.3@osu.edu>, "Scrape, Scott" <Scott.Scrape@osumc.edu>, "Gentry, James" <gentry.50@osu.edu>, "Mohler, Peter" <Peter.Mohler@osumc.edu>, "Shaffer, Rick" <shaffer.25@osu.edu>, "McAfee, Katherine A." <mcafee.83@osu.edu>, "Koletar, Susan" <Susan.Koletar@osumc.edu>, "Guerau, Mireia" <Mireia.Guerau@osumc.edu>, "Ranganathan, Parvathi" <Parvathi.Ranganathan@osumc.edu>, "Chung, Dongjun" <Dongjun.Chung@osumc.edu>, "raj.muthusamy@osumc.edu" <raj.muthusamy@osumc.edu>, "Lozanski, Gerard" <Gerard.Lozanski@osumc.edu>, "Wen, Haitao" <Haitao.Wen@osumc.edu>, "Ghoneim, Hazem" <Hazem.Ghoneim@osumc.edu>, "Dubey, Purnima" <purnima.dubey@osumc.edu>, "Rajaram, Murugesan" <Murugesan.Rajaram@osumc.edu>, "Robinson, Richard" <Richard.Robinson@osumc.edu>, "Schelhorn, Jean" <schelhorn.1@osu.edu>, "Shaffer, Rick" <shaffer.25@osu.edu>, "Polina.Shindiapina@osumc.edu" <Polina.Shindiapina@osumc.edu>
**Subject:** OSU COVID-19 working groups

Good morning everybody! Happy Friday.

Thank you for sharing your ideas and participating in the discussions. I know many of you (and me too) have questions regarding where and how to obtain patient's samples, how to quickly get IBC amendments approved and get to get access to BSL3 facilities on campus, etc. Those are indeed critical questions and issues at this time, and all have been discussed in the past week Zoom meeting organized by Gene Otlz. We hope to have some updates next week.

See below a link, and also attached, in yesterday's *Cell* regarding the origin and emergence of the SARS-CoV-2 that causes COVID-19. These authors released the first genome sequence of SARS-CoV-2 on January 10.

https://www.cell.com/cell/fulltext/S0092-8674(20)30328-7?dgcid=raven_jbs_aip_email

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor

Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

**From:** Liu, Shan-Lu

**To:** Zihai.Li@OSUMC.edu; Liu, Shan-Lu; Weller, Kevin; Vilgelm, Anna; Funderburg, Nicholas; Mitton-Fry, Mark J.; Gourapura, Renukaradhya; Larue, Ross; Kim, Sanggu; Garabed, Rebecca; Pomeroy, Laura; Bowman, Andrew; Li, Jianrong; Octavio.Ramilo@nationwidechildrens.org; Mejias, Asuncion; Peeples, Mark; Liyanage, Namal; Davis, Ian; Ghoneim, Hazem; Sullivan, Matthew; Tridandapani, Susheela; Artsimovitch, Irina; Moroi-Fetters, Sayoko; Kenney, Scott P.; Vlasova, Anastasia; Yount, Jacob; Behbehani, Gregory; Saif, Linda; Sullivan, Matthew; Boyaka, Prosper; Lee, Dean; Robins, Elizabeth; Riesenberg, Brian; Song, No joon; Wu, Xingjun; Bucci, Donna; Velegraki, Maria; Yang, Yiping - Division Director Hematology; Mardis, Elaine; Carson, William; Carbone, David; Li, Lang; Oglesbee, Michael; Hale, Vanessa; Lee, Jiyoung; Popovich, Phillip; Wang, Qiuhong; Reed, Erica; Ibba, Michael; Oltz, Eugene; Benson, Don; Baiocchi, Robert; Bednash, Joseph; El Boghdadly, Zeinab; Stiff, Andrew; Ahmer, Brian; Sharma, Amit; Kudryashov, Dmitri S.; Faith, Seth A.; Scrape, Scott; Gentry, James; Mohler, Peter; Shaffer, Rick; McAfee, Katherine A.; Koletar, Susan; Guerau, Mireia; Ranganathan, Parvathi; Chung, Dongjun; raj.muthusamy@osumc.edu; Lozanski, Gerard; Wen, Haitao; Ghoneim, Hazem; Dubey, Purnima; Rajaram, Murugesan; Robinson, Richard; Schelhorn, Jean; Shaffer, Rick; Polina.Shindiapina@osumc.edu

**Subject:** OSU COVID-19 working groups

**Date:** Friday, March 27, 2020 8:02:38 AM

**Attachments:** SARS-CoV-2-Emergence_review.pdf
image001.png

---

Good morning everybody! Happy Friday.

Thank you for sharing your ideas and participating in the discussions. I know many of you (and me too) have questions regarding where and how to obtain patient's samples, how to quickly get IBC amendments approved and get to get access to BSL3 facilities on campus, etc. Those are indeed critical questions and issues at this time, and all have been discussed in the past week Zoom meeting organized by Gene Otlz. We hope to have some updates next week.

See below a link, and also attached, in yesterday's *Cell* regarding the origin and emergence of the SARS-CoV-2 that causes COVID-19. These authors released the first genome sequence of SARS-CoV-2 on January 10.

https://www.cell.com/cell/fulltext/S0092-8674(20)30328-7?dgcid=raven_jbs_aip_email

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Please cite this article in press as: Zhang and Holmes, A Genomic Perspective on the Origin and Emergence of SARS CoV 2, Cell (2020), https://doi.org/10.1016/j.cell.2020.03.035



Cell

# A Genomic Perspective on the Origin and Emergence of SARS-CoV-2

Yong-Zhen Zhang[1] and Edward C. Holmes[1,2,*]
[1]Shanghai Public Health Clinical Center and School of Life Science, Fudan University, Shanghai, China
[2]Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Life and Environmental Sciences and School of Medical Sciences, The University of Sydney, Sydney, Australia
*Correspondence: edward.holmes@sydney.edu.au
https://doi.org/10.1016/j.cell.2020.03.035

The ongoing pandemic of a new human coronavirus, SARS-CoV-2, has generated enormous global concern. We and others in China were involved in the initial genome sequencing of the virus. Herein, we describe what genomic data reveal about the emergence SARS-CoV-2 and discuss the gaps in our understanding of its origins.

## A New Human Coronavirus

The first reports of a novel pneumonia (COVID-19) in Wuhan city, Hubei province, China, occurred in late December 2019, although retrospective analyses have identified a patient with symptom onset as early as December 1st. Because the number of SARS-CoV-2 cases is growing rapidly and spreading globally, we will refrain from citing the number of confirmed infections. However, it is likely that the true number of cases will be substantially greater than reported because very mild or asymptomatic infections will often be excluded from counts. Any under-reporting of case numbers obviously means that the case fatality rate (CFR) associated with COVID-19 in the worst-hit regions will be lower than that currently cited. CFRs will also vary geographically, between age groups and temporally. Although these uncertainties will likely not be resolved without large-scale serological surveys, from current data it is clear that the CFR for COVID-19 is substantially higher than that of seasonal influenza but lower than that of two closely related coronaviruses that have similarly recently emerged in humans: SARS-CoV, responsible for the SARS outbreak of 2002–2003, and MERS-CoV that since 2015 has been responsible for the ongoing outbreak of MERS largely centered on the Arabian peninsula. However, it is also evident that SARS-CoV-2 is more infectious than both SARS-CoV and MERS-CoV and that individuals can transmit the virus when asymptomatic or presymptomatic, although how frequently remains uncertain.

An important early association was observed between the first reported cases of COVID-19 and the Huanan seafood and wildlife market in Wuhan city (which we both visited several years ago) where a variety of mammalian species were available for purchase at the time of the outbreak (Figure 1). Given that SARS-CoV-2 undoubtedly has a zoonotic origin, the link to such a "wet" market should come as no surprise. However, as not all of the early cases were market associated, it is possible that the emergence story is more complicated than first suspected. Genome sequences of "environmental samples"—likely surfaces—from the market have now been obtained, and phylogenetic analysis reveals that they are very closely related to viruses sampled from the earliest Wuhan patients. While this again suggests that the market played an important role in virus emergence, it is not clear whether the samples were derived from people who inadvertently deposited infectious material or from animals or animal matter present at that location. Unfortunately, the apparent lack of direct animal sampling in the market may mean that it will be difficult, perhaps even impossible, to accurately identify any animal reservoir at this location.

After clinical cases began to appear, our research team, along with a number of others, attempted to determine the genome sequence of the causative pathogen (Lu et al., 2020; Wu et al., 2020; Zhou et al., 2020; Zhu et al., 2020). We focused on a patient admitted to the Central Hospital of Wuhan on December 26, 2019, six days after the onset of symptoms (Wu et al., 2020). This patient was experiencing fever, chest tightness, cough, pain, and weakness, along with lung abnormalities indicative of pneumonia that appear to be commonplace in COVID-19 (Huang et al., 2020). Fortunately, next-generation meta-transcriptomic sequencing enabled us to obtain a complete viral genome from this patient on January 5, 2020. Initial analysis revealed that the virus was closely related to those of SARS-like viruses (family *Coronaviridae*). This result was immediately reported to the relevant authorities, and an annotated version of the genome sequence (strain Wuhan-Hu-1) was submitted to NCBI/GenBank on the same day. Although the GenBank sequence (GenBank: MN908947) was the first of SARS-CoV-2 available, it was subsequently corrected to ensure its accuracy. With the help of Dr. Andrew Rambaut (University of Edinburgh), we released the genome sequence of the virus on the open access Virological website (http://virological.org/) early on January 11, 2020. Afterwards, the China CDC similarly released SARS-CoV-2 genome sequences (with associated epidemiological data) on the public access GISAID database (https://www.gisaid.org/). At the time of writing, almost 200 SARS-CoV-2 genomes are publicly available, representing the genomic diversity of the virus in China and beyond and providing a freely accessible global resource. Importantly, the release of the SARS-CoV-2 genome sequence data facilitated the rapid development of diagnostic tests (Corman et al., 2020) and now an

**Figure 1. The Huanan Seafood and Wildlife Market in Wuhan, China**

The photographs (credit: E.C.H.) were taken when both authors visited the market together in October 2014 and highlight some of the wide variety of wildlife on sale, providing a potent mechanism for zoonotic transmission. Importantly, although many of the early COVID 19 cases were linked to this market, its role in the initial emergence of SARS CoV 2 remains uncertain.

infectious clone (Thao et al., 2020). The race to develop an effective vaccine and antivirals is ongoing, with trails of the latter underway (Wang et al., 2020).

## Comparisons between SARS-CoV-2 and Other Coronaviruses

The earliest genomic genome sequence data made it clear that SARS-CoV-2 was a member of the genus *Betacoronavirus* and fell within a subgenus (*Sarbecovirus*) that includes SARS-CoV (MERS-CoV falls in a separate subgenus, *Merbecovirus*) (Lu et al., 2020; Wu et al., 2020; Zhou et al., 2020; Zhu et al., 2020). Indeed, initial comparisons revealed that SARS-CoV-2 was approximately 79% similar to SARS-CoV at the nucleotide level. Of course, patterns of similarity vary greatly between genes, and SARS-CoV and SARS-CoV-2 exhibit only ~72% nucleotide sequence similarity in the spike (S) protein, the key surface glycoprotein that interacts with host cell receptors.

Given these close evolutionary relationships, it is unsurprising that the genome structure of SARS-CoV-2 resembles those of other betacoronaviruses, with the gene order 5′-replicase ORF1ab-S-envelope(E)-membrane(M)-N-3′. The long replicase ORF1ab gene of SARS-CoV-2 is over 21 kb in length and contains 16 predicted non-structural proteins and a number of downstream open reading frames (ORFs) likely of similar function to those of SARS-CoV. Comparative genomic analysis has been greatly assisted by the availability of a related virus from a *Rhinolophus affinis* (i.e., horseshoe) bat sampled in Yunnan province, China, in 2013 (Zhou et al., 2020). This virus, denoted RaTG13, is ~96% similar to SARS-CoV-2 at the nucleotide sequence level. Despite this sequence similarity, SARS-CoV-2 and RaTG13 differ in a number of key genomic features, arguably the most important of which is that SARS-CoV-2 contains a polybasic (furin) cleavage site insertion (residues PRRA) at the junction of the S1 and S2 subunits of the S protein (Coutard et al., 2020). This insertion, which may increase the infectivity of the virus, is not present in related betacoronaviruses, although similar polybasic insertions are present in other human coronaviruses, including HCoV-HKU1, as well as in highly pathogenic strains of avian influenza virus. In addition, the receptor binding domain (RBD) of SARS-CoV-2 and RaTG13 are only ~85% similar and share just one of six critical amino acid residues. Both sequence and structural comparisons suggest that the SARS-CoV-2 RBD is well suited for binding to the human ACE2 receptor that was also utilized by SARS-CoV (Wrapp et al., 2020). Importantly, an independent insertion(s) of the amino acids PAA at the S1/S2 cleavage site was recently observed in a virus (RmYN02) sampled in mid-2019 from another *Rhinolophus* bat in Yunnan province, indicating that these insertion events reflect a natural part of ongoing coronavirus evolution (Zhou et al., 2020). While RmYN02 is relatively divergent from SARS-CoV-2 in the S protein (~72% sequence similarity), it is the closest relative (~97% nucleotide sequence similarity) of the human virus in the long replicase gene.

Although SARS-CoV and MERS-CoV are both closely related to SARS-CoV-2

and have bat reservoirs, the biological differences between these viruses are striking. As noted above, SARS-CoV-2 is markedly more infectious, resulting in very different epidemiological dynamics to those of SARS-CoV and MERS-CoV. In these latter two viruses, there was a relatively slow rise in case numbers, and MERS-CoV has never been able to fully adapt to human transmission: the majority of the cases are due to spillover from camels on the Arabian peninsula with only sporadic human-to-human transmission (Sabir et al., 2016). In contrast, the remarkable local and global spread of SARS-CoV-2 caught most by surprise. Determining the virological characteristics that underpin such transmissibility is clearly a priority.

## The Zoonotic Origins of SARS-CoV-2

The emergence and rapid spread of COVID-19 signifies a perfect epidemiological storm. A respiratory pathogen of relatively high virulence from a virus family that has an unusual knack of jumping species boundaries, that emerged in a major population center and travel hub shortly before the biggest travel period of the year: the Chinese Spring Festival. Indeed, it is no surprise that epidemiological modeling suggests that SARS-CoV-2 had already spread widely in China before the city of Wuhan was placed under strict quarantine (Chinazzi et al., 2020).

It was also no surprise that early genomic comparisons revealed that the most closely related viruses to SARS-CoV-2 came from bats (Zhou et al., 2020). Sampling in recent years has identified an impressive array of bat coronaviruses, including RaTG13 and RmYN02 (Hu et al., 2017; Yang et al., 2015). Hence, bats are undoubtedly important reservoir species for a diverse range of coronaviruses (Cui et al., 2019). Despite this, the exact role played by bats in the zoonotic origin of SARS-CoV-2 is not established. In particular, the bat viruses most closely related to SARS-CoV-2 were sampled from animals in Yunnan province, over 1,500 km from Wuhan. There are relatively few bat coronaviruses from Hubei province, and those that have been sequenced are relatively distant to SARS-CoV-2 in phylogenetic trees (Lin et al., 2017). The simple inference from this is that our sampling of bat viruses is strongly biased toward some geographical locations. This will need to be rectified in future studies. In addition, although sequence similarity values of 96%–97% make it sound like the available bat viruses are very closely related to SARS-CoV-2, in reality this likely represents more than 20 years of sequence evolution (although the underlying molecular clock may tick at an uncertain rate if there was strong adaptive evolution of the virus in humans). It is therefore almost a certainty that more sampling will identify additional bat viruses that are even closer relatives of SARS-CoV-2. A key issue is whether these viruses, or those from any other animal species, contain the key RBD mutations and the same furin-like cleavage site insertion as found in SARS-CoV-2.

Although bats are likely the reservoir hosts for this virus, their general ecological separation from humans makes it probable that other mammalian species act as "intermediate" or "amplifying" hosts, within which SARS-CoV-2 was able to acquire some or all of the mutations needed for efficient human transmission. In the case of SARS and MERS, civets and camels, respectively, played the role of intermediate hosts, although as MERS-CoV was likely present in camels for some decades before it emerged in humans during multiple cross-species events, these animals may be better thought of as true reservoir hosts (Sabir et al., 2016). To determine what these intermediate host species might be, it is imperative to perform a far wider sampling of animals from wet markets or that live close to human populations. This is highlighted by the recent discovery of viruses closely related to SARS-CoV-2 in Malayan pangolins (*Manis javanica*) illegally imported into southern China (Guangdong and Guangxi provinces). The Guangdong pangolin viruses are particularly closely related to SARS-CoV-2 in the RBD, containing all six of the six key mutations thought to shape binding to the ACE2 receptor and exhibiting 97% amino acid sequence similarity (although they are more divergent from SARS-CoV-2 in the remainder of the genome). Although pangolins are of great interest because of how frequently they are involved in illegal trafficking and their endangered status, that they carry a virus related to SARS-CoV-2 strongly suggests that a far greater diversity of related betacoronaviruses exists in a variety of mammalian species but has yet to be sampled.

While our past experience with coronaviruses suggests that evolution in animal hosts, both reservoirs and intermediates, is needed to explain the emergence of SARS-CoV-2 in humans, it cannot be excluded that the virus acquired some of its key mutations during a period of "cryptic" spread in humans prior to its first detection in December 2019. Specifically, it is possible that the virus emerged earlier in human populations than envisaged (perhaps not even in Wuhan) but was not detected because asymptomatic infections, those with mild respiratory symptoms, and even sporadic cases of pneumonia were not visible to the standard systems used for surveillance and pathogen identification. During this period of cryptic transmission, the virus could have gradually acquired the key mutations, perhaps including the RBD and furin cleavage site insertions, that enabled it to adapt fully to humans. It wasn't until a cluster of pneumonia cases occurred that we were able to detect COVID-19 via the routine surveillance system. Obviously, retrospective serological or metagenomic studies of respiratory infection will go a long way to determining whether this scenario is correct, although such early cases may never be detected.

Another issue that has received considerable attention is whether SARS-CoV-2 is a recombinant virus, and whether such recombination might have facilitated its emergence (Lu et al., 2020; Wu et al., 2020). The complicating factor here is that sarbeviruses, and coronaviruses more broadly, experience widespread recombination, so that distinguishing recombination that assisted virus emergence from "background" recombination events is not trivial. Recombination is visible at multiple locations across the sarbevirus genome, including in the S protein, and in bat viruses closely related to SARS-CoV-2. For example, there is some evidence for recombination among SARS-CoV-2, RaTG13, and the Guangdong pangolin CoVs (Lam et al., 2020), and the genome of RmYN02 has similarly been widely impacted by recombination (Zhou et al., 2020). However, trying to

determine the exact pattern and genomic ancestry of recombination events is difficult, particularly as many of the recombinant regions may be small and are likely to change as we sample more viruses related to SARS-CoV-2. To resolve these issues, it will again be necessary to perform a far wider sampling of viral diversity in animal populations.

## Ongoing Genomic Evolution of SARS-CoV-2

As the COVID-19 epidemic has progressed, so more viral genomes have been sequenced. As expected given their recent common ancestry, the earliest samples from Wuhan contained relatively little genetic diversity. While this can prevent detailed phylogenetic and phylogeographic inferences, it does show that the public health authorities in Wuhan did a remarkable job in detecting the first cluster of pneumonia cases. However, this seemingly recent common ancestry does not exclude a pre-outbreak period of cryptic transmission in humans. Although accumulating genetic diversity means that it is now possible to detect distinct phylogenetic clusters of SARS-CoV-2 sequences, it is difficult to determine using genomic comparisons alone whether the virus is fixing phenotypically important mutations as it spreads through the global population, and any such claims require careful experimental verification.

