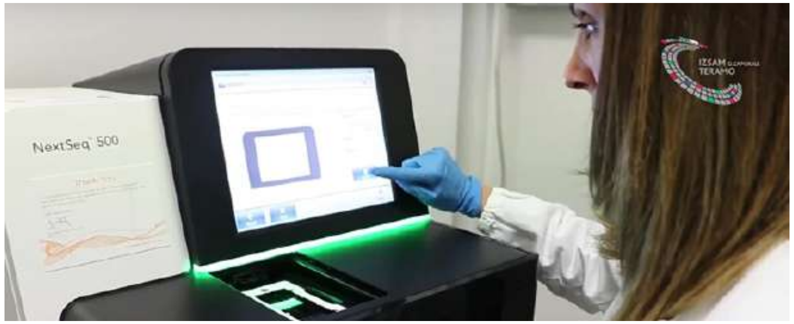
IZSAM G.CAPORALE
TERAMO

SARS-CoV-2: genesi ed evoluzione

Alessio Lorusso, 26 Novembre 2020

IZSAM G.CAPORALE
TERAMO

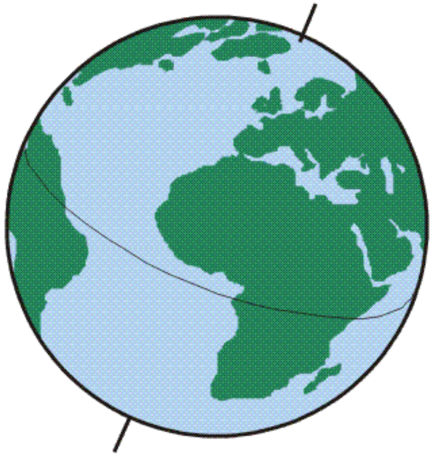
Centro di Referenza Nazionale
per Sequenze Genomiche di
microrganismi patogeni:
banca dati e analisi di Bioinformatica