Given the high mutation rates that characterize RNA viruses, it is obvious that many more mutations will appear in the viral genome and that these will help us to track the spread of SARS-CoV-2 (Grubaugh et al., 2019). However, as the epidemic grows, our sample size of sequences will likely be so small relative to the total number of cases that it will be very difficult, if not impossible, to detect individual transmission chains. Caution must therefore always be exercised when attempting to infer exact transmission events. As an aside, although coronaviruses likely have lower mutation rates than other RNA viruses because of an inherent capacity for some proof-reading activity due to a 3′-to-5′ exoribonuclease (Minskaia et al., 2006), their long-term rates of nucleotide substitution (i.e., of molecular evolution) fall within the distribution of those seen in other RNA viruses (Holmes et al., 2016). This suggests that lower mutation rates are to some extent compensated by high rates of virus replication within hosts. Although there is no evidence that this capacity to mutate (common to RNA viruses) will result in any radical changes in phenotype—such as in transmissibility and virulence—as these only rarely change at the scale of individual disease outbreaks (Grubaugh et al., 2020), it is obviously important to monitor any changes in phenotype as the virus spreads. In all likelihood, any drop in the number of cases and/or CFR of COVID-19 will likely be due to rising immunity in the human population and epidemiological context rather than mutational changes in the virus.

## Conclusions

It seems inevitable that SARS-CoV-2 will become the fifth endemic coronavirus in the human population (along with HKU1, NL63, OC43, and 229E) and one that is currently spreading in a totally susceptible population. Coronaviruses clearly have the capacity to jump species boundaries and adapt to new hosts, making it straightforward to predict that more will emerge in the future, although quite why coronaviruses possess this capacity in comparison to some other RNA viruses is unclear. Critically, the surveillance of animal coronaviruses should include animals other than bats, as the role of intermediate hosts is likely of major importance, providing a more direct pathway for the virus to emerge in humans. Given the enormous diversity of viruses in wildlife and their ongoing evolution, arguably the simplest and most cost-effective way to reduce the risk of future outbreaks is to limit our exposure to animal pathogens as much as possible. While our intimate relationship with the animal world means we cannot build impregnable barriers, stronger action against the illegal wildlife trade and removing all mammalian (and perhaps avian) wildlife from wet markets will provide an important buffer.

## ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (grants 81861138003 and 31930001), the Special National Project on investigation of basic resources of China (grant 2019FY101500), and the Australian Research Council (grant FL170100022).

## WEB RESOURCES

GISAID, https://www.gisaid.org/
Virological, http://virological.org/

## REFERENCES

Chinazzi, M., Davis, J.T., Ajelli, M., Gioannini, C., Litvinova, M., Merler, S., Pastore Y Piontti, A., Mu, K., Rossi, L., Sun, K., et al. (2020). The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID 19) outbreak. Science. eaba9757. Published online March 6, 2020. https://doi.org/10.1126/science.aba9757.

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K.W., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M.L., et al. (2020). Detection of 2019 novel coronavirus (2019 nCoV) by real time RT PCR. Euro Surveill. *25*, 2000045.

Coutard, B., Valle, C., de Lamballerie, X., Canard, B., Seidah, N.G., and Decroly, E. (2020). The spike glycoprotein of the new coronavirus 2019 nCoV contains a furin like cleavage site absent in CoV of the same clade. Antiviral Res. *176*, 104742.

Cui, J., Li, F., and Shi, Z. L. (2019). Origin and evo lution of pathogenic coronaviruses. Nat. Rev. Mi crobiol. *17*, 181 192.

Grubaugh, N.D., Ladner, J.T., Lemey, P., Pybus, O.G., Rambaut, A., Holmes, E.C., and Andersen, K.G. (2019). Tracking virus outbreaks in the twenty first century. Nat. Microbiol. *4*, 10 19.

Grubaugh, N.D., Petrone, M.E., and Holmes, E.C. (2020). We shouldn't worry when a virus mutates during disease outbreaks. Nat Microbiol. Pub lished online February 18, 2020. https://doi.org/10.1038/s41564 020 0690 4.

Holmes, E.C., Dudas, G., Rambaut, A., and Ander sen, K.G. (2016). The evolution of Ebola virus: In sights from the 2013 2016 epidemic. Nature *538*, 193 200.

Hu, B., Zeng, L.P., Yang, X.L., Ge, X.Y., Zhang, W., Li, B., Xie, J.Z., Shen, X.R., Zhang, Y.Z., Wang, N., et al. (2017). Discovery of a rich gene pool of bat SARS related coronaviruses provides new insights into the origin of SARS coronavirus. PLoS Pathog. *13*, e1006698.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet *395*, 497 506.

Lam, T.T. Y., Shum, M.H. H., Zhu, H. C., Tong, Y. G., Ni, X. B., Liao, Y. S., Wei, W., Cheung, W.Y. M., Li, W. J., Li, L. F., et al. (2020). Identifi cation of 2019 nCoV related coronaviruses in Malayan pangolins in southern China. bioRxiv. https://doi.org/10.1101/2020.02.13.945485.

Lin, X. D., Wang, W., Hao, Z. Y., Wang, Z. X., Guo, W. P., Guan, X. Q., Wang, M. R., Wang, H. W., Zhou, R. H., Li, M. H., et al. (2017). Extensive di versity of coronaviruses in bats from China. Virology *507*, 1 10.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet *395*, 565 574.

Minskaia, E., Hertzig, T., Gorbalenya, A.E., Campanacci, V., Cambillau, C., Canard, B., and Ziebuhr, J. (2006). Discovery of an RNA virus 3′ >5′ exoribonuclease that is critically involved in coronavirus RNA synthesis. Proc. Natl. Acad. Sci. USA *103*, 5108 5113.

Sabir, J.S.M., Lam, T.T. Y., Ahmed, M.M.A., Li, L., Shen, Y., Abo Aba, S.E.M., Qureshi, M.I., Abu Zeid, M., Zhang, Y., Khiyami, M.A., et al. (2016). Co circulation of three camel coronavirus species and recombination of MERS CoVs in Saudi Arabia. Science *351*, 81 84.

Thao, T.T.N., Labroussaa, F., Ebert, N., V'kovski, P., Stalder, H., Portmann, J., Kelly, J., Steiner, S., Holwerda, M., Kratzel, A., et al. (2020). Rapid reconstruction of SARS CoV 2 using a synthetic genomics platform. bioRxiv. https://doi.org/10.1101/2020.02.21.959817.

Wang, Y., Zhou, F., Zhang, D., Zhao, J., Du, R., Hu, Y., Cheng, Z., Gao, L., Jin, Y., Luo, G., et al. (2020). Evaluation of the Efficacy and Safety of Intravenous Remdesivir in Adult Patients with Severe Pneumonia caused by COVID 19 virus infection: study protocol for a Phase 3 Randomized, Double blind, Placebo controlled, Multicentre trial. BMC Trials. https://doi.org/10.21203/rs.2.24058/v1.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C. L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020). Cryo EM structure of the 2019 nCoV spike in the prefusion conformation. Science *367*, 1260 1263.

Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei, Y.Y., et al. (2020). A new coronavirus associated with human respiratory disease in China. Nature *579*, 265 269.

Yang, X.L., Hu, B., Wang, B., Wang, M.N., Zhang, Q., Zhang, W., Wu, L.J., Ge, X.Y., Zhang, Y.Z., Daszak, P., et al. (2015). Isolation and characterization of a novel bat coronavirus closely related to the direct progenitor of Severe Acute Respiratory Syndrome coronavirus. J. Virol. *90*, 3253 3256.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature *579*, 270 273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., et al.; China Novel Coronavirus Investigating and Research Team (2020). A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. *382*, 727 733.

**From:** Lee, Dean
**To:** Liu, Shan-Lu; Kevin.Weller@osumc.edu; Anna.Vilgelm@osumc.edu; Zihai.Li@OSUMC.edu; Nicholas.Funderburg@osumc.edu; Mitton-Fry, Mark J.; Gourapura, Renukaradhya; Larue, Ross; Kim, Sanggu; 6477@osu.edu; Garabed, Rebecca; Pomeroy, Laura; Bowman, Andrew; Li, Jianrong; Ramilo, Octavio; Mejias, Asuncion; Peeples, Mark; Namal.MalimbadaLiyanage@osumc.edu; Davis, Ian; Hazem.Ghoneim@osumc.edu; Sullivan, Matthew; Susheela.Tridandapani@osumc.edu; Artsimovitch, Irina; Sayoko.Moroi@osumc.edu; Kenney, Scott P.; Vlasova, Anastasia; Yount, Jacob; Behbehani, Gregory; Saif, Linda; Sullivan, Matthew; Boyaka, Prosper; Robins, Elizabeth; Riesenberg, Brian; Song, No joon; Wu, Xingjun; Bucci, Donna; Velegraki, Maria; Yang, Yiping - Division Director Hematology; Mardis, Elaine; Carson, William; Carbone, David; Li, Lang; Oglesbee, Michael; Hale, Vanessa; Lee, Jiyoung; Popovich, Phillip; Wang, Qiuhong; Reed, Erica; Ibba, Michael; Oltz, Eugene; Benson, Don; Baiocchi, Robert; Bednash, Joseph; El Boghdadly, Zeinab; Stiff, Andrew; Ahmer, Brian; Sharma, Amit; Kudryashov, Dmitri S.
**Subject:** Re: OSU COIVD-19 working groups
**Date:** Tuesday, March 24, 2020 10:34:25 PM
**Attachments:** image001.png
TrendsMolMed Sun 2020 preprint.pdf

---

The Trends in Molecular Med COVID review did not have canine ACE2 listed in Table 1. I did the alignment and added it to the table in the attached version, in case this is useful to anyone else. Overall homology was about 82%, and that of the proposed critical binding residues is 13/19.

---

**From:** "Liu, Shan-Lu" <liu.6244@osu.edu>
**Date:** Tuesday, March 24, 2020 at 8:28 AM
**To:** "Kevin.Weller@osumc.edu" <Kevin.Weller@osumc.edu>, "Anna.Vilgelm@osumc.edu" <Anna.Vilgelm@osumc.edu>, "Zihai.Li@OSUMC.edu" <Zihai.Li@osumc.edu>, "Nicholas.Funderburg@osumc.edu" <Nicholas.Funderburg@osumc.edu>, "Mitton-Fry, Mark J." <mitton-fry.1@osu.edu>, "Gourapura, Renukaradhya" <gourapura.1@osu.edu>, "Larue, Ross" <larue.22@osu.edu>, "Kim, Sanggu" <kim.6477@osu.edu>, "6477@osu.edu" <6477@osu.edu>, "Garabed, Rebecca" <garabed.1@osu.edu>, "Pomeroy, Laura" <pomeroy.26@osu.edu>, "Bowman, Andrew" <bowman.214@osu.edu>, "Li, Jianrong" <li.926@osu.edu>, "Ramilo, Octavio" <Octavio.Ramilo@nationwidechildrens.org>, "Mejias, Asuncion" <Asuncion.Mejias@nationwidechildrens.org>, "Peeples, Mark" <Mark.Peeples@nationwidechildrens.org>, "Namal.MalimbadaLiyanage@osumc.edu" <Namal.MalimbadaLiyanage@osumc.edu>, "Davis, Ian" <davis.2448@osu.edu>, "Hazem.Ghoneim@osumc.edu" <Hazem.Ghoneim@osumc.edu>, "Sullivan, Matthew" <sullivan.948@osu.edu>, "Susheela.Tridandapani@osumc.edu" <Susheela.Tridandapani@osumc.edu>, "Artsimovitch, Irina" <artsimovitch.1@osu.edu>, "Sayoko.Moroi@osumc.edu" <Sayoko.Moroi@osumc.edu>, "Kenney, Scott P." <kenney.157@osu.edu>, "Vlasova, Anastasia" <vlasova.1@osu.edu>, "Yount, Jacob" <Jacob.Yount@osumc.edu>, "Behbehani, Gregory" <Gregory.Behbehani@osumc.edu>, "Liu, Shan-Lu" <liu.6244@osu.edu>, "Saif, Linda" <saif.2@osu.edu>, "Sullivan, Matthew" <sullivan.948@osu.edu>, "Boyaka, Prosper" <boyaka.1@osu.edu>, Dean Lee <Dean.Lee@nationwidechildrens.org>, "Robins, Elizabeth" <Elizabeth.Robins@osumc.edu>, "Riesenberg, Brian" <Brian.Riesenberg@osumc.edu>, "Song, No joon" <Nojoon.Song@osumc.edu>, "Wu, Xingjun" <Xingjun.Wu@osumc.edu>, "Bucci, Donna" <Donna.Bucci@osumc.edu>, "Velegraki, Maria" <Maria.Velegraki@osumc.edu>, "Yang, Yiping - Division Director Hematology" <Yiping.Yang2@osumc.edu>, "Mardis, Elaine" <Elaine.Mardis@nationwidechildrens.org>, "William Carson III, MD"

<William.Carson@osumc.edu>, "Carbone, David" <David.Carbone@osumc.edu>, "Li, Lang" <Lang.Li@osumc.edu>, "Oglesbee, Michael" <oglesbee.1@osu.edu>, "Hale, Vanessa" <hale.502@osu.edu>, "Lee, Jiyoung" <lee.3598@osu.edu>, "Popovich, Phillip" <Phillip.Popovich@osumc.edu>, "Wang, Qiuhong" <wang.655@osu.edu>, "Reed, Erica" <reed.983@osu.edu>, "Ibba, Michael" <ibba.1@osu.edu>, "Oltz, Eugene" <Eugene.Oltz@osumc.edu>, "Benson, Don" <Don.Benson@osumc.edu>, "Baiocchi, Robert" <Robert.Baiocchi@osumc.edu>, "Bednash, Joseph" <Joseph.Bednash@osumc.edu>, "El Boghdadly, Zeinab" <Zeinab.ElBoghdadly@osumc.edu>, "Stiff, Andrew" <Andrew.Stiff@osumc.edu>, "Ahmer, Brian" <ahmer.1@osu.edu>, "Sharma, Amit" <sharma.157@osu.edu>, "Kudryashov, Dmitri S." <kudryashov.1@osu.edu>
**Subject:** OSU COIVD-19 working groups

[WARNING: External Email - Use Caution]

Good morning everybody!

Thank you for your interest in joining the OSU COIVD-18 discussion and working groups. I believe I have included everyone who expressed an interest, but if not, please let me know.

Today, I would like to share a new paper just appearing on the BioRxiv website. I thought this is a cool study.

https://www.biorxiv.org/content/10.1101/2020.03.22.002386v1

Attached also please find a review article, which I thought is comprehensive.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

Trends in Molecular Medicine

CellPress REVIEWS

Review

# COVID-19: Epidemiology, Evolution, and Cross-Disciplinary Perspectives

Jiumeng Sun,[1,8] Wan Ting He,[1,8] Lifang Wang,[2] Alexander Lai,[3] Xiang Ji,[4] Xiaofeng Zhai,[1] Gairu Li,[1] Marc A. Suchard,[4] Jin Tian,[5] Jiyong Zhou,[6] Michael Veit,[7,*] and Shuo Su[1,*]

**The recent outbreak of COVID-19 in Wuhan turned into a public health emergency of international concern. With no antiviral drugs nor vaccines, and the presence of carriers without obvious symptoms, traditional public health intervention measures are significantly less effective. Here, we report the epidemiological and virological characteristics of the COVID-19 outbreak. Originated in bats, 2019-nCoV/ severe acute respiratory syndrome coronavirus (SARS-CoV)-2 likely experienced adaptive evolution in intermediate hosts before transfer to humans at a concentrated source of transmission. Similarities of receptor sequence binding to 2019-nCoV between humans and animals suggest a low species barrier for transmission of the virus to farm animals. We propose, based on the One Health model, that veterinarians and animal specialists should be involved in a cross-disciplinary collaboration in the fight against this epidemic.**

## Highlights

The basic reproductive number ($R_0$) of 2019 nCoV is higher than $R_0$ of severe acute respiratory syndrome coronavirus (SARS CoV) and Middle East respiratory syndrome coronavirus (MERS CoV). COVID 19 presents with asymptomatic infections, with potential to propagate and perpetuate this epidemic.

2019 nCoV isolated from patients shows limited sequence diversity, suggesting that the interspecies transmission event was very recent and that the source of the virus was focused, possibly a point source event.

The amino acid sequence in the ACE2 receptor responsible for 2019 nCoV binding in farm animals and cats has only a few exchanges compared with the human receptor, suggesting that the species barrier for virus transmission is small.

## Emergence of COVID-19

In December 2019, a cluster of pneumonia with unknown etiology appeared in Wuhan City, Hubei Province of China. Several of the initial patients visited a wet seafood market where other wildlife species were also sold. Subsequent virus isolation from human patients and molecular analysis showed that the pathogen was a new coronavirus (CoV), first named 2019-nCoV, and subsequently this disease was renamed by WHO as COVID-19. A study group of the International Committee on Taxonomy of Viruses (ICTV) proposed the name SARS-CoV-2, but this name remains to be officially approved [1]. This new CoV is now the seventh member of the *Coronaviridae* known to infect humans. With the explosive increase of confirmed cases, the WHO declared this outbreak a public health emergency of international concern (PHEIC) on January 30, 2020.

CoVs are a class of genetic diverse viruses found in a wide range of host species, including birds and mammals. Many CoVs cause intestinal and respiratory infections in animals and in humans [2–5]. CoV came into the spotlight in 2002–2003, when clusters of 'atypical pneumonia' were first reported in Guangdong Province, subsequently spreading to Hong Kong. Researchers in Hong Kong isolated a novel CoV virus (SARS-CoV) and the disease was later renamed **severe acute respiratory syndrome (SARS)** (see Glossary). Because of international travel, the virus spread from Hong Kong to the rest of the world and more than 8000 people in 26 countries became infected, with a case fatality rate of approximately 10% (https://www.who.int/csr/sars/country/table2004_04_21/en/). SARS posed a serious public health threat to the world at that time, with a significant negative impact on the economy in affected areas. Subsequent studies found that SARS-CoV originated from bats and interspecies transmission to humans took place via an intermediate host: Himalayan palm civets (*Paguma larvata*) or raccoon dogs (*Nyctereutes procyonoides*) [5–7]. Another well-known CoV of animal origin is **Middle East respiratory syndrome coronavirus (MERS-CoV)**, which has an even higher case fatality rate, but it is rarely transmitted between humans.

[1]MOE Joint International Research Laboratory of Animal Health and Food Safety, Jiangsu Engineering Laboratory of Animal Immunology, Institute of Immunology and College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China
[2]College of Veterinary Medicine, China Agricultural University, Beijing, China
[3]College of Natural, Applied, and Health Sciences, Kentucky State University, Frankfort, KY, USA
[4]Department of Biomathematics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA, USA
[5]State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China
[6]Key Laboratory of Animal Virology of Ministry of Agriculture, Zhejiang University, Hangzhou, China

[7]Institute for Virology, Center for Infection Medicine, Veterinary Faculty, Free University Berlin, Berlin, Germany
[8]These authors are co first authors

*Correspondence:
shuosu@njau.edu.cn (S. Su) and Michael.Veit@fu berlin.de (M. Veit).

As major natural reservoir species of *Alphacoronavirus* and *Betacoronavirus*, bats carry highly diverse SARS-like-CoVs. These bats are distributed in many provinces of China. The genetic diversity of these SARS-like-CoVs and their molecular evolution within their natural host species have been studied intensively [2,8–11]. Here, we review the recent but still very limited facts about the current epidemiology of COVID-19 and discuss viral characteristics of 2019-nCoV on the backdrop of our knowledge about the previous epidemic of SARS and MERS.

## Epidemiology of COVID-19

As of 24:00 February 20, 2020 (UTC+8), there are a total of 75 995 confirmed cases, including 2239 fatalities in China (mainland: 75 891; Hong Kong: 68; Macao: 10; and Taiwan: 26), and 1200 confirmed cases, including eight fatal ones outside China, in all five continents (Figure 1). The epidemiology curve can roughly be divided into three phases.

i. The local outbreak by exposure in the aforementioned food wholesale market marks the first phase. From the first case in December 2019 to the emergence of new cases outside Wuhan by January 13, 2020, a total of 41 cases were confirmed. Epidemiologic analysis showed that already in this initial phase, person-to-person transmission had occurred by close contact [12].
ii. The second phase started on January 13, marked by rapid expansion and spread of the virus within hospitals (nosocomial infection) and by family transmission (close-contact transmission). In this phase the epidemic spread from Wuhan to other areas [12–18]. The first case outside of China was reported in Thailand on January 13, caused by a Wuhan resident travelling to this country. On January 19 cases were reported from outside Wuhan, in Beijing City, and in the Guangdong Province, indicating that the virus had spread within China, and the total number of confirmed cases rose to 205. Already by January 23, 29 provinces, plus six foreign countries, had reported a total of 846 confirmed cases, an approximately 20-fold increase from the first phase. Meanwhile, Wuhan city implemented a 'lock-down' (i.e., shutting down all movement within and out of the city). Unfortunately, this period coincided with the traditional mass movement of people, a form of 'home-coming', before Chinese New Year and thus more than 5 million people had already left Wuhan.
iii. The third phase started on January 26, which is marked by the rapid increase of cluster cases. On February 10, retrospective analysis showed that the number of clustered cases accounted for 50–80% of all confirmed cases in Beijing, Shanghai, Jiangsu, and Shandong [19]. On January 30, the number increased 240-fold, reaching 9826 confirmed cases, and the WHO declared this epidemic a PHEIC. By February 11, 44 730 confirmed cases and 16 067 suspected cases were reported in about 1386 counties and districts in China [20]. However, there were only 441 confirmed cases in 24 countries outside of China. The fatality rate remained high in China, with a total of 1114 deaths, but with just one fatality outside China, in the Philippines. By February 12, due to adoption of a new clinical definition for diagnosis in Hubei province, newly confirmed cases jumped to 14 840, of which 13 332 cases were based only on clinical diagnosis. By that time, 25 countries had reported 60 329 infections, with 1471 times the initial number (Figure 1A). Of note, February 3 seems to be a tipping point of the epidemic, from which time the daily number of confirmed cases outside Hubei began to decline. Whether it reflects a success of the 'Wuhan lock-down' and other public health measures, or virus transmission reduced for other reasons, remains unclear.

Furthermore, 85.8% of 37 269 confirmed cases had either lived in or traveled to Wuhan, or had close contact with persons who had been to Wuhan [20,21]. Unfortunately, as of February 11, 1716 medical-related staff from 422 medical institutions were infected, of which 1688 confirmed

cases were analyzed. Among them, 64% were infected in Wuhan city and 23.3% in the rest of Hubei, excluding Wuhan [20]. The specific causes of the infection of medical staff and the failure of protection need further investigation.

Initial evaluation of COVID-19 transmission dynamics showed that the **basic reproductive number ($R_0$)** of 2019-nCoV is estimated to be 1.4–3.9 [12]. The $R_0$ of SARS-CoV in the absence of interventions was 2.3–3.7 [22,23]. Breban *et al.* estimated MERS-CoV $R_0$ to be 0.50–0.92 by analysis of 55 of the first 64 laboratory-confirmed cases [24]. With the implementation of rapid diagnosis, coupled with effective isolation of patients, the $R_0$ of SARS-CoV dropped to less than 1, explaining why the SARS-CoV outbreak could eventually be controlled [25–27]. However, it is worth noting that $R_0$ estimates may vary upon numerous biologic, socio-behavioral, and environmental factors, and must be interpreted with caution [28].

## Clinical Phenotype of COVID-19

Major initial symptoms of COVID-19 include fever, cough, muscular soreness, and dyspnea. Some patients showed atypical symptoms, such as diarrhea and vomiting. However, the clinical phenotype is confounded by the fact that 25.2% patients had at least one other underlying medical condition [13,15,29–32]. The overall clinical characteristics of COVID-19 were also influenced by the different phases of this epidemic [12,13,21,29,33]. Patients in the first and second phase of the epidemic were older, more likely to be male, and likely to have exposure to the seafood market. Clinically, they had more bilateral patchy shadows, or ground glass opacity in the lungs [13,21,29,33–36]. In addition, the mortality rate of the first and second phases of the epidemic was 4.3–15% and thus significantly higher than the 1.36% determined for the later phase of the epidemic [13,21,29,33,34]. This higher mortality rate was either due to: (i) more people with underlying medical conditions, such as high blood pressure and diabetes [12,13,19,20,29,31,33]; (ii) during the early phase of this epidemic the virus was more pathogenic; or (iii) the lower mortality rate was skewed by a larger sample size at the later phase of this epidemic. Importantly, 889 asymptomatic or subclinically symptomatic infected cases were reported [20,37]. Asymptomatic infection was also documented in Germany: two asymptomatic patients' throat samples were tested positive by reverse transcription (RT)-PCR and by virus isolation, while both patients remained well and afebrile for 7 days [38]. Importantly, the asymptomatic manifestation jeopardizes the screening of infected people by temperature measurements or by overt signs and symptoms [12,13,19,20,29,31,33]. Virus infection is not selective in age, as it was reported even in a 1-month-old infant [20,21,37]. Of the 44 672 confirmed cases, 77.8% are between 30 and 69 years old and 51.4% are male [20]. Until now, there is no evidence for intrauterine infection by vertical transmission in women who developed COVID-19 during late pregnancy and no evidence that pregnant women are more susceptible compared with other adult patients [34,39]. Although currently the number of new infections is decreasing, the COVID-19 epidemic is still ongoing. The order to Chinese citizens to return to work, which is accompanied by massive population movement, will likely increase the risk of transmission again. Overall, the current mortality rate of COVID-19 in China is 2.9% and in foreign countries 0.7%. The overall mortality rate remains the highest in Hubei (3.4%), 4.9 times higher than in other provinces (0.7%). For comparison, SARS-CoV exhibited a case fatality rate of 9.6% (774/8096) and MERS-CoV had a fatality rate of 34.4% (858/2494) (https://www.who.int/csr/sars/country/table2004_04_21/en/; https://www.who.int/emergencies/mers-cov/en/). However, 2019-nCoV is more infectious than SARS-CoV or MERS-CoV [40,41].

## Origin and Evolution of 2019-nCoV

As animal markets had been implicated in the SARS-CoV outbreak of 2002–2003, and initial 2019-nCoV infections are also related to the seafood market with wildlife trading, it was soon assumed that wild animals were also involved in the emergence of 2019-nCoV. Yet, from

## Glossary

**Avian influenza virus:** influenza viruses that circulate in birds, mainly in water fowl, without causing clinical symptoms (low pathogenic influenza virus). Occasionally they are introduced into poultry, where they might acquire a polybasic cleavage site within their main glycoprotein hemagglutinin (HA). HA is then cleaved by the ubiquitous protease furin and the now highly pathogenic virus causes a systemic and hence deadly infection ('bird flu').

**Basic reproductive number ($R_0$):** an epidemiologic metric to describe the contagiousness or transmissibility of infectious agents. It refers to the expected number of secondary infections that one infected person generates on average in an entirely susceptible population. It allows estimation of the potential of an agent to cause an epidemic, the extent of transmission without control measures, and the efficiency of control measures to reduce transmission.

**Enfuvirtide:** antiviral drug (trade name Fuzeon), licensed for the treatment of HIV infection, that inhibits the membrane fusion activity of its glycoprotein and hence cell entry of the virus.

**Middle East respiratory syndrome coronavirus (MERS-CoV):** a highly lethal and zoonotic pathogen that was first identified in Saudi Arabia in 2012. Since 2012, MERS has been reported in 27 countries. Scientific evidence suggests that people are infected through direct or indirect contact with infected dromedary camels.

**Plaque:** a plaque is an area of dead cells within a cell monolayer. The plaque is caused by an infection of a single cell by one virus that then spreads to neighboring cells. Plaque assays are used to determine the number of infectious virus particles.

**Severe acute respiratory syndrome (SARS):** caused by SARS coronavirus (SARS CoV), which first occurred in Guangdong province, China, and became a global epidemic disease in 2002–2003. The disease was reported by 26 countries, with a case fatality rate of approximately 10%. Studies showed that SARS CoV originated from bats and was transmitted to humans via palm civets or raccoon dogs.

**ZDHHC family:** family of polytopic membrane proteins that are characterized by the amino acid motif DHHC, which is located within a cysteine rich domain in one of its cytoplasmic loops. Many of the family

which species and under what circumstance the virus crossed the species barrier to infect humans remains to be clarified. Early investigations about the origin of COVID-19 suggested that the 2019-nCoV may have jumped from bats to human [42,43]. This is not unprecedented since bat viruses have been shown to 'jump' the species barrier frequently to infect new species [44–50]. However, since bats were in hibernation when the outbreak occurred, and it was uncertain whether bats were sold at the market, the virus is more likely to have been transmitted via

members have been shown to transfer long chain fatty acids to cysteine residues of cellular and viral proteins.

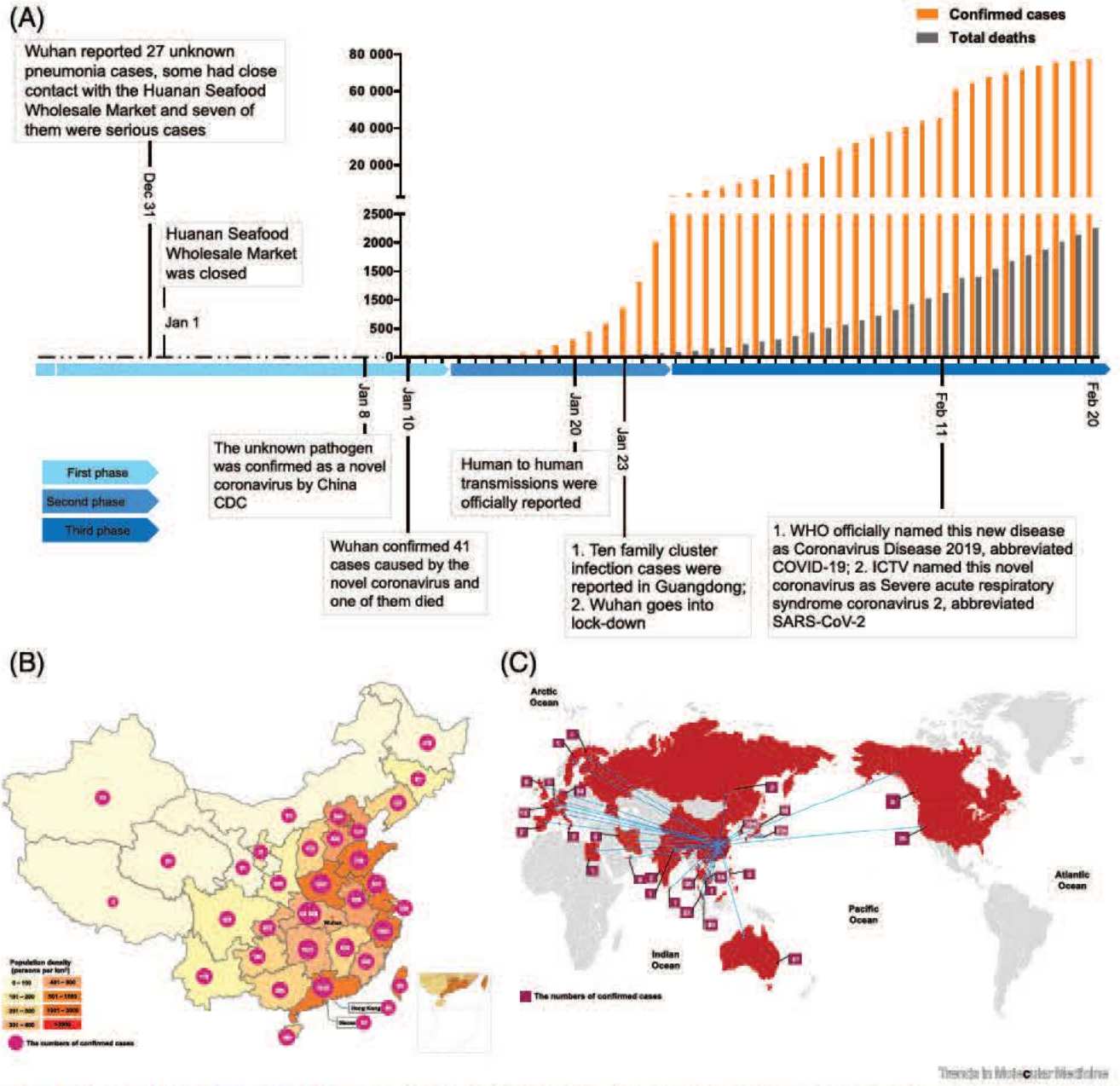



Figure 1. Spreading of the 2019 nCoV Epidemic. (A) Timeline of events during the 2019 nCoV epidemic. (B) Human confirmed cases of 2019 nCoV infection in China. (C) Human confirmed cases of 2019 nCoV infection in the world (Last update on 24:00 UTC+8, 20 February 2020). Abbreviations: CDC, Centers for Disease Control; ICTV, International Committee on Taxonomy of Viruses.

### Box 1. Evolution Analysis Methods

Sequences analyzed: 18 betacoronavirus sequences and 95 full length 2019 nCoV genomes kindly made available from GISAID (https://www.gisaid.org/) and from the National Center for Biotechnology Information GenBank (https://www.ncbi.nlm.nih.gov/) platforms. Some sequences were omitted, as they were too short, contained sequencing artefacts, resulted from resequencing of the same sample, or had insufficient annotations.

Sequence alignment and potential recombination analysis: sequences were aligned using MAFFT [83] and manually adjusted in MEGA7 [84]. The breakpoints were detected using the phylogenetic incongruence among segments in sequence alignments using GARD and are shown by using the Simplot version 3.5.1 and Kimura model. Slide windows were set as 1000 bp, with each step 500 bp.

Phylogenetic analysis: all ML trees were reconstructed using the general time reversible substitution model with gamma distributed rate heterogeneity and 1000 bootstraps by RAxML (v4.8.10) [85].

other species on the market. Genomic analyses of 2019-nCoV demonstrate a 96% nucleotide identity with a CoV isolated from a bat: BetaCoV/RaTG13/2013 [42]. Previous reports showed that species from the bat genera *Rhinolophus* in southern China are a rich pool of SARS-like-CoVs, which belong to the subgenera *Sarbecovirus*. These viruses exhibit rich genetic diversity and frequent recombination events, which may increase the potential for cross-species transmission [7,42,51–55]. Here, we reconstructed the evolutionary history of the 2019-nCoV cluster (Box

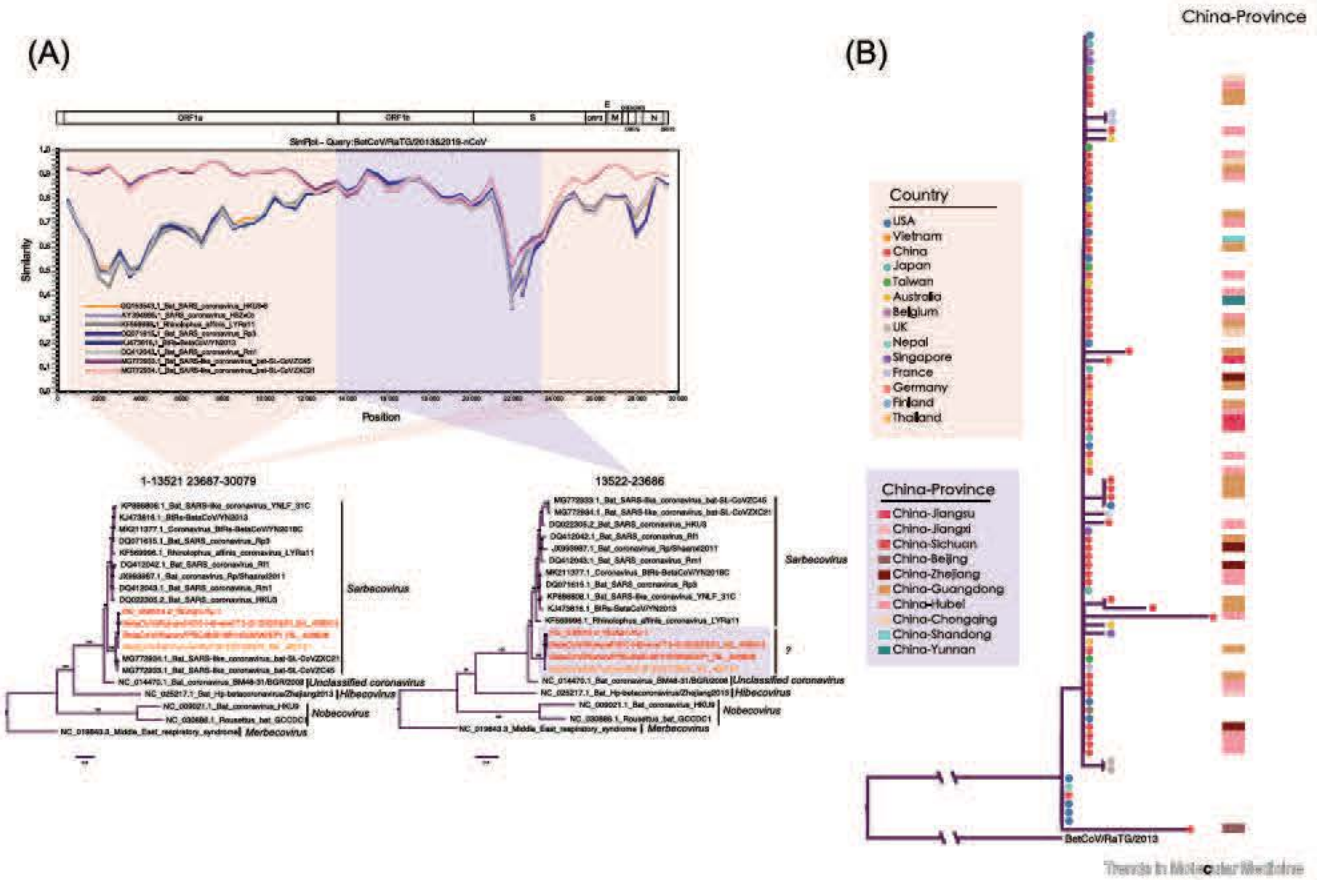



Figure 2. Structure of the 2019 nCoV Genome. (A) Recombination analysis of 2019 nCoV. A rescaled structure of the 2019 nCoV genome (top) and similarity recombination analysis with reference sequences using Simplot v3.5.1 (accession number BetaCoV/Wuhan/WIV02/2019|EPI_ISL_402127 EPI_ISL_402131, KJ473816, DQ071615, DQ412043, GQ153543, AY394995, KF569996, MG772933, MG772934). Sequences were separated based on potential recombination breakpoint on nucleotides 13 522 and 23 686. Maximum likelihood (ML) phylogenetic trees inferred for the pink and purple regions confirm different topologies and recombination. (B) ML tree of 2019 nCoV spike protein gene. The ML tree was reconstructed using the general time reversible substitution model with gamma distributed rate heterogeneity and 1000 bootstraps using RAxML (v4.8.10).