COLUMBUS
RISTORANTE - PIZZERIA

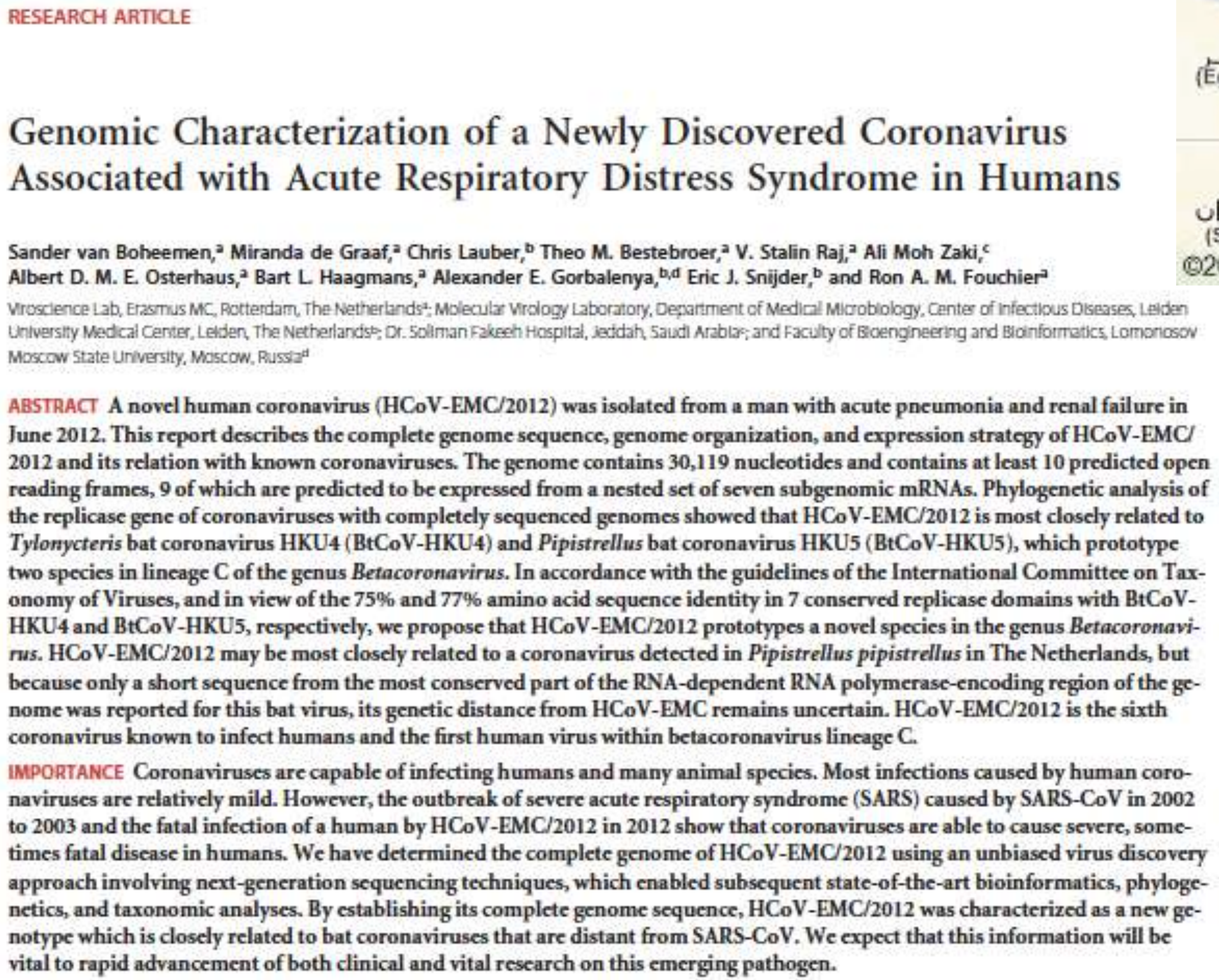

GELAIRE
JUPITER

2002-2003-2004, SARS-CoV-1



Coronavirus diventano noti al grande pubblico

2012, MERS-CoV



RESEARCH ARTICLE

Genomic Characterization of a Newly Discovered Coronavirus Associated with Acute Respiratory Distress Syndrome in Humans

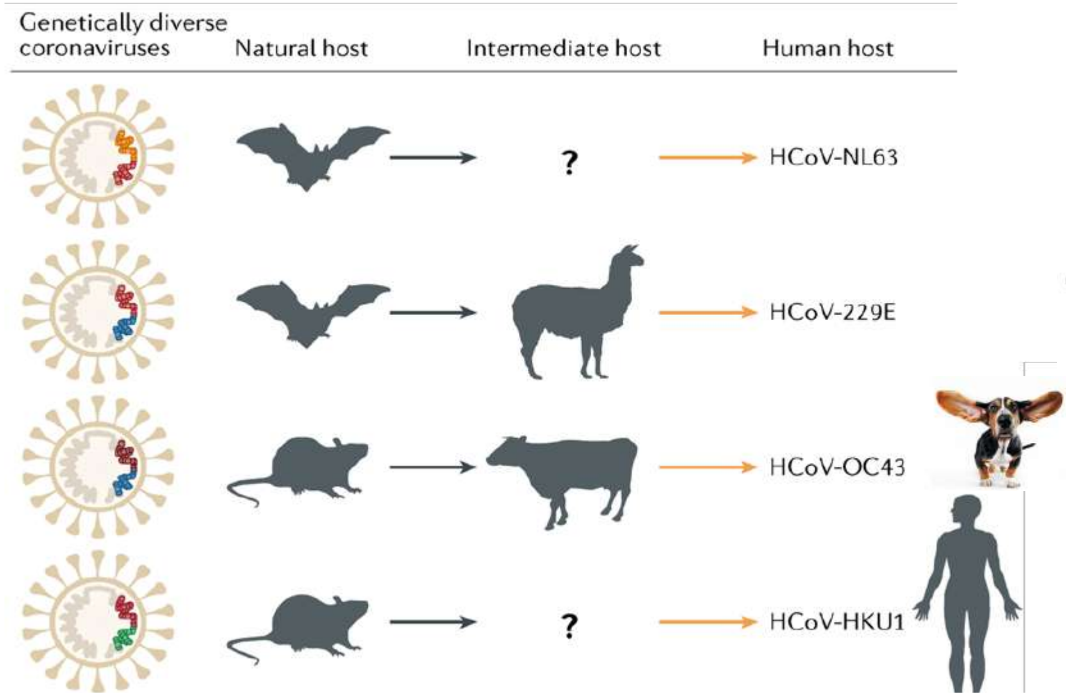
Sander van Boheemen,[a] Miranda de Graaf,[a] Chris Lauber,[b] Theo M. Bestebroer,[a] V. Stalin Raj,[a] Ali Moh Zaki,[c] Albert D. M. E. Osterhaus,[a] Bart L. Haagmans,[a] Alexander E. Gorbalenya,[b,d] Eric J. Snijder,[b] and Ron A. M. Fouchier[a]

Viroscience Lab, Erasmus MC, Rotterdam, The Netherlands[a]; Molecular Virology Laboratory, Department of Medical Microbiology, Center of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands[b]; Dr. Soliman Fakeeh Hospital, Jeddah, Saudi Arabia[c]; and Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia[d]