1). Based on recombination analysis and phylogenetic trees (Figure 2A), we found that 2019-nCoV shares a most recent common ancestor with BetaCoV/RaTG13/2013 (EPI ISL 402131), because both viruses are in the same cluster. However, our results indicate that this cluster may be the result of convergent evolution or complex recombination events involving at least two virus species with differing evolutionary histories (Figure 2A). The two external segments of this clustered viral genome, encompassing nucleotide (nt) 1 to nt 13 521, and nt 23 687 to nt 30 079, are similar to bat CoVs ZC45 and ZXC21. The first segment includes ORF1a and the second segment includes the C terminus of the S protein, ORF3, E, M, ORF6, ORF7a, ORF8, N, and ORF10 (Figure 2A). This finding is also supported by reconstructing maximum likelihood (ML) phylogenetic trees, which reveal that segments from nt 1 to nt 13 521 and from nt 23 687 to nt 30 079 are clustered with *Sarbecovirus*. However, based on the ML tree result, the middle segment from nt 13 522 to nt 23 686 of 2019-nCoV genome and RaTG13 does not cluster with *Sarbecovirus*. It forms a new branch in the phylogenetic tree, located between *Sarbecovirus* and an Unclassified CoV. In addition, a recent preliminary report showed that the receptor-binding motif (RBM) of these two genomes shares a very low sequence similarity [56]. This divergence indicates a possible alternative source for the RBM encoding sequence in 2019-nCoV, as suggested by other preliminary reports [52,57]. Interestingly, Lam *et al.* found several putative pangolin CoV sequences with 85.5% to 92.4% similarity to 2019-nCoV [52]. Further preliminary studies showing the existence of multiple lineages of pangolin CoVs with genetic similarity to 2019-nCoV further support the hypothesis that pangolins served as a potential intermediate host [52,58]. The currently available data do not fully elucidate if the virus was directly transmitted from bats to humans or indirectly through an intermediate host, nor do they currently rule out convergent evolution as an alternative hypothesis to recombination to explain the discordant phylogenetic trees. Consequentially, more sequence data are needed to confirm the specific source and origin of the 2019-nCoV, which can only be achieved by enhanced collection and monitoring of bat and other wild animal samples.

The topology of a phylogenetic tree with all the currently available spike protein gene sequences of 2019-nCoV shows high similarities between human isolates (Figure 2B), indicating only minimal genetic variation, which is rather unexpected for fast evolving RNA viruses [42]. However, these similarities could be the result of a relatively recent common ancestor, suggesting that the emergence of the virus was a recent event. Furthermore, results are similar to the finding from other preliminary reports that indicate that the virus source of interspecies transmission was highly concentrated or limited, possibly a single event [14,42,43,59]. In addition, the high sequence similarity among the viruses isolated from patients indicates a recent introduction to humans [60]. In all, these results further support the role of Wuhan as the epicenter of the outbreak and there is no evidence for other sources of this 2019-nCoV.

## Structure and Function of the Spike Protein of 2019-nCoV, the Major Determinant of Cell Tropism

The spike protein (S) is the major determinant of cell tropism and hence interspecies transmission of CoVs, since it binds the virus to a cellular receptor and subsequently catalyzes virus entry by membrane fusion. The 3D structure of the viral S of 2019-nCoV determined by electron microscopy (Figure 3A, [61]) revealed its similarity to S of other CoVs. This allows deduction of further features from other CoVs. S is a type I trimeric transmembrane protein with an N terminal cleavable signal peptide, one large and heavily *N*-glycosylated ectodomain (60–90 carbohydrates per trimer), a transmembrane region, and a cytoplasmic tail containing a cluster of *S*-acylated cysteine residues. The ectodomain is cleaved by proteases into the between genera highly variable S1 domain, carrying the receptor-binding activities, and the more conserved S2 domain that catalyzes membrane fusion. The S1 domain is further divided into

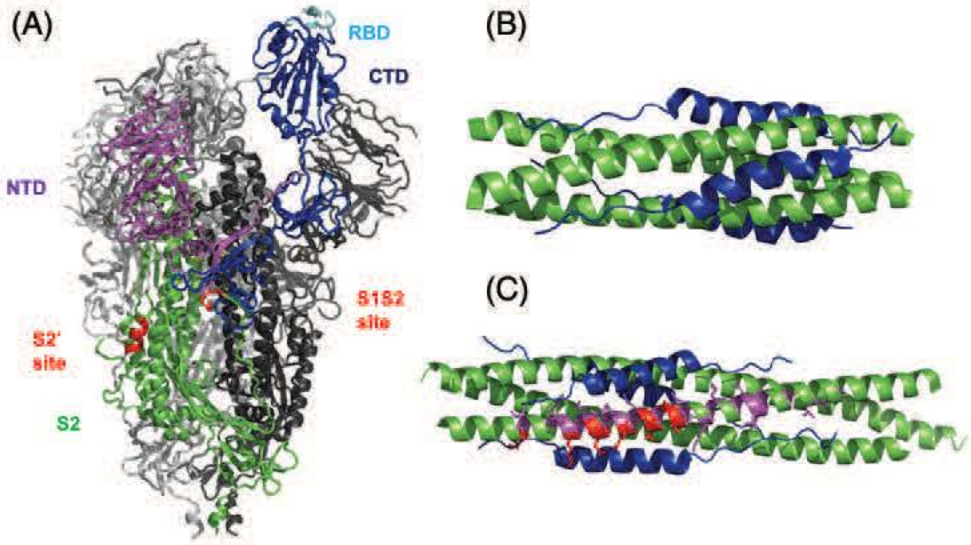



Figure 3. Structure of Spike Protein (S) Before and After Membrane Fusion. (A) Structure of the trimeric ectodomain of S from 2019 nCoV. The S2 subunit in one monomer is shown in green, the N terminal domain (NTD) of S2 in magenta, and the C terminal domain (CTD) of S2 in blue. The CTD is in the 'up conformation', exposing the binding domain for the angiotensin converting enzyme 2 (ACE2) receptor (cyan). The S1/S2 and S2' cleavage sites are indicated in red. The figure was created with Pymol from Protein Data Bank (PDB) file 6VSB. (B) Structure of the heptad repeat (HR) domains of S from severe acute respiratory syndrome coronavirus (SARS CoV). Heptad repeat region 1 (HR1) is labeled green and repeat region 2 (HR2) in blue. Formation of this six helix bundle is supposed to drive membrane fusion. The figure was created with Pymol from PDB file 1ZV8. (C) Structure of the HR1 of S from SARS CoV (green) bound to the pan coronavirus peptide inhibitor EK1 (blue). The amino acids in S essential for binding to EK1 are shown as magenta sticks in one helix. The amino acids in S from 2019 nCoV not conserved in S from SARS CoV are shown as red sticks. Since the nonconserved amino acids are apparently not required for binding to EK1, the fusion inhibitor is likely to prevent cell entry of 2019 nCoV. The figure was created with Pymol from PDB file 5ZVM. Abbreviations: RBD, receptor binding domain.

an N terminal domain (NTD) and a C terminal domain (CTD). The NTD exhibits a structural fold as human galectins, galactose-binding lectins, and hence, in most CoVs, a sugar present at the cell surface serves as an attachment factor. The CTD is responsible for binding to the host receptor angiotensin-converting enzyme 2 (ACE2) in the case of SARS-CoV and 2019-nCoV. The CTD contains two subdomains: a core structure (a five-stranded antiparallel β-sheet) and the actual RBM, which determines the receptor binding specificity. The recently released structure of the RBM ACE2 complex (Figure 4A) revealed that most S residues contacting ACE2 are identical between SARS-CoV and 2019-nCoV. However, some are unique, including an important salt bridge that involves different amino acids in ACE2 to bind S of SARS-CoV and 2019-nCoV. These slight differences might explain the more efficient binding of S from 2019-nCoV to ACE2, but this has not been observed in other preliminary studies [61,62].

The CTD of S has basically the same folding in other CoVs, even if they use different host receptors, such as dipeptidyl peptidase 4 for MERS-CoV. The diversity of receptor usage is an outstanding feature of CoVs and (assuming that they all have derived from a common ancestor) already indicates that they have changed their receptor binding specificity multiple times during evolution [63–65].

After binding to its receptor, S catalyzes fusion of the viral and cellular membrane to allow access of the viral genome to the cytosol. A prerequisite for this activity is the cleavage of S into subunits, a process called priming. The first cleavage site is located at the S1/S2 boundary

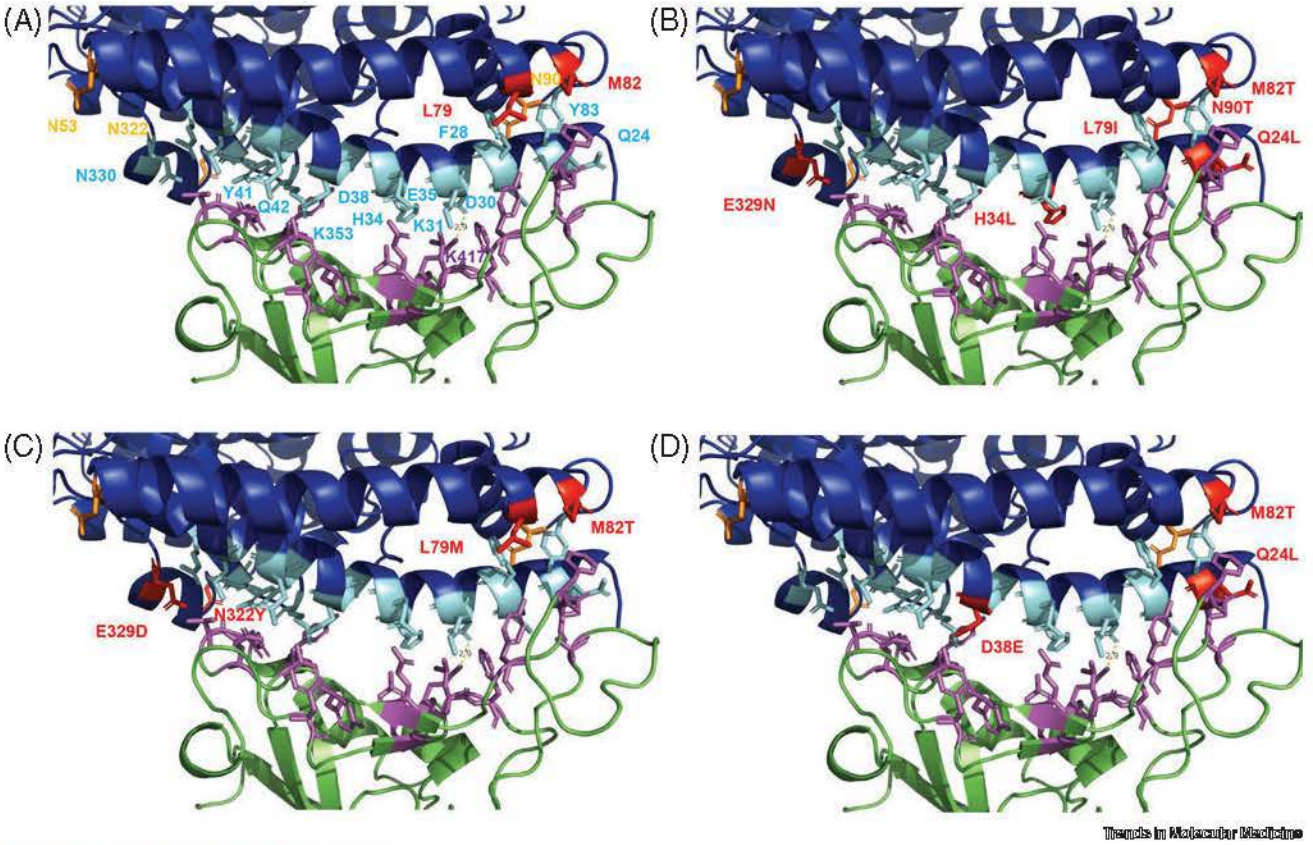



Figure 4. Spike Protein (S) and Its Receptor. (A) Structure of the receptor binding domain of S from 2019 nCoV (green) bound to human angiotensin converting enzyme 2 (ACE2) (blue). Most amino acids involved in binding are highlighted as magenta (S) and cyan (ACE2) sticks. Asparagine (N) that are *N* glycosylation sites (motif N X S/T) in human ACE2 are shown as orange sticks. Amino acids in human ACE2 that are involved in binding, but encode a potential *N* glycosylation site in ACE2 from other species, are shown as red sticks. The dotted line indicates the salt bridge between D30 and K417 (generated with Pymol from Protein Data Bank file6VSB). (B) Amino acid exchanges between human ACE2 and pig ACE2. Amino acid exchanges in ACE2 from pig compared with human ACE2 are highlighted in red. The exchange N90T destroys the *N* glycosylation site in human ACE2. (C) Amino acid exchanges between human ACE2 and cattle ACE2. Amino acid exchanges in ACE2 from cattle compared with human ACE2 are highlighted in red. The exchange N322Y destroys the *N* glycosylation site in human ACE2. ACE2 from sheep exhibits identical amino acid exchanges. (D) Amino acid exchanges between human and cat ACE2. Amino acid exchanges in ACE2 from cat compared with human ACE2 are highlighted in red. All relevant glycosylation sites in human ACE2 are conserved.

and another site (called S2') within S2. CoVs have evolved multiple strategies for proteolytic activation of S, and a large number of host proteases, such as furin, trypsin, trans-membrane protease/serine (TMPRSS), and cathepsins have been identified to process the spike protein. As a rule, furin cleaves S at a polybasic cleavage site (minimal motif R-X-X-R) during its biosynthesis in the trans-Golgi compartments or during virus entry in endosomes. Cleavage by trypsin and TMPRSS family members occurs at monobasic cleavage sites and likely takes place in the extracellular space and at the cell surface. Cathepsins, ubiquitous lysosomal enzymes with a rather broad substrate specificity, cleave S during virus entry [66]. For 2019-nCoV, it was shown that TMPRSS 2 primes S, the cathepsins B and L are only required in the absence of this protease [67]. Interestingly, S of 2019-nCoV has acquired a polybasic motif at the S1/S2 boundary, which is not present in S of the bat CoVs and SARS-CoV [68]. Preliminary data showed that S of 2019-nCoV is cleaved by furin during its biosynthesis [69]. This is reminiscent of low-pathogenic **avian influenza viruses**, which, if introduced into a poultry farm, may acquire a polybasic cleavage motif that causes a deadly outbreak of highly

pathogenic virus. S of MERS-CoV has a similar motif, which is cleaved by furin during biosynthesis of S. The availability and activity of the proteases in a certain cell, tissue, and host species regulates the tropisms of CoVs. However, the fact that S can easily acquire new protease cleavage sites and that various (some of them ubiquitous) proteases can fulfil the same task suggests that CoVs are naturally equipped or can easily adapt to multiply in several cell types.

Cleavage at the internal S2′ site occurs just upstream of the sequence S-F-I-E-D-L-L-F, which is highly conserved between S proteins of CoVs. It likely functions as a fusion peptide that inserts into the cellular membrane once the conformational change that catalyzes membrane fusion has been initiated. What triggers the refolding of S is unclear; the low pH prevailing in the endosome during virus entry is only required to activate cathepsins and binding to the receptor causes only minor conformational changes, but might be required to expose a previously hidden proteolytic cleavage site. The structure of parts of the S2 subunit from SARS-CoV in the postfusion conformation (Figure 3B) revealed a six helix bundle between two heptad repeats (a motif of seven amino acids in which amino acid 1 and 4 are hydrophobic), which is a typical feature of class I fusion proteins, such as hemagglutinin (HA) of influenza virus and Gp160 of HIV. However, the six helix bundle formed by S is longer, indicating its formation released more energy that drives the fusion of two lipid bilayers [70,71]. In summary, an amazingly large number of experimental data have already been worked out for S of 2019-nCoV and these models are still evolving.

## Molecular Differences in the ACE2 Receptor between Human and Animal Species

The identification of the contact residues between the receptor-binding domain of S from 2019-nCoV and human ACE2 allows estimation of whether 2019-nCoV could infect other species (Figure 4A) [72]. To do so, we aligned all available ACE2 amino acid sequences with human ACE2. We placed emphasis on the presence of *N*-glycosylation motifs near the binding site, since they might affect attachment of S. Human ACE2 is glycosylated at N53, N90, and N322 (Figure 4A, orange sticks). N53 is conserved in all species. N90 is not a glycosylation site in ACE2 of mouse, pig, *N. procyonoides*, raccoon, civet, ferret, fox, *E. telfairi*, and chicken. N322 is not a glycosylation site in ACE2 of mouse, rat, cattle, sheep, *E. telfairi*, and pangolin. However, ACE2 of some species contain an additional glycosylation motif in this region. Residue L79 is a potential *N*-glycosylation site in chicken and M82 is a potential glycosylation site in *Rhinolophus sinicus*, pangolin, and rat. Notably, glycosylation of residue 82 has been show to prevent binding of S from SARS-CoV to rat ACE2 [73].

Some amino acids in ACE2 affect binding to S of 2019-nCoV are depicted for various species in Table 1. The S binding site of ACE2 from macaque and chimpanzees is identical to human ACE2. ACE2 from other species revealed eleven (chicken), nine and ten (rodents), or only three (cat) amino acid differences compared with human ACE2. Of special interest are ACE2 proteins from farm animals and a pet cat, since they might become another possible reservoir for 2019-nCoV. ACE2 from pig contains six exchanges, but they are mostly located at the periphery of the binding site (Figure 4B). N90T causes the loss of the glycosylation site. E329 forms a salt bridge with R426 in S of SARS-CoV, but S of 2019-nCoV forms a salt bridge with another residue (D30) in ACE2. Thus, the exchange of E329 by N in porcine ACE2 might affect binding to S of SARS-CoV, but not to S from 2019-nCoV. A similar pattern emerges for amino acid differences between human and cattle ACE2 (Figure 4C) and cat ACE2 (Figure 4D). The few exchanges are also located peripheral to the core of the binding region and thus their exchange might not represent a large obstacle for infection of cells from these species with 2019-nCoV.

Table 1. Comparison of Some Important ACE2 Residues among Different Species That Affect Binding to 2019 nCoV Receptor Binding Domain (RBD)

| Species | Amino acids (19) in different species ACE2 that affect binding to 2019 nCoV RBD, corresponding positions are based on human ACE2 numbering | | | | | | | | | | | | | | | | | | | Similarity to human ACE2 (based on 19 amino acids) | GenBank accession number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | 31 | 34 | 35 | 38 | 41 | 42 | 53 | 79 | 82 | 83 | 90 | 322 | 325 | 329 | 330 | 353 | 652 | 710 | | |
| Human | Q | K | H | E | D | Y | Q | N | L | M | Y | N | N | Q | E | N | K | R | R | 19/19 | AAT45083.1 |
| Pig | **L** | K | **L** | E | D | Y | Q | N | **I** | **T** | Y | **T** | N | Q | **N** | N | K | R | R | 13/19 | XP 020935033.1 |
| Cat | **L** | K | H | E | **E** | Y | Q | N | L | **T** | Y | N | N | Q | E | N | K | R | R | 16/19 | XP 023104564.1 |
| Macaque | Q | K | H | E | D | Y | Q | N | L | M | Y | N | N | Q | E | N | K | R | R | 19/19 | XP 011733505.1 |
| Chimpanzee | Q | K | H | E | D | Y | Q | N | L | M | Y | N | N | Q | E | N | K | R | R | 19/19 | XP 016798468.1 |
| Mouse | **N** | **N** | **Q** | E | D | Y | Q | N | **T** | **S** | **F** | **T** | **H** | Q | **A** | N | **H** | R | R | 9/19 | ABN80106.1 |
| Rat | **K** | K | **Q** | E | D | Y | Q | N | **I** | **N** | **F** | N | **Q** | **P** | **T** | N | **H** | R | R | 10/19 | AAW78017.1 |
| *Rhinolophus sinicus* | **E** | K | **T** | **K** | D | **H** | Q | N | L | **N** | Y | N | N | **E** | **N** | N | K | R | R | 12/19 | AGZ48803.1 |
| Horse | **L** | K | **S** | E | **E** | **H** | Q | N | L | **T** | Y | N | N | Q | E | N | K | R | R | 14/19 | XP 001490241.1 |
| Cattle | Q | K | H | E | D | Y | Q | N | **M** | **T** | Y | N | **Y** | Q | **D** | N | K | R | R | 15/19 | XP 005228485.1 |
| Sheep | Q | K | H | E | D | Y | Q | N | **M** | **T** | Y | N | **Y** | Q | **D** | N | K | R | R | 15/19 | XP 011961657.1 |
| *Nyctereutes procyonoides* | **L** | K | **Y** | E | **E** | Y | Q | N | L | **T** | Y | **D** | N | Q | E | N | **R** | R | R | 13/19 | ABW16956.1 |
| Raccoon | **L** | **N** | **N** | E | **E** | Y | Q | N | **Q** | **T** | Y | **D** | N | Q | E | N | K | R | R | 12/19 | BAE72462.1 |
| Camel | **L** | **E** | H | E | D | Y | Q | N | **T** | **T** | Y | N | N | Q | **D** | N | K | R | R | 14/19 | XP 031301717.1 |
| Civet | **L** | **T** | **Y** | E | **E** | Y | Q | N | L | **T** | Y | **D** | N | Q | E | N | K | R | R | 13/19 | AAX63775.1 |
| Ferret | **L** | K | **Y** | E | **E** | Y | Q | N | **H** | **T** | Y | **D** | N | **E** | **Q** | N | K | R | R | 11/19 | BAE53380.1 |
| Fox | **L** | K | **Y** | E | **E** | Y | Q | N | L | **T** | Y | **D** | N | Q | E | N | K | R | R | 14/19 | XP 025842513.1 |
| *Echinops telfairi* | Q | **T** | **N** | E | **N** | Y | Q | N | L | **K** | **F** | **D** | **P** | Q | **D** | **K** | **L** | R | R | 9/19 | XP 004710002.1 |
| Chicken | **E** | **E** | **V** | **R** | D | Y | **E** | N | **N** | **R** | **F** | **D** | N | **E** | **T** | N | K | R | R | 8/19 | XP 416822.2 |
| Pangolin | **E** | K | **S** | E | **E** | Y | Q | N | **I** | **N** | Y | N | **K** | Q | E | N | K | R | R | 13/19 | XP 017505752.1 |

## Potential Drug Targets in S of 2019-nCoV

No approved antiviral agents are available against the current outbreak, but convalescent sera or monoclonal antibodies inhibit SARS-CoV or MERS-CoV *in vitro* or in animal models. However, sufficient sera and antibodies can hardly be produced during a large outbreak. Moreover, monoclonal antibodies neutralizing SARS-CoV are not (or only poorly) reactive against 2019-nCoV, indicating that the antibody epitopes are highly variable [74]. Inhibitors of the proteases that prime S for fusion also have antiviral activity. However, since S can use various proteases for priming, more than one inhibitor is required.

More promising are drugs directed against the highly conserved S2 subunit, such as peptides that inhibit membrane fusion. The proof of principle is **enfuvirtide**, a 20 amino acid peptide that is identical in sequence to a part of the heptad repeat region 2 (HR2) that forms a six helix bundle with heptad repeat region 1 (HR1). The peptide binds to HR1, which saturates the binding site for HR2, thereby preventing the conformational change that catalyzes membrane fusion. Peptides with a similar mode of action have been developed for the S2 subunit of SARS-CoV and MERS-CoV. They inhibit virus entry, reduce formation of **plaques** *in vitro*, and had beneficial effects in a mouse model. The

most promising peptide is called E1, which binds with high affinity to the HR1 region of S from SARS-CoV [75]. Sequence comparison between HR1 of S from SARS-CoV and 2019-nCoV shows various amino acid exchanges, but none of them is involved in binding to E1 (Figure 3C), indicating that E1 could also be effective against 2019-nCoV.

Another potential drug target might be the cellular enzyme(s) that attach fatty acids to a cluster of cysteines in the cytoplasmic tail of S. The fatty acids are required for S to fuse with the host cell and affect virus assembly, similar to what has been described for other spike proteins, such as HA of influenza virus. Enzymes that attach acyl chains to S have not been identified, but cellular proteins are acylated by one or several of the 23 members of the **ZDHHC family**, which have distinct, only partly overlapping substrate specificities. If only a few of them might acylate S in airway cells of the lung, their blockade might result in suppression of viral replication, while acylation of cellular proteins will not be (or very little) compromised. Although more research is required, targeting acyltransferases might be promising, since the cluster of cysteines is present in S from all CoV genera, regardless of their origin. Acylation might thus be required for a very basic function of S, arguing that even newly emerged CoVs probably will also rely on this modification of S to replicate efficiently [76]. However, since key proteins of the innate immune response are also palmitoylated, acylation inhibitors might be limited if the proteins of the innate immune response are modified by the same enzymes as viral proteins.

## Concluding Remarks

Previous studies showed that CoVs genomes display a high degree of plasticity in terms of gene content and recombination. Furthermore, the relatively large CoV genome increases the probabilities for adaptive mutations, with it being relative easy for the spike protein to exploit multiple cellular receptors for virus attachment and entry [52,77–79]. These features are likely the cause of this alarming propensity of CoVs for host-species expansion. Unfortunately, China has seen a number of interspecies transmissions by CoV in recent years [80–82]. Whether this current COVID-19 epidemic 'frizzles out' or expands into a full-blown pandemic remains to be seen. It might also be desirable to monitor farm animals and pet cats for infection with 2019-nCoV, since their ACE2 receptor responsible for 2019-nCoV binding differs in only a few amino acids from human ACE2. Surveillance might prevent the virus establishing itself in another animal species that is in close contact to humans. In addition, in light of the fact that there are multiple species of CoVs circulating in wildlife species and that these animals are constantly interacting with each other, host-species expansion or interspecies transmission of new CoV to humans seems to be inevitable. Major knowledge gaps regarding the emergence of 2019-nCoV remain exists but worldwide scientists are working with unprecedented speed to investigate the virus, rushing to develop targeted therapeutics (see Outstanding Questions). Notwithstanding, a global surveillance network involving veterinarians and animal biologists is urgently needed to monitor, and possibly to predict, potential sources for the emergence of another highly pathogenic CoV. We propose the concept of 'One Health' to facilitate scientific exchange across disciplines, sharing of data, and coordinated efforts in order to prevent future outbreaks.

## Outstanding Questions

When and how did COVID 19 emerge? What is or are the natural and intermediate host species for 2019 nCoV? What is the distribution of 2019 nCoV in different mammalian species? Will it infect farm animals or pets?

From surveillance and evolutionary studies on animal viruses, can their zoonotic potential be identified before interspecies transmission occurs?

What are the key interactions between the spike protein (S) of 2019 nCoV and its receptor angiotensin converting enzyme 2 (ACE 2)? Which amino acids in ACE2 determine whether S can bind? Is efficient binding to ACE2 the only determinant that decides whether an animal species can be infected?

Is expression of the trans membrane protease/serine another decisive factor for infection of a cell? Is the newly acquired polybasic cleavage site in S associated with cross species transmission of 2019 nCoV?

What are the similarities and differences of COVID 19 epidemiology in comparison with SARS and MERS? What is the basic reproductive number ($R_0$), the real incubation period, and the morbidity and mortality rate? Can COVID 19 develop into an endemic or seasonal infectious disease, like the flu?

With the experience of mitigating the outbreaks of SARS and avian influenza, what strategies can be applied in mitigating COVID 19 and future CoV outbreaks? Should veterinarians play more important roles in the prevention and control of emerging zoonoses in the future?

### Acknowledgments

We thank Professor Changchun Tu, Institute of Military Veterinary Medicine, Academy of Military Medical Sciences, for his guidance and help. This work was financially supported by the National Key Research and Development Program of China (2017YFD0500101), the Fundamental Research Funds for the Central Universities Y0201900459, the Young Top Notch Talents of National Ten Thousand Talents Program, Sino German cooperation and exchange project in international cooperation and Cultivation Project in 2019, and the Bioinformatics Center of Nanjing Agricultural University. X.J. and M.A.S. are partially supported through National Institutes of Health grant U19 AI135995. Research in the lab of M.V. is funded by the

German Research Foundation (DFG). WT.H., JY.Z., V.M., and S.S. are co senior authors. We thank Professor Jason S. McLellan and his team, Department of Molecular Biosciences, The University of Texas at Austin, for providing us with the coordinates of the 2019 nCoV spike protein.

## References

1. Gorbalenya, A.E. *et al.* (2020) Severe acute respiratory syndrome-related coronavirus: the species and its viruses – a statement of the Coronavirus Study Group. *bioRxiv*. Published online February 11, 2020. https://doi.org/10.1101/2020.02.07.937862
2. Resta, S. *et al.* (2018) Isolation and propagation of a human enteric coronavirus. *Science* 4717, 978–981
3. Arabi, Y.M. *et al.* (2017) Middle East Respiratory Syndrome. *N. Engl. J. Med.* 376, 584–594
4. Zhong, N.S. *et al.* (2003) Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* 362, 1353–1358
5. Drosten, C. *et al.* (2003) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* 348, 1967–1976
6. Guan, Y. *et al.* (2003) Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 302, 276–278
7. Song, H.D. *et al.* (2005) Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2430–2435
8. Cohen, J. *et al.* (2019) New SARS-like virus in China triggers alarm. *Science* 6475, 234–235
9. Woo, P.C. *et al.* (2012) Discovery of seven novel mammalian and avian coronaviruses in the genus *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. *J. Virol.* 86, 3995–4008
10. Chinese SARS Molecular Epidemiology Consortium (2004) Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 303, 1666–1669
11. He, B. *et al.* (2014) Identification of diverse alphacoronaviruses and genomic characterization of a novel severe acute respiratory syndrome-like coronavirus from bats in China. *J. Virol.* 88, 7070–7082
12. Li, Q. *et al.* (2020) Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *N. Engl. J. Med.* Published online January 29, 2020. https://doi.org/10.1056/NEJMoa2001316
13. Wang, D. *et al.* (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. Published online February 7, 2020. https://doi.org/10.1001/jama.2020.1585
14. Chan, J.F-W. *et al.* (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395, 514–523
15. Yang, Y. *et al.* (2020) Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. *medRxiv*. Published online February 21, 2020. https://doi.org/10.1101/2020.02.10.20021675
16. Kang, M. *et al.* (2020) Evidence and characteristics of human-to-human transmission of SARS-CoV-2. *medRxiv*. Published online February 17, 2020. https://doi.org/10.1101/2020.02.03.20019141
17. Liu, Y.-C. *et al.* (2020) A locally transmitted case of SARS-CoV-2 infection in Taiwan. *N. Engl. J. Med.* Published online February 12, 2020. https://doi.org/10.1056/NEJMc2001573
18. Chang, D. *et al.* (2020) Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. *JAMA*. Published online February 7, 2020. https://doi.org/10.1001/jama.2020.1623
19. Li, L. *et al.* (2020) An update on the epidemiological characteristics of novel coronavirus pneumonia (COVID-19). *Zhonghua Liu Xing Bing Xue Za Zhi* 41, 139–144
20. Novel Coronavirus Pneumonia Emergency Response Epidemiology Team (2020) The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. *Zhonghua Liu Xing Bing Xue Za Zhi* 41, 145–151
21. Guan, W.-j. *et al.* (2020) Clinical characteristics of 2019 novel coronavirus infection in China. *medRxiv*. Published online February 9, 2020. https://doi.org/10.1101/2020.02.06.20020974
22. Lipsitch, M. *et al.* (2003) Transmission dynamics and control of severe acute respiratory syndrome. *Science* 300, 1966–1970
23. Riley, S. *et al.* (2003) Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions. *Science* 300, 1961–1966
24. Breban, R. *et al.* (2013) Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 382, 694–699
25. Chowell, G. *et al.* (2004) Model parameters and outbreak control for SARS. *Emerg. Infect. Dis.* 10, 1258–1263
26. Swerdlow, D.L. and Finelli, L. (2020) Preparation for possible sustained transmission of 2019 novel coronavirus: lessons From previous epidemics. *JAMA*. Published online February 11, 2020. https://doi.org/10.1001/jama.2020.1960
27. Park, J.E. *et al.* (2018) MERS transmission and risk factors: a systematic review. *BMC Public Health* 18, 574
28. Paul, L.D. *et al.* (2019) Complexity of the basic reproduction number ($R_0$). *Emerg. Infect. Dis.* 25, 1–4
29. Chen, N. *et al.* (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513
30. Pongpirul, W.A. *et al.* (2020) Journey of a Thai taxi driver and novel coronavirus. *N. Engl. J. Med.* Published online February 12, 2020. https://doi.org/10.1056/NEJMc2001621
31. Bastola, A. *et al.* (2020) The first 2019 novel coronavirus case in Nepal. *Lancet Infect. Dis.* 20, 279–280
32. Holshue, M.L. *et al.* (2020) First case of 2019 novel coronavirus in the United States. *N Engl. J. Med.* Published online January 31, 2020. https://doi.org/10.1016/S1473-3099(20)30067-0
33. Huang, C., *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497-506
34. Chen, H., *et al.* Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 20, 30360-30363
35. Xu, X. *et al.* (2020) Imaging features of 2019 novel coronavirus pneumonia. *Eur. J. Nucl. Med. Mol. Imaging*. Published online February 14, 2020. https://doi.org/10.1007/s00259-020-04720-2
36. Lin, X. and Gong, Z. (2020) Novel coronavirus pneumonia outbreak in 2019: computed tomographic findings in two cases. *Korean J. Radiol.* 21, 365–368
37. Wei, M. *et al.* (2020) Novel coronavirus infection in hospitalized infants under 1 year of age in China. *JAMA*. Published online February 14, 2020. https://doi.org/10.1001/jama.2020.2131
38. Hoehl, S. *et al.* (2020) Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China. *N. Engl. J. Med.* Published online February 18, 2020. https://doi.org/10.1056/NEJMc2001899
39. Zhu, H. *et al.* (2020) Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. *Transl. Pediatr.* 9, 51–60
40. Anderson, R.M. *et al.* (2004) Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 359, 1091–1105
41. Chowell, G. *et al.* (2015) Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC Med.* 13, 210
42. Zhou, P. *et al.* (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. Published online February 3, 2020. https://doi.org/10.1038/s41586-020-2012-7
43. Wu, F. *et al.* (2020) A new coronavirus associated with human respiratory disease in China. *Nature*. Published online February 3, 2020. https://doi.org/10.1038/s41586-020-2008-3
44. Li, G. *et al.* (2018) Organ, genetic diversity, and evolutionary dynamics of novel porcine circovirus 3. *Adv. Sci. (Weinh)* 5, 1800275

45. Huang, Y.-W. *et al.* (2013) Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *mBio* 4, e00737-00713
46. Tao, Y. *et al.* (2017) Surveillance of bat coronaviruses in Kenya identifies relatives of human coronaviruses NL63 and 229E and their recombination history. *J. Virol.*, e01953-16
47. Yang, L. *et al.* (2019) Broad cross-species infection of cultured cells by bat HKU2-related swine acute diarrhea syndrome coronavirus and identification of its replication in murine dendritic cells in vivo highlight its potential for diverse interspecies transmission. *J. Virol.* 93, e01448-19
48. Karakus, U. *et al.* (2019) MHC class II proteins mediate cross-species entry of bat influenza viruses. *Nature* 7746, 109–112
49. He, W. *et al.* (2019) Genetic analysis and evolutionary changes of porcine circovirus 2. *Mol. Phylogenet. Evol.* 139, 106520
50. Chen, W. *et al.* (2005) SARS-associated coronavirus transmitted from human to pig. *Emerg. Infect. Dis.* 11, 446–448
51. Hu, D. *et al.* (2018) Genomic characterization and infectivity of a novel SARS-like coronavirus in Chinese bats. *Emerg. Microbes Infect.* 7, 154
52. Lam, T.T.-Y. *et al.* (2020) Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China. *bioRxiv.* Published online February 18, 2020. https://doi.org/10.1101/2020.02.13.945485
53. Menachery, V.D. *et al.* (2015) A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat. Med.* 21, 1508–1513
54. Paraskevis, D. *et al.* (2020) Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect. Genet. Evol.* 79, 104212
55. Luk, H.K.H. *et al.* (2019) Molecular epidemiology, evolution and phylogeny of SARS coronavirus. *Infect. Genet. Evol.* 71, 21–30
56. Wong, M.C. *et al.* (2020) Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019. *bioRxiv.* Published online February 13, 2020. https://doi.org/10.1101/2020.02.07.939207
57. Xiao, K. *et al.* (2020) Isolation and characterization of 2019-nCoV-like coronavirus from Malayan pangolins. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.17.951335
58. Liu, P. *et al.* (2020) Are pangolins the intermediate host of the 2019 novel coronavirus (2019-nCoV)? *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.18.954628
59. Lu, R. *et al.* (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395, 565–574
60. Hui, K.P. *et al.* (2017) Tropism and innate host responses of influenza A/H5N6 virus: an analysis of ex vivo and in vitro cultures of the human respiratory tract. *Eur. Respir. J.* 49, 1601710
61. Wrapp, D. *et al.* (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* Published online February 19, 2020. https://doi.org/10.1126/science.abb2507
62. Sun, C. *et al.* (2020) SARS-CoV-2 and SARS-CoV spike-RBD structure and receptor binding comparison and potential implications on neutralizing antibody and vaccine development. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.16.951723
63. Li, F. (2016) Structure, function, and evolution of coronavirus spike proteins. *Annu. Rev. Virol.* 3, 237–261
64. Cui, J. *et al.* (2019) Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.* 17, 181–192
65. Lu, G. *et al.* (2015) Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. *Trends Microbiol.* 23, 468–478
66. Millet, J.K. and Whittaker, G.R. (2015) Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. *Virus Res.* 202, 120–134
67. Hoffmann, M. *et al.* (2020) The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *bioRxiv.* Published online January 31, 2020. https://doi.org/10.1101/2020.01.31.929042
68. Coutard, B. *et al.* (2020) The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antivir. Res.* 176, 104742
69. Walls, A.C. *et al.* (2020) Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.19.956581
70. Deng, Y. *et al.* (2006) Structures and polymorphic interactions of two heptad-repeat regions of the SARS virus S2 protein. *Structure* 14, 889–899
71. Heald-Sargent, T. and Gallagher, T. (2012) Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. *Viruses* 4, 557–580
72. Lan, J. *et al.* (2020) Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor. *bioRxiv.* Published online February 20, 2020. https://doi.org/10.1101/2020.02.19.956235
73. Li, F. *et al.* (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 309, 1864–1868
74. Zumla, A. *et al.* (2016) Coronaviruses - drug discovery and therapeutic options. *Nat. Rev. Drug Discov.* 15, 327–347
75. Xia, S. and Yan, L. (2019) A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci. Adv.* 5, eaav4580
76. Gadalla, M.R. and Veit, M. (2020) Toward the identification of ZDHHC enzymes required for palmitoylation of viral protein as potential drug targets. *Expert Opin. Drug Discov.* 15, 159–177
77. Wang, N. *et al.* (2019) Structural definition of a neutralization-sensitive epitope on the MERS-CoV S1-NTD. *Cell Rep.* 28, 3395–3405
78. Letko, M. *et al.* (2018) Adaptive evolution of MERS-CoV to species variation in DPP4. *Cell Rep.* 24, 1730–1737
79. Zhang, S. *et al.* (2018) Structural definition of a unique neutralization epitope on the receptor-binding domain of MERS-CoV spike glycoprotein. *Cell Rep.* 24, 441–452
80. Su, S. *et al.* (2016) Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends Microbiol.* 24, 490–502
81. Zhou, P. *et al.* (2018) Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* 556, 255–258
82. Hu, B. *et al.* (2017) Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog.* 13, e1006698
83. Katoh, K. *et al.* (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30, 3059–3066
84. Kumar, S. *et al.* (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874
85. Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313

**From:** Liu, Shan-Lu
**To:** Saif, Linda; Yount, Jacob
**Subject:** Columbus Dispatch letter or commentary
**Date:** Saturday, March 21, 2020 4:03:36 PM
**Attachments:** Dispatch_commentary .docx
image001.png

---

Hi Linda and Jacob:

Last few days, I have received numerous requests for interview, including local news media and even fire departments. I had to decline all of them for a variety of reasons. But I thought that it would be helpful for three of us to write a letter or commentary addressing some common questions and concerns people may have regarding the virus (not too much the COIVD-19 disease). With this mind, I just had a draft and would share with you. I would appreciate your comments, edits, etc.