ABSTRACT A novel human coronavirus (HCoV-EMC/2012) was isolated from a man with acute pneumonia and renal failure in June 2012. This report describes the complete genome sequence, genome organization, and expression strategy of HCoV-EMC/2012 and its relation with known coronaviruses. The genome contains 30,119 nucleotides and contains at least 10 predicted open reading frames, 9 of which are predicted to be expressed from a nested set of seven subgenomic mRNAs. Phylogenetic analysis of the replicase gene of coronaviruses with completely sequenced genomes showed that HCoV-EMC/2012 is most closely related to *Tylonycteris* bat coronavirus HKU4 (BtCoV-HKU4) and *Pipistrellus* bat coronavirus HKU5 (BtCoV-HKU5), which prototype two species in lineage C of the genus *Betacoronavirus*. In accordance with the guidelines of the International Committee on Taxonomy of Viruses, and in view of the 75% and 77% amino acid sequence identity in 7 conserved replicase domains with BtCoV-HKU4 and BtCoV-HKU5, respectively, we propose that HCoV-EMC/2012 prototypes a novel species in the genus *Betacoronavirus*. HCoV-EMC/2012 may be most closely related to a coronavirus detected in *Pipistrellus pipistrellus* in The Netherlands, but because only a short sequence from the most conserved part of the RNA-dependent RNA polymerase-encoding region of the genome was reported for this bat virus, its genetic distance from HCoV-EMC remains uncertain. HCoV-EMC/2012 is the sixth coronavirus known to infect humans and the first human virus within betacoronavirus lineage C.

IMPORTANCE Coronaviruses are capable of infecting humans and many animal species. Most infections caused by human coronaviruses are relatively mild. However, the outbreak of severe acute respiratory syndrome (SARS) caused by SARS-CoV in 2002 to 2003 and the fatal infection of a human by HCoV-EMC/2012 in 2012 show that coronaviruses are able to cause severe, sometimes fatal disease in humans. We have determined the complete genome of HCoV-EMC/2012 using an unbiased virus discovery approach involving next-generation sequencing techniques, which enabled subsequent state-of-the-art bioinformatics, phylogenetics, and taxonomic analyses. By establishing its complete genome sequence, HCoV-EMC/2012 was characterized as a new genotype which is closely related to bat coronaviruses that are distant from SARS-CoV. We expect that this information will be vital to rapid advancement of both clinical and vital research on this emerging pathogen.

Coronavirus nell'uomo



SERBATOIO ANIMALE

In Medicina Veterinaria

Murine hepatitis virus (MHV)

Infectious bronchitis virus (IBV)

Feline infectious peritonitis virus (FIPV)

….e tanti altri negli
animali domestici e
selvatici….

Veterinary Microbiology 244 (2020) 108693

Contents lists available at ScienceDirect

Veterinary Microbiology

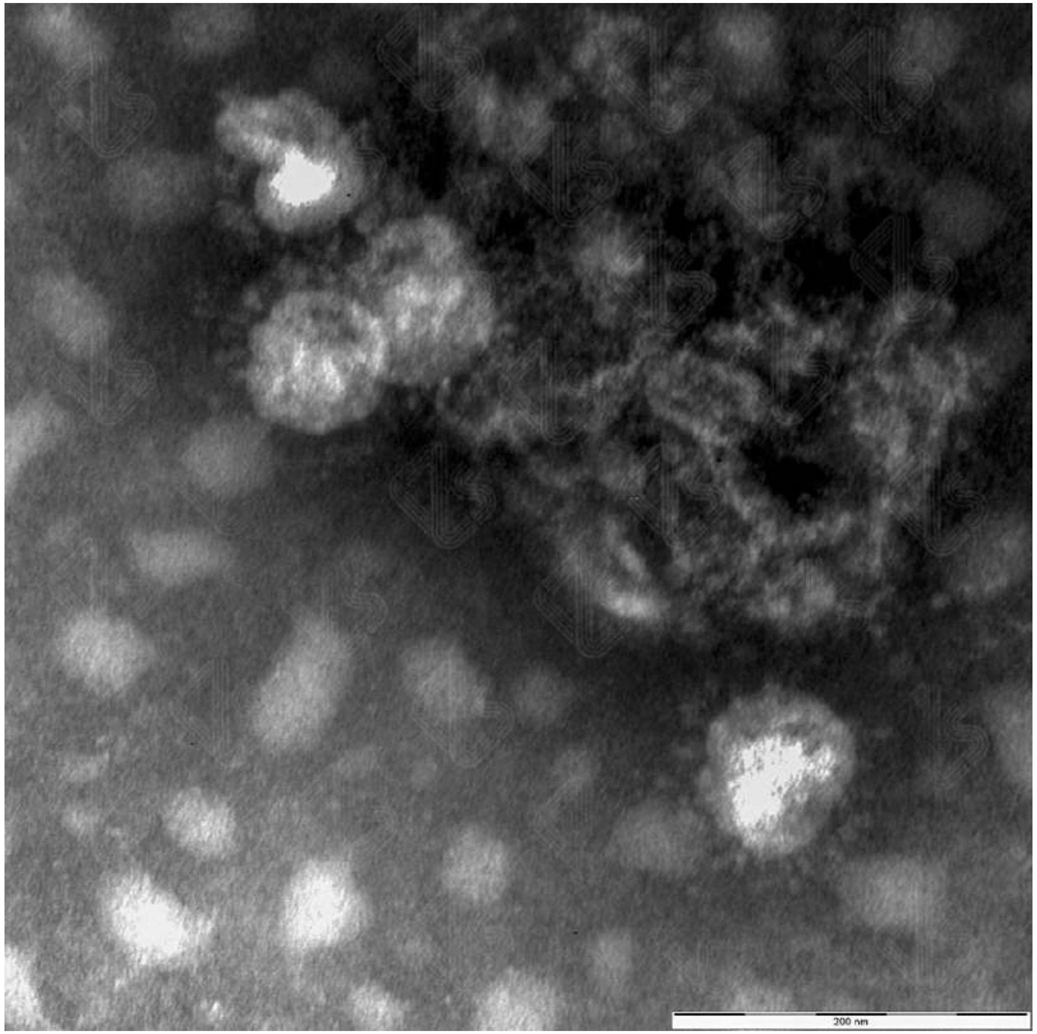
journal homepage: www.elsevier.com/locate/vetmic

Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses

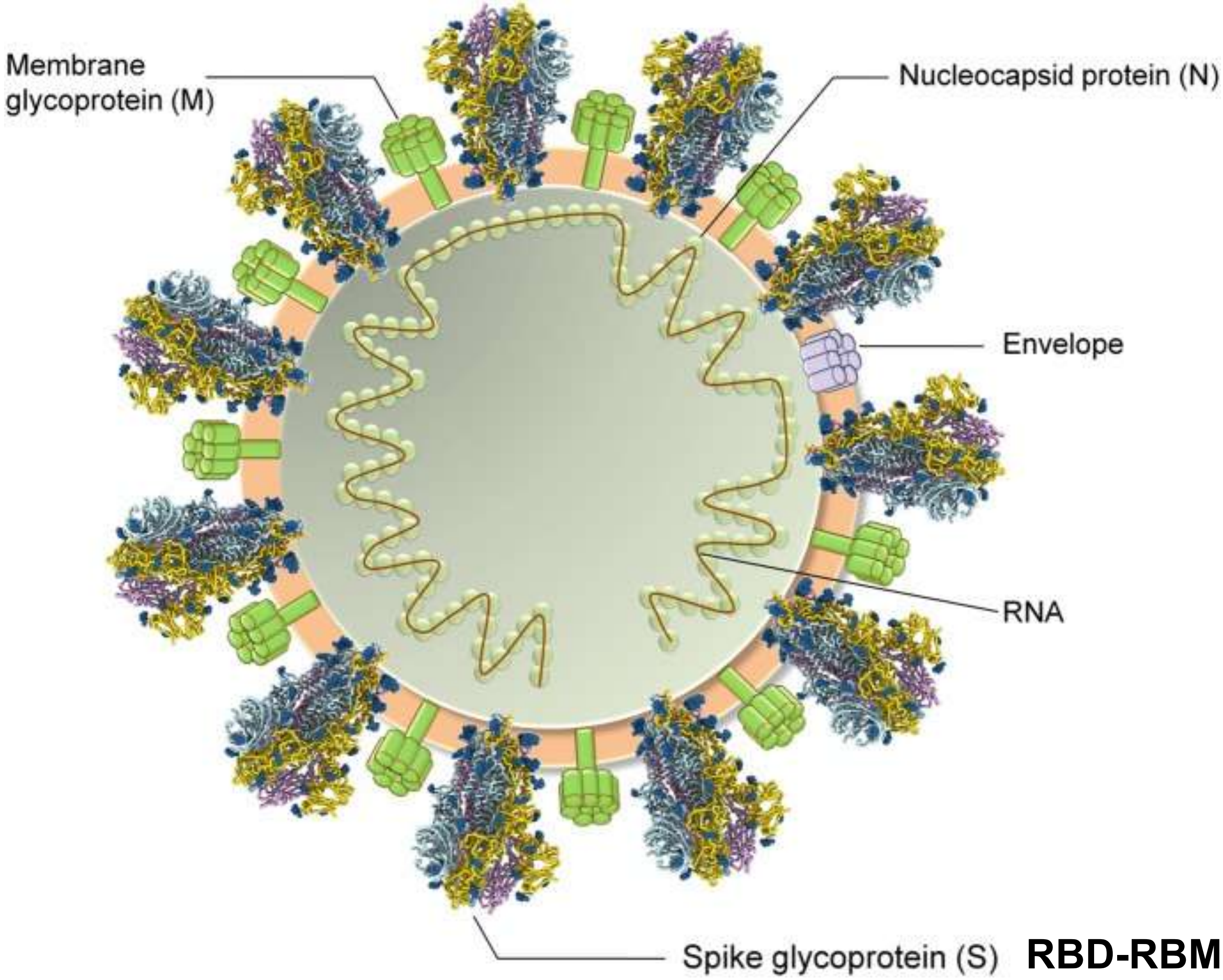
Nicola Decaro[a,*], Alessio Lorusso[b]