Again, this is just an idea and the draft is rough, kind of outline…

Thanks.

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

# SARS-CoV-2: The Virus that Causes COIVD-19

Shan-Lu Liu, Jacob Yount, and Linda Saif

The Ohio State University

COIVD-19 (coronavirus diseases 2019) is now a global pandemic. The disease originated in Wuhan, the capital city of Hubei Province in China in November 2019. A Huanan seafood wholesale market in the city is thought to be the original source of the virus where wild animals were sold, resulting in the transmission of the virus to humans. As of March 21, 2020, more than XXX,000 confirmed cases of COIVD-19 were reported worldwide, affecting at least XX countries and causing XXX deaths. In the US, there are XXXX confirmed cases, including XX cases in the state of Ohio.

The virus causing COIVD-19 has been named by the International Committee on Taxonomy of Viruses as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The natural reservoir of the virus SARS-CoV-2 is believed to be bats, the only flying animal that harbors many other viruses, including the SARS coronavirus, Ebola virus and Zika virus. Viral phylogenetic analyses show that SARS-CoV-2 shares over 96 % similarity to one of the bat coronaviruses known as RaTG13 found in *Rhinolophus affinis*. However, the intermediate animal species, if there is one, that directly transmit the virus to human is currently clear. Notably, SARS-CoV-2 shares about 90% overall nucleotide sequence identity to another related coronavirus found in the endangered species of small mammals known as pangolins, and both likely use the same receptor

ACE2 to enter the host cell. Recombination between coronaviruses in different animal species may account for the origin of SARS-CoV-2.

Viruses in their natural hosts do not normally cause diseases because of mutual coadaptation. However, when the virus jumps to a new species, including humans, severe infection occurs that results in pathogenesis even deaths. This has been proven to be the case for HIV that causes AIDS pandemic and many viruses. One critical question is whether or not the continued spread of SARS-CoV-2 in humans would result in changes in transmission rates and diseases severity. If the transmission is weakened over time, the outbreak would ultimately end and the virus SARS-CoV-2 be eradicated from humans. However, if effective transmission is sustained, the viral infection will become community-acquired human coronaviruses, such as 229E, OC43, HKU1 and NL63, which are known to cause flu-like common cold. One measurement of the viral transmission rate is the viral reproductive number ($R_0$); for SARS-CoV-2, it is currently estimated to be 2.7, corresponding to an epidemic doubling time of about 6.4 days. This rate is relatively high compared to that of SARS-CoV, the virus that caused SARS outbreak in 2003 ($R_0$ less than 2.0). Accurately defining and monitoring the $R_0$ values should provide informed guidance for the effective control of the SARS-CoV-2 spread.

While SARS-CoV-2 causes severe pulmonary syndromes and even deaths, many infected individuals remain asymptomatic, which constitutes a dangerous source of viral transmission. Hence, social distancing currently taken by the US and other COIVD-19 outbroken countries is critical and the most effective way to contain the viral and

disease spread. In addition to transmission by droplets and close contact, fecal–oral transmission of SARS-CoV has been recently reported; thus, frequent handwashing and clean sanitation may be important. There have also been reports of ocular infection in SARS-CoV-2 infected individuals, so eye protection is needed under certain circumstances.

Animal coronavirus and implications for COIVD-19: Linda please add.

Vaccination is the most effective strategy to prevent occurrence of infectious diseases. Unfortunately, an FDA-approved vaccine for SARS-CoV-2-induced COIVD-19 is currently not available. Encouragingly, a viral mRNA-based vaccine has just entered the first phase of human trial, and if successful, this vaccine, along with many others in the pipeline, will become powerful in the fight of COIVD-19.

*The authors of this commentary, SLL, JY and LS, are co-directors of the Viruses and Emerging Pathogens Program, The Infectious Diseases Institute, The Ohio State University.*

**From:** Liu, Shan-Lu
**To:** rbaric@email.unc.edu
**Cc:** Saif, Linda; tcbaric@med.unc.edu
**Subject:** Visit to The Ohio State University and commentary
**Date:** Thursday, February 27, 2020 2:20:10 AM
**Attachments:** No credible evidence supporting claims of the laboratory engineering of SARS CoV 2.pdf
image001.png

---

Hi Ralph,

See below the link and also the attached PDF file of our newly published commentary.

https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1733440

Kindly let us know your preferred date of the visit to OSU.

Best.

Shan-Lu

---

**From:** "Liu, Shan-Lu" <Shan-Lu.Liu@osumc.edu>
**Date:** Tuesday, February 25, 2020 at 6:34 PM
**To:** "rbaric@email.unc.edu" <rbaric@email.unc.edu>
**Cc:** "Saif, Linda" <saif.2@osu.edu>
**Subject:** Visit to The Ohio State University for a distinguished seminar

Dear Ralph,

It was great to see you at the VirB meeting last week, and I truly enjoyed our discussion, although it was short.

As I mentioned, Linda and I would like to invite you to The Ohio State for a distinguished seminar this year for our Infectious Diseases Institute seminar series. I just looked at our schedule and realized that we will have a workshop focusing on emerging viral pathogenesis and vaccine development on April 15. If you are able to make this time, we will arrange your talk in the morning opening session as a distinguished keynote address. In the afternoon, Dan Barouch from Harvard Medical School will give another keynote lecture.

If the date of April 15 does not work for you, I will discuss with Linda and try to find another time suitable for you. Perhaps you may also suggest some preferred dates from March -June that will work for you.

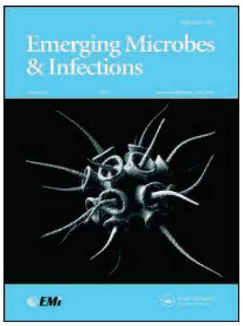
As promised, I will send you're the link to our Commentary in EMI once it becomes available online – should be online tomorrow or on Thursday.

Best wishes!

Shan-Lu

THE OHIO STATE UNIVERSITY

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu



Emerging Microbes & Infections

ISSN: (Print) 2222-1751 (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su

**To cite this article:** Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su (2020) No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2, Emerging Microbes & Infections, 9:1, 505-507, DOI: 10.1080/22221751.2020.1733440

**To link to this article:** https://doi.org/10.1080/22221751.2020.1733440



Published online: 26 Feb 2020.

Emerging Microbes & Infections
2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

OPEN ACCESS Check for updates

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan R. Weiss [f] and Lishan Su[g]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [b]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [c]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA; [d]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA; [e]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [f]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [g]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

**ARTICLE HISTORY** Received 13 February 2020; Accepted 13 February 2020

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1,100 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It was proposed that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary

**CONTACT** Shan Lu Liu Liu.6244@osu.edu; Lishan Su lsu@med.unc.edu

evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to MA15 chimeric virus with the original human SARS S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## ORCID

*Susan R. Weiss* http://orcid.org/0000 0002 8155 4528

## References

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1585

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

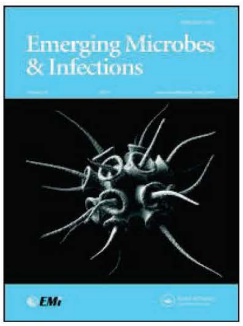
[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426(6965): 450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

| | |
|---|---|
| **From:** | Liu, Shan-Lu |
| **To:** | Saif, Linda; Lishan Su; Susan Weiss |
| **Date:** | Thursday, February 27, 2020 1:52:04 AM |
| **Attachments:** | No credible evidence supporting claims of the laboratory engineering of SARS CoV 2.pdf<br>ATT00001.txt |

---

https://www.tandfonline.com/doi/pdf/10.1080/22221751.2020.1733440?needAccess=true



Emerging Microbes & Infections

ISSN: (Print) 2222-1751 (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su

**To cite this article:** Shan-Lu Liu, Linda J. Saif, Susan R. Weiss & Lishan Su (2020) No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2, Emerging Microbes & Infections, 9:1, 505-507, DOI: 10.1080/22221751.2020.1733440

**To link to this article:** https://doi.org/10.1080/22221751.2020.1733440



Published online: 26 Feb 2020.

Emerging Microbes & Infections
2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

OPEN ACCESS Check for updates

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan R. Weiss [f] and Lishan Su[g]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [b]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [c]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA; [d]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA; [e]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [f]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [g]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

**ARTICLE HISTORY** Received 13 February 2020; Accepted 13 February 2020

The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1,100 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

It was proposed that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe bats for entry [8]. Combined with evolutionary

**CONTACT** Shan Lu Liu Liu.6244@osu.edu; Lishan Su lsu@med.unc.edu

evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SHC014-MA15 chimeric virus relative to MA15 chimeric virus with the original human SARS S gene in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## ORCID

*Susan R. Weiss* http://orcid.org/0000 0002 8155 4528

## References

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1585

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections invol ving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. doi:10.1001/jama.2020.1623

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coro navirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30;395(10223):507 513.

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia out break associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2012 7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24;382(8):727 733.

[6] Song HD, Tu CC, Zhang GW, et al. Cross host evol ution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430 2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508 1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characteriz ation of a bat SARS like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535 538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse adapted SARS coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3 (1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS corona virus spike receptor binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864 1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin con verting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426(6965): 450 454.
[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and charac terization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276 278.
[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2 utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350 6353.
[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associ ated with human respiratory disease in China. Nature. 2020 Feb 3. doi:10.1038/s41586 020 2008 3
[15] Xiao C, Li X, Liu S, et al. HIV 1 did not contribute to the 2019 nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378 381.

**From:** Liu, Shan-Lu
**To:** Su, Lishan; Saif, Linda; Susan Weiss
**Subject:** Re: Your article proofs for review (ID# TEMI 1733440)
**Date:** Friday, February 21, 2020 10:04:43 AM
**Attachments:** image001.png

---

We agreed to add this link to the proof to the third paragraph regarding RaTG13.

**The Proximal Origin of SARS-CoV-2**
http://virological.org/t/the-proximal-origin-of-sars-cov-2/398

Shan-Lu Liu, M.D., Ph.D.
Professor
Co-Director, Viruses and Emerging Pathogens Program
Infectious Diseases Institute
Center for Retrovirus Research
Departments of Veterinary Biosciences, Microbial Infection and Immunity, and Microbiology
The Ohio State University
1900 Coffey Rd, Room 480 VMAB
Columbus, Ohio 43210
Phone: (614) 292-8690
Fax: (614) 292-6473
Email: liu.6244@osu.edu; shan-lu.liu@osumc.edu

---

**From:** Shan-Lu Liu <liu.6244@osu.edu>
**Date:** Friday, February 21, 2020 at 4:46 AM
**To:** "Su, Lishan" <lishan_su@med.unc.edu>, "Saif, Linda" <saif.2@osu.edu>, Susan Weiss <weisssr@pennmedicine.upenn.edu>
**Subject:** FW: Your article proofs for review (ID# TEMI 1733440)

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

---

**From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
**Reply-To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
**Date:** Friday, February 21, 2020 at 4:13 AM
**To:** Shan-Lu Liu <liu.6244@osu.edu>

**Subject:** Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear Shan-Lu Liu,

Your article proofs are now available for review through the Central Article Tracking System (CATS) at: https://cats.informa.com/PTS/in?ut=B2AB6692AA414D96905B59E6C51FA240 .

PLEASE NOTE: The CATS system only supports Internet Explorer 6 (and later), or Firefox 3 (and later) browser software. Popup blockers should be disabled. If you have any difficulty using CATS, please contact me.

• Your User Name is: LIUS876

• If you do not know your password, you may reset it here: http://cats.informa.com/PTS/forgottenPassword.do

1. Click on 'Review Proofs'.

2. Select 'Download PDF'.

3. Follow the guidance on the proof cover sheet to return your corrections. Please limit changes to answering any author queries and to correcting errors. We would not expect to receive more than 30 corrections.

Please check your proofs thoroughly before submitting your corrections as once they have been submitted we are unable to accept further corrections. If you have any queries, please email me.

To avoid delaying publication of your article, please approve these proofs or return any corrections by 26 Feb 2020.

Reprint and issue orders may be placed by logging in to your CATS account and accessing the order form on the "Additional Actions" menu. If you have any questions on this process, please contact me or visit our author services site https://authorservices.taylorandfrancis.com/ordering-print-copies-of-your-article/

• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link: https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

**From:** Liu, Shan-Lu
**To:** Su, Lishan; Saif, Linda; Susan Weiss
**Subject:** FW: Your article proofs for review (ID# TEMI 1733440)
**Date:** Friday, February 21, 2020 7:46:49 AM
**Attachments:** TEMI_A_1733440_Proof.pdf

---

All,

See message below and also the attached proof.

Please mark your changes in the attached PDF file, and Lishan and I will incorporate to finalize.

Thanks.

Shan-Lu

---

**From:** "TEMI-production@journals.tandf.co.uk" <cats@taylorandfrancis.com>
**Reply-To:** "TEMI-production@journals.tandf.co.uk" <TEMI-production@journals.tandf.co.uk>
**Date:** Friday, February 21, 2020 at 4:13 AM
**To:** Shan-Lu Liu <liu.6244@osu.edu>
**Subject:** Your article proofs for review (ID# TEMI 1733440)

Article: No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Journal: *Emerging Microbes & Infections* (TEMI)

Article ID: TEMI 1733440

Dear Shan-Lu Liu,

Your article proofs are now available for review through the Central Article Tracking System (CATS) at: https://cats.informa.com/PTS/in?ut=B2AB6692AA414D96905B59E6C51FA240 .

PLEASE NOTE: The CATS system only supports Internet Explorer 6 (and later), or Firefox 3 (and later) browser software. Popup blockers should be disabled. If you have any difficulty using CATS, please contact me.

• Your User Name is: LIUS876

• If you do not know your password, you may reset it here: http://cats.informa.com/PTS/forgottenPassword.do

1. Click on 'Review Proofs'.

2. Select 'Download PDF'.

3. Follow the guidance on the proof cover sheet to return your corrections. Please limit changes to answering any author queries and to correcting errors. We would not expect to receive more than 30 corrections.

Please check your proofs thoroughly before submitting your corrections as once they have been submitted we are unable to accept further corrections. If you have any queries, please email me.

To avoid delaying publication of your article, please approve these proofs or return any corrections by 26 Feb 2020.

Reprint and issue orders may be placed by logging in to your CATS account and accessing the order form on the "Additional Actions" menu. If you have any questions on this process, please contact me or visit our author services site
https://authorservices.taylorandfrancis.com/ordering-print-copies-of-your-article/

• The DOI of your paper is: 10.1080/22221751.2020.1733440. Once your article has published online, it will be available at the following permanent link:
https://doi.org/10.1080/22221751.2020.1733440 .

Thank you,

Malathi Boopalan

Email:TEMI-production@journals.tandf.co.uk

TEMI1733440 VOL 0, ISS 0

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Shan-Lu Liu, Linda J. Saif, Susan Weiss, and Lishan Su

## QUERY SHEET

This page lists questions we have about your paper. The numbers displayed at left are hyperlinked to the location of the query in your paper.

The title and author names are listed on this sheet as they will be published, both on your paper and on the Table of Contents. Please review and ensure the information is correct and advise us if any changes need to be made. In addition, please review your paper as a whole for typographical and essential corrections.

Your PDF proof has been enabled so that you can comment on the proof directly using Adobe Acrobat. For further information on marking corrections using Acrobat, please visit http://journalauthors.tandf.co.uk/production/acrobat.asp; https://authorservices.taylorandfrancis.com/how-to-correct-proofs-with-adobe/

The CrossRef database (www.crossref.org/) has been used to validate the references. Changes resulting from mismatches are tracked in red font.

## AUTHOR QUERIES

| QUERY NO. | QUERY DETAILS |
|---|---|
| Q1 | Please note that the ORCID has/have been created from information provided through CATS. Please correct if this is inaccurate. |
| Q2 | The disclosure statement has been inserted. Please correct if this is inaccurate. |
| Q3 | The CrossRef database (www.crossref.org/) has been used to validate the references. Mismatches between the original manuscript and CrossRef are tracked in red font. Please provide a revision if the change is incorrect. Do not comment on correct changes. |
| Q4 | Please provide missing volume number and page range for reference "[1]" references list entry. |
| Q5 | Please provide missing volume number and page range for reference "[2]" references list entry. |
| Q6 | Please provide missing volume number and page range for reference "[3]" references list entry. |
| Q7 | Please provide missing volume number and page range for reference "[4]" references list entry. |
| Q8 | Please provide missing volume number and page range for reference "[5]" references list entry. |
| Q9 | Please provide missing volume number and page range for reference "[14]" references list entry. |

Emerging Microbes & Infections
https://doi.org/10.1080/22221751.2020.1733440

COMMENTARY

OPEN ACCESS

# No credible evidence supporting claims of the laboratory engineering of SARS-CoV-2

Q1 Shan-Lu Liu[a,b,c,d], Linda J. Saif[d,e], Susan Weiss[f] and Lishan Su[g]

[a]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [b]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [c]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA; [d]Viruses and Emerging Pathogens Program, Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA; [e]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA; [f]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [g]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

**ARTICLE HISTORY** Received 13 February 2020; Accepted 13 February 2020



The emergence and outbreak of a newly discovered acute respiratory disease in Wuhan, China, has affected greater than 40,000 people, and killed more than 1,000 as of Feb. 10, 2020. A new human coronavirus, SARS-CoV-2, was quickly identified, and the associated disease is now referred to as coronavirus disease discovered in 2019 (COVID-19) (https://globalbiodefense.com/novel-coronavirus-covid-19-portal/).