[a] *Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy*
[b] *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy*

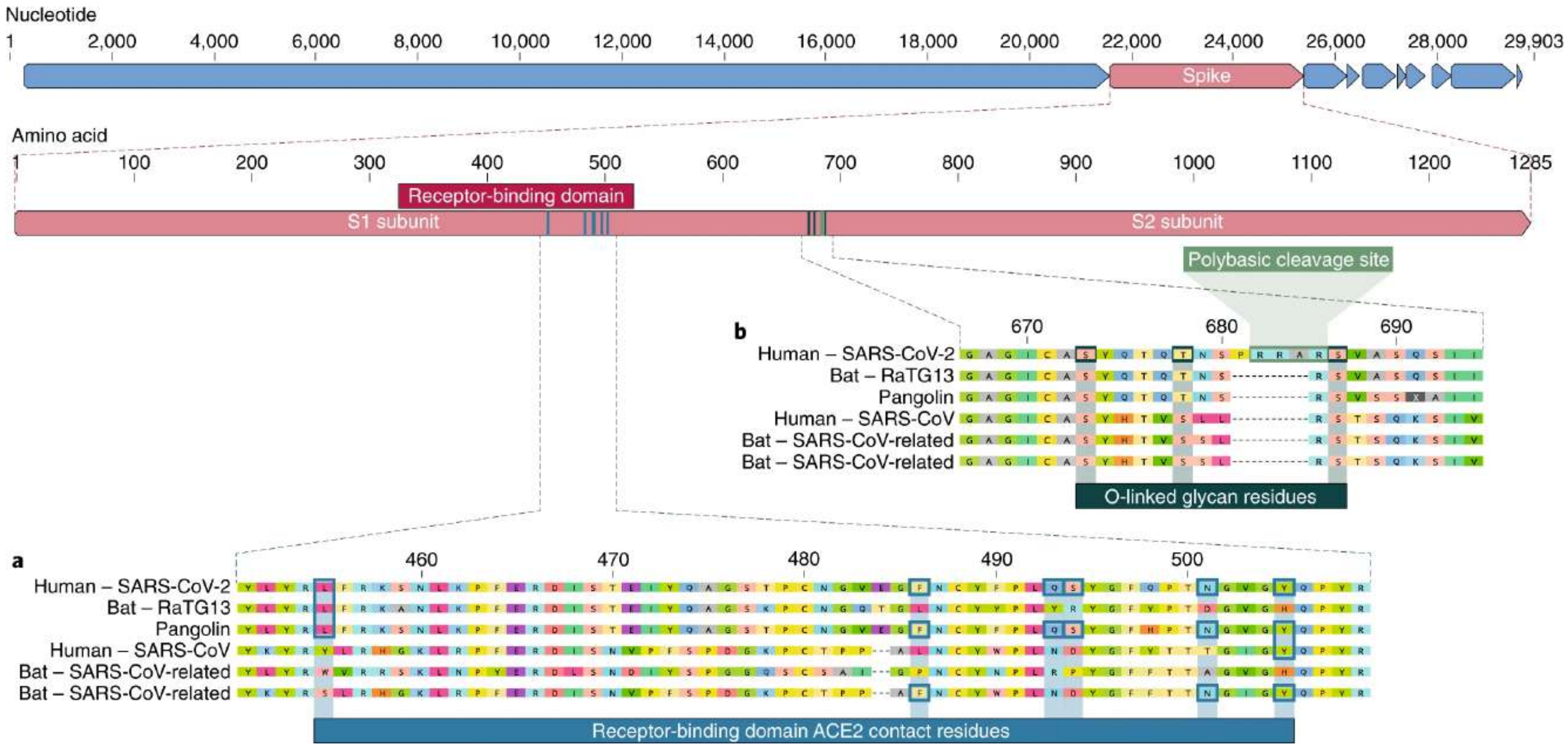
SARS-CoV-2



Antonio Lavazza, IZSLER


Membrane glycoprotein (M)
Nucleocapsid protein (N)
Envelope
RNA
Spike glycoprotein (S)

RBD-RBM

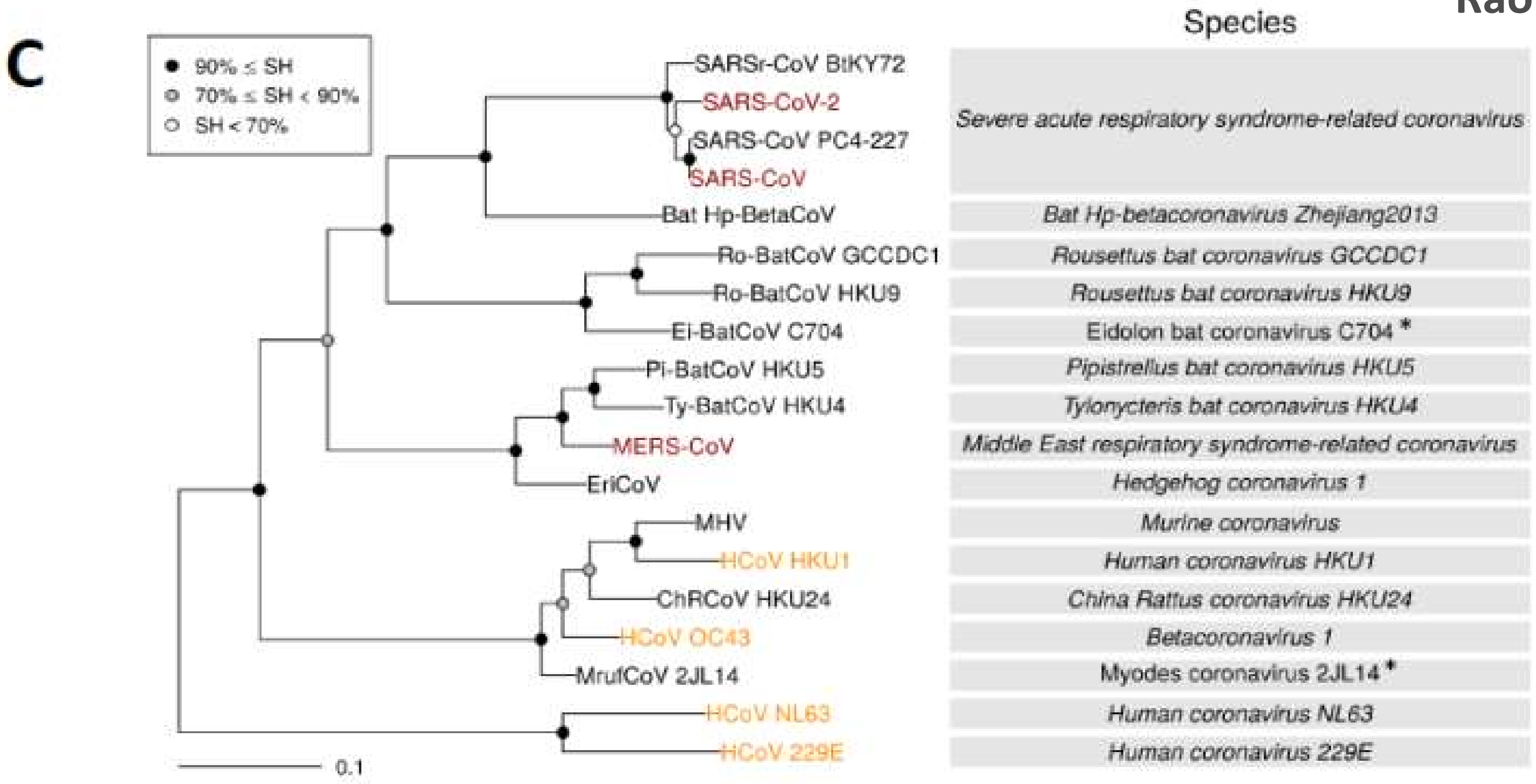


RBM: L455, F486, Q493, S494, N501 and Y505

Sito Clivaggio S1/S2 (uncommon): naturale? artificiale? complotto

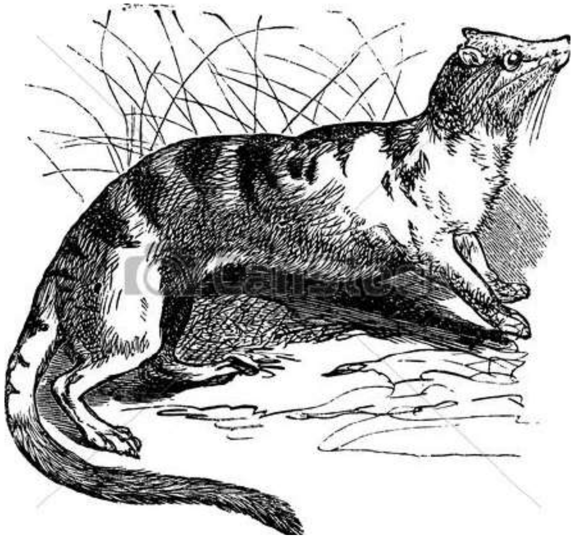
SARS-CoV-2

Raoul de Groot



Coronavirus study group, Feb 2020, subgenus *Sarbecovirus*, species *SARSr-CoV*

SARS-CoV-1



…..Mai trovato nel pipistrello un virus identico a SARS-CoV-1…..