According to what has been reported [1–3], COVID-2019 seems to have similar clinical manifestations to that of the severe acute respiratory syndrome (SARS) caused by SARS-CoV. The SARS-CoV-2 genome sequence also has ~80% identity with SARS-CoV, but it is most similar to some bat beta-coronaviruses, with the highest being >96% identity [4,5].

Currently, there are speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. Some people have alleged that the human SARS-CoV-2 was leaked directly from a laboratory in Wuhan where a bat CoV (RaTG13) was recently reported, which shared ~96% homology with the SARS-CoV-2 [4]. However, as we know, the human SARS-CoV and intermediate host palm civet SARS-like CoV shared 99.8% homology, with a total of 202 single-nucleotide (nt) variations (SNVs) identified across the genome [6]. Given that there are greater than 1000 nt differences between the human SARS-CoV-2 and the bat RaTG13-CoV [4], which are distributed throughout the genome in a naturally occurring pattern following the evolutionary characteristics typical of CoVs, it is highly unlikely that RaTG13 CoV is the immediate source of SARS-CoV-2. The absence of a logical targeted pattern in the new viral sequences and a close relative in a wildlife species (bats) are the most revealing signs that SARS-CoV-2 evolved by natural evolution. A search for an intermediate animal host between bats and humans is needed to identify animal CoVs more closely related to human SARS-CoV-2. There is speculation that pangolins might carry CoVs closely related to SARS-CoV-2, but the data to substantiate this is not yet published (https://www.nature.com/articles/d41586-020-00364-2).

Another claim in Chinese social media points to a Nature Medicine paper published in 2015 [7], which reports the construction of a chimeric CoV with a bat CoV S gene (SHC014) in the backbone of a SARS CoV that has adapted to infect mice (MA15) and is capable of infecting human cells [8]. However, this claim lacks any scientific basis and must be discounted because of significant divergence in the genetic sequence of this construct with the new SARS-CoV-2 (>5,000 nucleotides).

The mouse-adapted SARS virus (MA15) [9] was generated by serial passage of an infectious wildtype SARS CoV clone in the respiratory tract of BALB/c mice. After 15 passages in mice, the SARS-CoV gained elevated replication and lung pathogenesis in aged mice (hence M15), due to six coding genetic mutations associated with mouse adaptation. It is likely that MA15 is highly attenuated to replicate in human cells or patients due to the mouse adaptation.

When the original SARS-CoV was isolated, it was concluded that the S gene from bat-derived CoV, unlike that from human patients- or civets-derived viruses, was unable to use human ACE2 as a receptor for entry into human cells [10,11]. Civets were proposed to be an intermediate host of the bat-CoVs, capable of spreading SARS CoV to humans [6,12]. However, in 2013 several novel bat coronaviruses were isolated from Chinese horseshoe bats and the bat SARS-like or SL-CoV-WIV1 was able to use ACE2 from humans, civets and Chinese horseshoe

CONTACT Shan-Lu Liu lsu@med.unc.edu; Lishan Su Liu.6244@osu.edu

bats for entry [8]. Combined with evolutionary evidence that the bat ACE2 gene has been positively selected at the same contact sites as the human ACE2 gene for interacting with SARS CoV [13], it was proposed that an intermediate host may not be necessary and that some bat SL-CoVs may be able to directly infect human hosts. To directly address this possibility, the exact S gene from bat coronavirus SL-SHC014 was synthesized and used to generate a chimeric virus in the mouse adapted MA15 SARS-CoV backbone. The resultant SL-SHC014-MA15 virus could indeed efficiently use human ACE2 and replicate in primary human airway cells to similar titres as epidemic strains of SARS-CoV. While SL-SHC014-MA15 can replicate efficiently in young and aged mouse lungs, infection was attenuated, and less virus antigen was present in the airway epithelium as compared to SARS MA15, which causes lethal outcomes in aged mice [7].

Due to the elevated pathogenic activity of the SL-SHC014-MA15 chimeric virus relative to the SARS-MA15 CoV in mice, such experiments with SL-SHC014-MA15 chimeric virus were later restricted as gain of function (GOF) studies under the US government-mandated pause policy (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/nih-lifts-funding-pause-gain-function-research). The current COVID-2019 epidemic has restarted the debate over the risks of constructing such viruses that could have pandemic potential, irrespective of the finding that these bat CoVs already exist in nature. Regardless, upon careful phylogenetic analyses by multiple international groups [5,14], the SARS-CoV-2 is undoubtedly distinct from SL-SHC014-MA15, with >6,000 nucleotide differences across the whole genome. Therefore, once again there is no credible evidence to support the claim that the SARS-CoV-2 is derived from the chimeric SL-SHC014-MA15 virus.

There are also rumours that the SARS-CoV-2 was artificially, or intentionally, made by humans in the lab, and this is highlighted in one manuscript submitted to BioRxiv (a manuscript sharing site prior to any peer review), claiming that SARS-CoV-2 has HIV sequence in it and was thus likely generated in the laboratory. In a rebuttal paper led by an HIV-1 virologist Dr. Feng Gao, they used careful bioinformatics analyses to demonstrate that the original claim of multiple HIV insertions into the SARS-CoV-2 is not HIV-1 specific but random [15]. Because of the many concerns raised by the international community, the authors who made the initial claim have already withdrawn this report.

Evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. In our view, there is currently no credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. It is more likely that SARS-CoV-2 is a recombinant CoV generated in nature between a bat CoV and another coronavirus in an intermediate animal host. More studies are needed to explore this possibility and resolve the natural origin of SARS-CoV-2. We should emphasize that, although SARS-CoV-2 shows no evidence of laboratory origin, viruses with such great public health threats must be handled properly in the laboratory and also properly regulated by the scientific community and governments.

## Disclosure statement

No potential conflict of interest was reported by the author(s). Q2

## ORCID

*Susan Weiss* http://orcid.org/0000-0002-8155-4528

## References Q3

[1] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020 Feb 7. Q4

[2] Chang LM, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA. 2020 Feb 7. Q5

[3] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Jan 30. Q6

[4] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Feb 3. Q7

[5] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24. Q8

[6] Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA. 2005 Feb 15;102(7):2430–2435.

[7] Menachery VD, Yount Jr. BL, Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015 Dec;21(12):1508–1513.

[8] Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535–538.

[9] Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.

[10] Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005 Sep 16;309(5742):1864–1868.

[11] Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the

SARS coronavirus. Nature. 2003 Nov 27;426 (6965):450–454.

[12] Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276–278.

[13] Demogines A, Farzan M, Sawyer SL. Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats. J Virol. 2012 Jun;86(11):6350–6353.

[14] Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Feb 3.

[15] Xiao C, Li X, Liu S, et al. HIV-1 did not contribute to the 2019-nCoV genome. Emerg Microbes Infect. 2020 Dec;9(1):378–381.

**From:** Greg Gray, M.D.

**To:** Bailey, Emily "TTUHC"; Barnes, Amber; Blair, Patrick; Brown, Ian; Bui, Anh Bui; Callahan, Johnny; Cooper, Michael (NIH/NIAID); Dao, Duy Tung; Dawson, Erica; Dean Erdman - CDC (Derdman05@gmail.com); DeRisi, Joe; Everett, Helen; Franz, David; Garcia-Rivera, Jose; Gerhard, John; Gonchigoo, Battsetseg (Batty); Greer, Annette; Hii, KingChing; Lindsley, William; Ma, Mai-Juan (MJ); Mazet, Jonna; Nguyen-Viet, Hung (ILRI); Nyunt, Myaing; John Perfect, M.D.; Plowe, Chris; Saif, Linda; Salman,Mo; Sanchez, Jose L; Suhaili, Mohammed Raili; Tamerius, John; Toh, Teck-Hock; Vugng, Bùi Nghia (Vuong); Wang, Guo-Lin; Weinberger, Ari; Wickenkamp, Natalie VT; Wijayaratne, Gaya; Dr Cameron Wolfe, M.D.; Zhang, Dingmei; Benjamin Anderson, Ph.D; Ricardo Oi; Xinye Wang; Anfal Abdelgadir; Natalie Alaria; Benjamin Anderson, Ph.D; Raquel Binder, Ph.D.; Chen Chen; Sumana Goli; Kara Kochek; Arthi Kozhumam; Cassandra Lautredou; Lucas Rocha Melogno; Emily Robie; Abby Turner; Leshan Xiu; Coleman, Kristen; Than, Son T.

**Subject:** Important commentary to consider!

**Date:** Saturday, July 25, 2020 8:03:24 AM

**Attachments:** Origins of COVID-19 (ASTMH 2019 Morens).pdf

---


## Gregory C. Gray, MD, MPH, FIDSA

Duke Medicine
Professor, Division of Infectious Diseases, Global Health Institute, & Nicholas School of the Environment, Duke University

Duke-NUS Medical School, Singapore
Professor, Program in Emerging Infectious Diseases
8 College Road, Singapore 169857

Duke Kunshan University, China
Professor, Global Health
No. 8 Duke Avenue, Kunshan, Jiangsu, China

Duke One Health: http://sites.globalhealth.duke.edu/dukeonehealth/ Email: Gregory.gray@duke.edu

*Am. J. Trop. Med. Hyg.*, 00(0), 2020, pp. 1–5
doi:10.4269/ajtmh.20-0849




**Perspective Piece**

# The Origin of COVID-19 and Why It Matters

David M. Morens,[1,2*] Joel G. Breman,[3] Charles H. Calisher,[4] Peter C. Doherty,[5] Beatrice H. Hahn,[6,7] Gerald T. Keusch,[8,9,10] Laura D. Kramer,[11,12] James W. LeDuc,[13] Thomas P. Monath,[3,14] and Jeffery K. Taubenberger[15]

[1]*American Committee on Arthropod-Borne Viruses, American Society of Tropical Medicine and Hygiene, Arlington, Virginia;* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland;* [3]*American Society of Tropical Medicine and Hygiene, Arlington, Virginia;* [4]*Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology & Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado;* [5]*Department of Microbiology and Immunology, University of Melbourne at the Doherty Institute, Melbourne, Australia;* [6]*Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;* [7]*Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;* [8]*Department of Medicine, Boston University School of Medicine, Boston, Massachusetts;* [9]*Department of Global Health, Boston University School of Public Health, Boston, Massachusetts;* [10]*National Emerging Infectious Diseases Laboratory at Boston University, Boston, Massachusetts;* [11]*Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, Albany, New York;* [12]*Department of Biomedical Sciences, School of Public Health, State University of New York at Albany, Albany, New York;* [13]*Galveston National Laboratory and Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas;* [14]*Crozet BioPharma LLC, Devens, Massachusetts;* [15]*Viral Pathogenesis and Evolution Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland*

*Abstract.* The COVID-19 pandemic is among the deadliest infectious diseases to have emerged in recent history. As with all past pandemics, the specific mechanism of its emergence in humans remains unknown. Nevertheless, a large body of virologic, epidemiologic, veterinary, and ecologic data establishes that the new virus, SARS-CoV-2, evolved directly or indirectly from a β-coronavirus in the sarbecovirus (SARS-like virus) group that naturally infect bats and pangolins in Asia and Southeast Asia. Scientists have warned for decades that such sarbecoviruses are poised to emerge again and again, identified risk factors, and argued for enhanced pandemic prevention and control efforts. Unfortunately, few such preventive actions were taken resulting in the latest coronavirus emergence detected in late 2019 which quickly spread pandemically. The risk of similar coronavirus outbreaks in the future remains high. In addition to controlling the COVID-19 pandemic, we must undertake vigorous scientific, public health, and societal actions, including significantly increased funding for basic and applied research addressing disease emergence, to prevent this tragic history from repeating itself.

In 2007, scientists studying coronaviruses warned: "The presence of a large reservoir of SARS-CoV–like viruses in horseshoe bats... is a time bomb. The possibility of the re-emergence of SARS and other novel viruses... should not be ignored."[1]

Few paid attention following the disappearance of SARS after the initial outbreak in 2002. Now, 18 years later, COVID-19 has emerged as the deadliest respiratory disease pandemic since 1918, when the "Spanish" influenza pandemic killed an estimated 50 million people.[2] We need to understand what happened so that we can prevent it from happening again, and be better prepared to contain similar pandemics at their outsets.

## EMERGENCE OF THE COVID-19 PANDEMIC

The agent of COVID-19, SARS-CoV-2, was named after the genetically related SARS-CoV (more recently distinguished by some as SARS-CoV-1), which caused a deadly near-pandemic in 2002–2003.[3] Before 2019, neither SARS-CoV-2 nor its genetic sequences had ever been identified in viruses of humans or animals.

Even so, scientific research conducted over the last two decades provides clues about how and why the COVID-19 pandemic appeared. We must understand these critically important scientific findings, described in the following text, so that we can better address significant existential risks we will continue to face for the foreseeable future.

* Address correspondence to David M. Morens, Room 7A-03, Building 31, 31 Center Drive, Bethesda, Maryland 20892-2520. Email: dm270q@nih.gov

## HOW VIRAL DISEASES EMERGE

Viruses are compact nucleic acid packages of either DNA or (in the case of coronaviruses) RNA associated with proteins, and in some cases with lipids. Viruses are not living organisms and can only reproduce inside living cells susceptible to viral entry and with the capacity to replicate viral nucleic acids and translate nucleic acid signals into amino acids to build viral proteins. Viruses are therefore nonliving self-contained genetic programs capable of redirecting a cell's machinery to produce more of themselves.

It follows that when a virus enters a human cell for the first time, it has very recently been transmitted from cells of some other host, that is, from another animal or, for example, an insect vector. Emergence of a pathogen between a vertebrate or an insect has been referred to as host-switching, sometimes described as a spillover event. Most of the human viral and nonviral infectious diseases that have existed for centuries—measles, influenza, cholera, smallpox (eradicated in 1980), falciparum malaria,[4] dengue, HIV, and many others—originated by animal-to-human host-switching.[5] The complex genetic events that underlie host-switching differ greatly from pathogen to pathogen, but general mechanisms have been recognized for many.[6–9]

Host-switching determinants prominently include social, environmental, and biological factors providing the opportunity for host–species interaction; shared host cell receptors; genetic distance between transmitting and receiving hosts; and characteristics and complexity of the viral quasi-species or viral swarm. (RNA viruses in particular are not transmitted to multiple cells as identical virions, but as collections of thousands of different genetically related virions. The ever-changing complexity of the viral swarm varies between infections of genetically distinct but related hosts and in single hosts over time.)

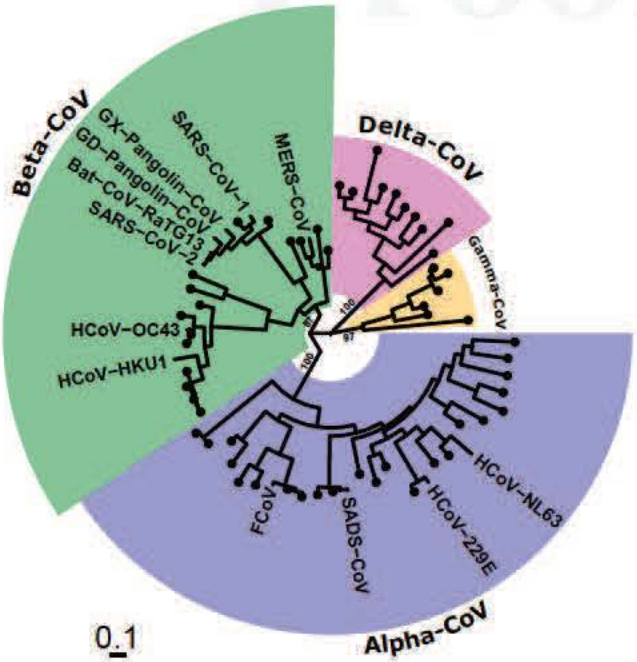



FIGURE 1. Phylogenetic relationships of selected coronaviruses of medical and veterinary importance. Human SARS-CoV and SARS-CoV-2 are closely related to numerous bat and pangolin coronaviruses in a viral genetic grouping called sarbecoviruses, which contains many other viruses very closely related to SARS-CoV and SARS-CoV-2. These viruses belong to the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae* and the four genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. The betacoronaviruses are comprised of two subgenera, *Sarbecovirus* and *Merbecovirus*. The former include SARS-CoV and SARS-CoV-2; the latter includes Middle East respiratory syndrome-related coronavirus (MERS-CoV). Image created by Sebastian M. Gygli, Ph.D., NIAID, NIH, and used with permission.

Studying animal viruses that have previously spilled over into humans provides clues about host-switching determinants. A well-understood example is influenza virus emergence into humans and other mammals.[2] Human pandemic and seasonal influenza viruses arise from enzootic viruses of wild waterfowl and shore birds. From within this natural reservoir, the 1918 pandemic "founder" virus somehow host-switched into humans. We know this from genetic studies comparing avian viruses, the 1918 virus, and its descendants, which have caused three subsequent pandemics, as well as annual seasonal influenza in each of the 102 years since 1918. Similarly, other avian influenza viruses have host-switched into horses, dogs, pigs, seals, and other vertebrates, with as yet unknown pandemic potential.[2,10,11] Although some molecular host-switching events remain unobserved, phylogenetic analyses of influenza viruses allow us to readily characterize evolution and host-switching as it occurs in nature.[2]

## CORONAVIRUSES

Coronaviruses are RNA viruses globally distributed in a large but unknown number of animal species. Coronaviruses important for humans are found within phylogenetically distinct taxonomic subgroups, labeled as the α- and β-coronaviruses (Figure 1).[12] Four endemic human coronaviruses, which emerged at some undetermined time in the past, cause (mostly) mild self-limited upper respiratory tract infections (Figure 1).

## RECENT CORONAVIRUS EMERGENCES FROM ANIMALS INTO HUMANS

Until recently, relatively little was known about coronaviruses, and research interest in these common cold viruses was minimal. Eighteen years ago, a previously unknown β-coronavirus named SARS-CoV suddenly emerged. Following its initial appearance in China it spread to 29 other countries, causing a near-pandemic and killing 813 of the 8,809 people with confirmed infection before being controlled by aggressive public health measures. It has not been seen since. In 2012, however, another previously unknown β-coronavirus named Middle East respiratory syndrome coronavirus (MERS-CoV), and closely related to SARS-CoV, emerged to cause high case-fatality human infections. Fortunately, this virus does not efficiently transmit between humans, and cases have been largely limited to the Middle East where its intermediary host, the dromedary camel, is present in relatively high numbers. In 2016, yet another novel bat-origin coronavirus, an α-coronavirus, emerged in China to cause a novel epizootic disease in pigs, termed swine acute diarrhea syndrome coronavirus (SADS-CoV). And most recently, at least as early as late November 2019, SARS-CoV-2 was recognized and became the third fatal bat virus–associated human disease

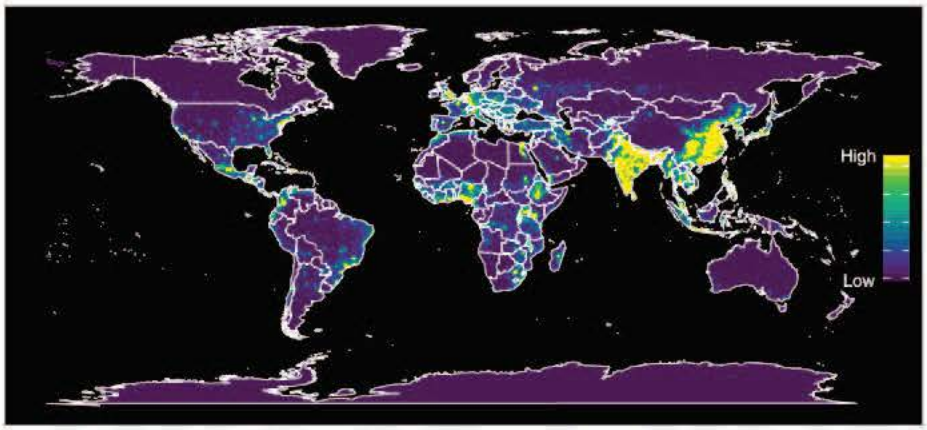



FIGURE 2. Predicted global hotspots for disease emergence, showing estimated risks, adjusted for reporting bias. From a comprehensive global study combining multiple data sources. Reproduced with permission from Allen et al.[14]

emergence and the fourth bat virus–associated mammalian emergence in 18 years.

## CORONAVIRUS EMERGENCE RISKS

An enormous reservoir of coronaviruses infects hundreds of bat species distributed globally. SARS-CoV, MERS-CoV, and SARS-CoV-2 are closely related β-coronaviruses clustering in two adjacent phylogenetic groupings: sarbecovirus (SARS-like viruses) and merbecovirus (MERS-like viruses) (Figure 1). The two SARS viruses, as well as SADS-CoV, are descended from viruses enzootic in rhinolophid (genus, *Rhinolophus*), or horseshoe bats.

Over the past 15 years, scientists have also identified global animal reservoirs of coronaviruses (in Africa, the Americas, the Middle East, Asia and Southeast Asia, and particularly China, the location of three of the four most recent emergences). These efforts have revealed much about coronaviral ecosystems, reservoir hosts, viral movement between hosts, viral evolution, and risk of emergence into humans and other mammals.

Bats of numerous globally distributed genera and species are now known to be the major reservoir of animal coronaviruses. One 20-country study of more than 19,000 animals (predominantly nonhuman primates, bats, and rodents) revealed that bats accounted for more than 98% of coronavirus detections, and that almost 9% of > 12,000 randomly studied bats were infected with one or more coronavirus.[13] Significant interspecies viral transmission between closely and distantly related bats also appears to be important. Bats of some species, including rhinolophids, co-roost with bats of other species, facilitating viral exchanges and enhanced viral evolution associated with genetic recombination. In fact, many such bat coronaviruses have genetic sequences similar to SARS-CoV and SARS-CoV-2.

Investigators have also mapped global hotspots for potential infection emergence, prominently in south/southwest China and contiguous regions and countries (Figure 2),[14] and have identified numerous human–animal interactions that constitute emergence risk factors, for example bat tourism, wet markets, wildlife supply chains for human consumption,[15] land management practices, and environmental perturbations.[16–18] Virologic and risk mapping studies indicate a very high risk of further coronavirus outbreaks.[19–21]

SARS-CoV and SARS-CoV-2 emerged in China, home to bats of more than 100 species, many of which carry α- and/or β-coronaviruses. In one study, more than 780 partial coronavirus genetic sequences were identified from bats of 41 species infected by α- and of 31 species infected by β-coronaviruses.[21] Within the sarbecovirus lineage, encompassing SARS and SARS-like viruses, many identified genetic sequences are very similar to SARS-CoV and SARS-CoV-2.[21–23] One such virus is more than 96% identical to SARS-CoV-2 in its whole genome[23]; another shares more than 97% identity in the 1ab replicase gene, as well as a furin cleavage site insertion.[24] Nature is clearly a cauldron for intense and dangerous coronavirus evolution.

## WAS COVID-19 PREDICTED?

A clearer, more worrisome picture of the coronavirus ecosystem has recently come together. A contiguous area encompassing parts of south/southwest China, Laos, Myanmar, and Vietnam constitutes a bat coronavirus "hotspot," featuring intense interspecies viral transmission. In such hotspots, a rich diversity of SARS-like viruses has been found, not only in rhinolophid bats but also in bats of other genera and species to which these viruses had host-switched. The same rhinolophid bats are also implicated in the emergence of SADS-CoV in southern China. Many of these SARS-like viruses bind to human angiotensin-converting enzyme-2 (ACE2) receptors and infect human respiratory epithelial cells in vitro, suggesting their pandemic potential.[19,25]

Ominously, bat-to-human transmission of SARS-like viruses has already been detected,[20] perhaps representing pandemic near-misses. Even the more genetically distant SADS-CoV infects cells of humans and numerous other vertebrates, raising concern about indirect coronavirus emergences. This seems to have occurred with the bat-to-camel-to-human emergence of MERS, and possibly with SARS-CoV emergence into humans, which may have resulted from bat virus infection of masked palm civet cats (*Paguma larvata*), with subsequent human spillover.[12] As a byproduct of the important international surveillance work described above, in 2017, the therapeutic benefit of the antiviral drug remdesivir was suggested; it is now, in 2020, being widely used to treat persons infected with SARS-CoV-2.[26]

Since 2007, when alarming predictions about threatened coronavirus emergences began to appear,[1] understanding of coronavirus ecosystems has become far more complete. Over the past 5 years, Chinese, American, European, and other scientists have begun to renew warnings that humans are intensively interacting with coronavirus-infected bats, that enzootic SARS-related bat coronaviruses have all of the essential components of the SARS virus, that some of these SARS-like viruses can infect laboratory-humanized mice to cause SARS-like disease, that SARS-like viruses have the ability to directly infect and be transmitted between humans, and, therefore, that these viruses are poised for human emergence.[19,21,22] Many scientists have proposed aggressive monitoring of known hotspots to try to predict and prevent viral emergence that might impact human health, including early warning of host-switching events.[19,20,27]

Unfortunately, outside of some members of the scientific community, there has been little interest and no sense of urgency. In 2020, we learned, tragically, what 12 years of unheeded warnings have led to: a bat-derived sarbecovirus—from the very same SARS-like bat virus group that had been warned about by multiple voices for over a decade—emerged and proceeded to cause the COVID-19 pandemic that now sweeps the globe.

SARS-CoV-2 emerged essentially as predicted: a natural event associated with either direct transmission of a bat coronavirus to humans or indirect transmission to humans via an intermediate host such as a Malaysian pangolin (*Manis javanica*) or another, yet-to-be-identified mammal.[28–31]

It should be clarified that theories about a hypothetical man-made origin of SARS-CoV-2 have been thoroughly discredited by multiple coronavirus experts.[21,28,29] SARS-CoV-2 contains neither the genetic fingerprints of any of the reverse genetics systems that have been used to engineer coronaviruses nor does it contain genetic sequences that would have been "forward engineered" from preexisting viruses, including the genetically closest sarbecoviruses. That is, SARS-CoV-2 is unlike any previously identified coronavirus from which it could have been engineered. Moreover, the SARS-CoV-2

receptor-binding domain, which has affinity for cells of various mammals, binds to human ACE2 receptors via a novel mechanism.

Engineering such a virus would have required 1) published or otherwise available scientific knowledge that did not exist until after COVID-19 recognition; 2) a failure to follow obvious engineering pathways, resulting in an imperfectly constructed virus; and 3) an ability to genetically engineer a new virus without leaving fingerprints of the engineering. Furthermore, the 12 amino acid furin-cleavage site insertion between the SARS-CoV-2 spike protein's S1 and S2 domains, which some have alleged to be a sign of genetic engineering, is found in other bat and human coronaviruses in nature, probably arising via naturally occurring recombination.[24]

It is also highly unlikely that SARS-CoV-2 was released from a laboratory by accident because no laboratory had the virus nor did its genetic sequence exist in any sequence database before its initial GenBank deposition (early January 2020). China's laboratory safety practices, policies, training, and engineering are equivalent to those of the United States and other developed countries,[32] making viral "escape" extremely unlikely, and of course impossible without a viral isolate present. SARS-CoV-2 shares genetic properties with many other sarbecoviruses, lies fully within their genetic cluster, and is thus a virus that emerged naturally.

## COVID-19 EMERGENCE MECHANISMS: WHY THEY MATTER

Understanding how COVID-19 emerged is of great importance. We now know that the viruses causing SARS, MERS, and COVID-19 are all members of enormous groups of bat coronaviruses distributed globally, and that many of these viruses are functionally preadapted to human emergence. This preadaptation can be thought of as "accidental" because it must have occurred in nature in the absence of human infection and does not rule out further human adaptation to enable pandemicity. Molecular mechanisms of preadaptation are not fully known, but are undoubtedly related to functional similarities between ACE2 receptors on the cells of numerous mammals (bats, humans, minks, cats, and other domestic and wild animals).[33,34]

The ability of coronaviruses to evolve at a high rate, illustrated by extreme phylogenetic diversity, coupled with the dispersion of new viral variants within an enormous array of wild animal species that can serve as hosts, portends poorly for the future of coronavirus disease emergence. We are already seeing coronavirus mutants with altered affinity for human ACE2. Whether bat coronaviruses evolve independently or by "sampling" various mammalian ACE2 receptors, the result is the same. That bat sarbecoviruses so easily switch between multiple hosts suggests a many-pronged human risk: directly from bats and indirectly from other mammals infected by bat viruses. Because we have only just begun to sample, sequence, and study bat/mammalian coronaviruses, we can be certain that what we now know is but the tip of a very large iceberg.

The findings described earlier reaffirm what has long been obvious: that future coronavirus transmissions into humans are not only possible, but likely. Scientists knew this years ago and raised appropriate alarm. Our prolonged deafness now exacts a tragic price.

The story of COVID-19 emergence sends a powerful message. A quantum leap in bat coronavirus surveillance and research is urgently needed. This work must emphasize virologic and behavioral field studies of humans and animals wherever they interface, and especially in disease hotspots, as well as virologic studies related to human and animal spillover risks and the means of reducing them.[35]

Important research that has languished, been underfunded, or discontinued should be greatly expanded to deal with the urgency of the situation, and more scientists, including scientists working in China and other hotspot countries (Figure 2), should be recruited to these efforts, especially in international research partnerships. Full, open international collaboration involving many countries is essential. In particular, field research on the prevalence and virus-host relationships of coronaviruses, development of platform technologies for diagnostics, vaccines, and animal models for studies of pathogenesis and potential therapeutics is essential to permit, for example, modeling structure/function relationships of specific binding domains from newly identified agents to create critical tools for disease control.

In addition to robust expansion of surveillance and research, there are things that we can do now to lower our risks. We know much about coronavirus hotspots, not only in China but also globally; we can more aggressively surveil these locations to learn more about the local viral ecology and identify initial human spillover events. We also know much about human behaviors that directly and indirectly bring us into contact with bats, including risks from wet markets, bat cave tourism, capturing and eating bats, and perturbing the environment in ways that alter bat habitats and habits. These are behaviors that we can and must change.

We can also strengthen basic public health, including hygiene and sanitation, so that emerging viruses do not have a fertile field in which to amplify replication, and we must build and maintain strong public health infrastructure to respond quickly and efficiently to pathogen emergence. For viruses that have emerged, such as SARS-CoV-2, we need to develop effective antivirals and, ideally, broadly protective vaccines. Education and communication with populations where spillover events occur is also an important component of risk reduction.

We must also realize that the problem is larger than just coronaviruses. In recent years, we have seen emergences and reemergences of numerous other human infectious diseases such as Ebola fever, Lassa fever, hantavirus pulmonary syndrome, human monkeypox, HIV, dengue, chikungunya, Zika, and epizootic avian influenza. We have entered a new pandemic era,[36] one in which epidemic and pandemic emergences are becoming commonplace; some are likely to be highly pathogenic. In 2020, our science is sufficiently robust to have a good chance of controlling pandemic viral emergences within 2–3 years, but dramatically insufficient to prevent and control their emergences in the first place.

We should begin developing broadly protective vaccines and broadly therapeutic antiviral/antimicrobial agents against pathogens within taxonomic groups likely to emerge in the future, including coronaviruses, henipaviruses, and filoviruses, among others. Organizations like the Coalition for Epidemic Preparedness Innovations, among others, should be extended and strengthened, emphasizing, in addition to vaccine development, therapeutics as well as prevention tools. Pandemic prevention should be a global effort on a par with chemical and nuclear weapon prevention.

Unless we reset the equation; invest more in critical and creative laboratory, field, and behavioral research; and start finding

ways to prevent these emergences, we will soon see additional coronavirus pandemics, as well as global spread of other types of infectious agents not yet imagined, caused by some of the millions of viruses in the natural world, many of which we have not yet had the time and funding to identify and study.[27]

Understanding how COVID-19 emerged is a critical point on a steep learning curve we must quickly master. As we face the mounting deaths and societal upheavals of the COVID-19 pandemic, we must not lose sight of how this pandemic began, how and why we missed the warning signs, and what we can do to prevent it from happening again—and again.

Received July 3, 2020. Accepted for publication July 13, 2020.

Published online July 22, 2020.

Acknowledgements: Publication charges for this article were waived due to the ongoing pandemic of COVID-19.

Financial support: This work was funded in part by the intramural research program of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH).

Disclosure: The views in this article are those of the authors and not of their institutions, or the NIAID, NIH, DHHS.

Authors' addresses: David M. Morens, American Committee on Arthropod-Borne Viruses, American Society of Tropical Medicine and Hygiene, Arlington, VA, and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, E-mail: dm270q@nih.gov. Joel G. Breman, American Society of Tropical Medicine and Hygiene, Arlington, VA, E-mail: jgbreman@gmail.com. Charles H. Calisher, Colorado State University, Fort Collins, CO, E-mail: calisher@cybersafe.net. Peter C. Doherty, Department of Microbiology and Immunology, University of Melbourne at the Doherty Institute, Melbourne, Australia, E-mail: pcd@unimelb.edu.au. Beatrice H. Hahn, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, E-mail: bhahn@pennmedicine.upenn.edu. Gerald T. Keusch, Boston University School of Medicine, Boston, MA, E-mail: keusch@bu.edu. Laura D. Kramer, Wadsworth Center, New York State Department of Health, Albany, NY, E-mail: laura.kramer@health.ny.gov. James LeDuc, University of Texas Medical Branch, Galveston, TX, E-mail: jwleduc@utmb.edu. Thomas P. Monath, Crozet BioPharma LLC, Devens, MA, E-mail: tom.monath@crozetbiopharma.com. Jeffery K. Taubenberger, Viral Pathogenesis and Evolution Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, E-mail: taubenbergerj@niaid.nih.gov.



## REFERENCES

1. Cheng VCC, Lau SKP, Woo PCY, Yuen KY, 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin Microbiol Rev 20:* 660–694.
2. Taubenberger JK, Kash JC, Morens DM, 2019. The 1918 influenza pandemic: 100 years of questions answered and unanswered. *Sci Transl Med 11:* eeaau5485.
3. Ksiazek TG et al., 2003. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med 348:* 1953–1966.
4. Sharp PM, Plenderleith LJ, Hahn BH, 2020. Ape origins of human malaria. *Ann Rev Microbiol 74:* 39–63.
5. Morens DM, Folkers GK, Fauci AS, 2008. Emerging infections: a perpetual challenge. *Lancet Infect Dis 8:* 710–719.
6. Culliton BJ, 1990. Emerging viruses, emerging threat. *Science 247:* 279–280.
7. Kuiken T, Holmes EC, McCauley J, Rimmelzwaan GF, Williams CS, Grenfell BT, 2006. Host species barriers to influenza virus infections. *Science 312:* 394–397.
8. Parrish CR, Holmes EC, Morens DM, Park E-C, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P, 2008. Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiol Mol Biol Rev 72:* 457–470.
9. Geoghegan JL, Holmes EC, 2018. Evolutionary virology at 40. *Genetics 210:* 1151–1162.
10. Morens DM, Taubenberger JK, 2011. Pandemic influenza: certain uncertainties. *Rev Med Virol 21:* 262–284.
11. Sun H et al., 2020. Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. *Proc Natl Acad Sci U S A*, doi: 10.1073/pnas.1921186117.
12. Corman VM, Muth D, Niemeyer D, Drosten C, 2018. Hosts and sources of endemic human coronaviruses. *Adv Virus Res 100:* 163–188.
13. Anthony SJ et al., 2017. Global patterns in coronavirus diversity. *Virus Evol 3:* vex012.
14. Allen T, Murray KA, Zambtana-Torrelio C, Morse SS, Rondinini C, Marco MD, Breit N, Olival NJ, Daszak P, 2017. Global hotspots and correlates of emerging zoonotic diseases. *Nat Comm 8:* 1124.
15. Huong NQ et al., 2020. Coronavirus testing indicates transmission risk along wildlife supply chains for human consumption in Viet Nam, 2013–2014. bioRxiv, doi: 10.1101/2020.06.05.098590.
16. Li H et al., 2019. Human-animal interactions and bat coronavirus spillover potential among rural residents in Southern China. *Biosaf Health 1:* 84–90.
17. Monagin C et al., 2018. Serologic and behavioral risk survey of workers with wildlife contact in China. *PLoS One 13:* e0194647.
18. Li H-Y et al., 2020. A qualitative study of zoonotic risk factors among rural communities in southern China. *Int Health 12:* 77–85.
19. Hu B et al., 2017. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog 13:* e1006698.
20. Wang N et al., 2018. Serological evidence of bat SARS-related coronavirus infection in humans, China. *Virol Sin 33:* 104–107.
21. Latinne A et al., 2020. Origin and cross-species transmission of bat coronaviruses in China. *Nat Commun* (In press).
22. Menachery VD et al., 2016. SARS-like WIV1-CoV poised for human emergence. *Proc Natl Acad Sci U S A 113:* 3048–3053.
23. Zhou P et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature 579:* 270–2734.
24. Zhou H et al., 2020. A novel bat coronavirus reveals natural insertions at the S1/S2 cleavage site of the Spike protein and a possible recombinant origin of HCoV-19. bioRxiv, doi: 10.1101/2020.03.02.974139.
25. Ge XY et al., 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature 503:* 535–538.
26. Sheahan TP et al., 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med 9:* eaal3653.
27. Carroll D, Daszak P, Wolfe ND, Gao GF, Morel CM, Morzaria S, Pablos-Méndez A, Tomori O, Mazet JAK, 2018. The Global Virome Project. *Science 359:* 872–974.
28. Anderson KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF, 2020. The proximal origin of SARS-CoV-2. *Nat Med 26:* 450–452.
29. Zhang Y-Z, Holmes EC, 2020. A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell 181:* 223–226.
30. Lu R et al., 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet 395:* 565–574.
31. Li X, Giorgi EE, Marichann MH, Foley B, Xiao C, Kong X-P, Chen Y, Krober B, Gao F, 2020. Emergence of SARS-CoV-2 through recombination and strong purifying selection. *Sci Adv 6:* eabb9153.
32. Xia H, Huang Y, Ma H, Liu B, Xie W, Song D, Yuan Z, 2019. Biosafety level 4 laboratory user training program, China. *Emerg Infect Dis 25:* e180220.
33. Oreshkova N et al., 2020. SARS-CoV2 infection in farmed mink, Netherlands. *Euro Surveill 25:* pii 2001005.
34. Halfman PJ et al., 2020. Transmission of SARS-CoV-2 in domestic cats. *N Engl J Med*, doi: 10.1056/NEJMc2013400.
35. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ, 2020. Bat-borne virus diversity, spillover, and emergence. *Nat Rev Microbiol 18:* 461–471.
36. Morens DM, Daszak P, Markel H, Taubenberger JK, 2020. Pandemic COVID-19 joins history's pandemic legion. *mBio 11:* e00812-20.