…..ma una serie enorme di Bat SARS-rCoVs

REVIEWS

Origin and evolution of pathogenic coronaviruses

Jie Cui[1], Fang Li[2] and Zheng-Li Shi[1]*

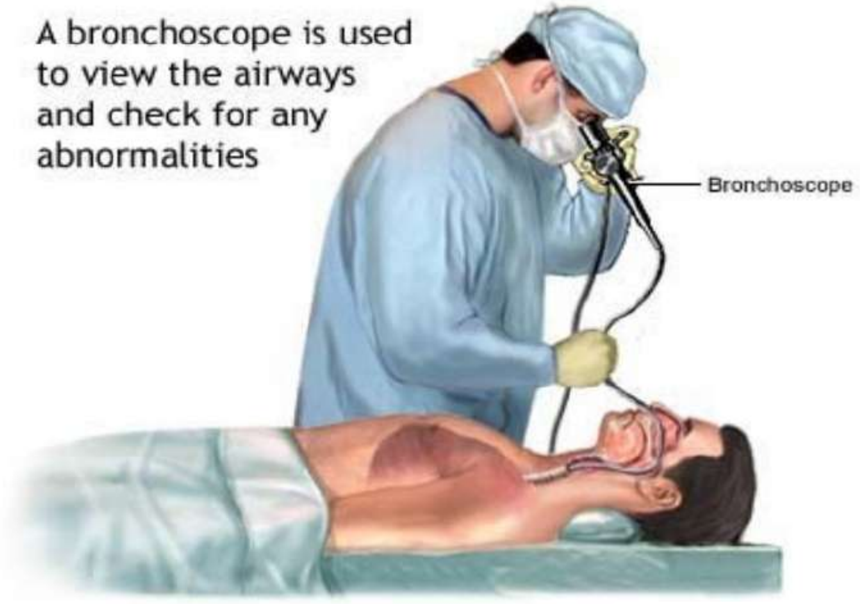
….given the prevalence and great genetic diversity of bat SARSr-CoVs, their close coexistence and the frequent recombination of CoVs,
it is expected that novel variants will emerge in the future…..

Nat Rev Microbiol. 2019 Mar;17(3):181-192.
doi: 10.1038/s41579-018-0118-9. Review.

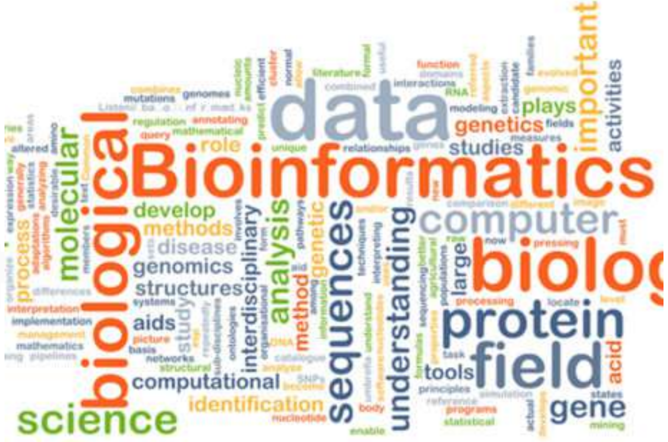

武汉华南海鲜批发市场
WUHAN HUANAN HAIXIAN PIFA SHICHANG
车场
P
杨智辉大闸蟹
杨智辉大闸蟹
孙氏泥鳅鳝鱼
金祥龙虾螃蟹
永
市场停车收费标准

Influenza e altri patogeni respiratori assenti

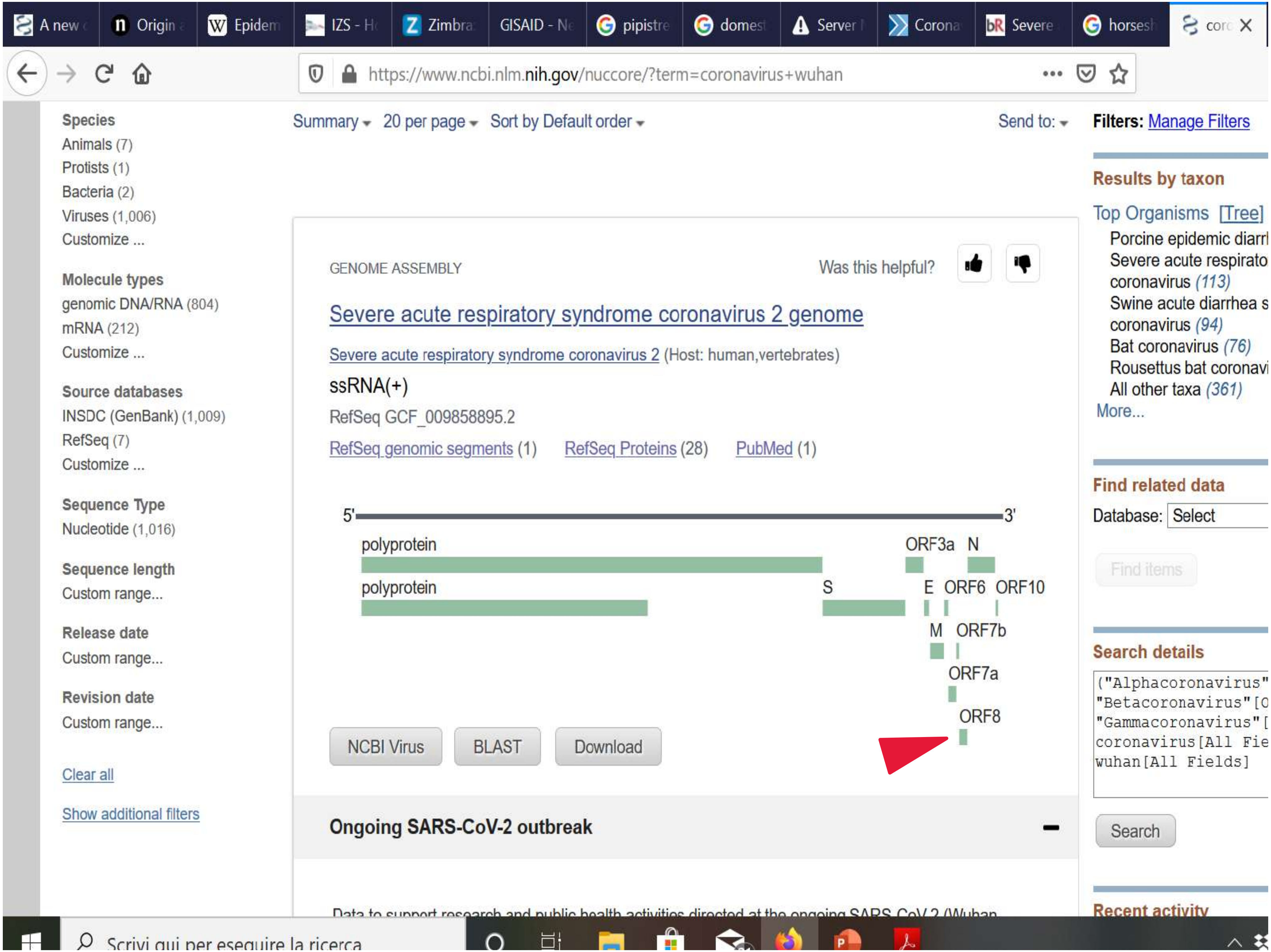
Next Generation Sequencing



…..Of the 384,096
contigs assembled by Megahit8, the longest
(30,474 nucleotides [nt])
had high abundance and was closely related
to a bat SARS-like coronavirus
isolate - bat-SL-CoVZC45 (GenBank Accession
MG772933) - previously
sampled in China, with a nt identity of
89.1%………



….a leader transcription regulatory
sequence (TRS) and nine putative body TRSs
which appeared in two forms – the ACGAAC
or CUAAAC



Species
Animals (7)
Protists (1)
Bacteria (2)
Viruses (1,006)
Customize ...

Molecule types
genomic DNA/RNA (804)
mRNA (212)
Customize ...

Source databases
INSDC (GenBank) (1,009)
RefSeq (7)
Customize ...

Sequence Type
Nucleotide (1,016)

Sequence length
Custom range...

Release date
Custom range...

Revision date
Custom range...

Clear all

Show additional filters

GENOME ASSEMBLY

Was this helpful?

Severe acute respiratory syndrome coronavirus 2 genome

Severe acute respiratory syndrome coronavirus 2 (Host: human,vertebrates)

ssRNA(+)

RefSeq GCF_009858895.2

RefSeq genomic segments (1) RefSeq Proteins (28) PubMed (1)

Ongoing SARS-CoV-2 outbreak

Results by taxon

Top Organisms [Tree]
Porcine epidemic diarr
Severe acute respirato coronavirus (113)
Swine acute diarrhea s coronavirus (94)
Bat coronavirus (76)
Rousettus bat coronavi
All other taxa (361)
More...

Find related data

Database: Select

Search details

```
("Alphacoronavirus"
"Betacoronavirus"[O
"Gammacoronavirus"[
coronavirus[All Fie
wuhan[All Fields]
```

Recent activity

Likely bat is the animal reservoir of SARS-CoV-2

- Campioni di archivio
- Bat SARS-rCoV RaTG13, frammento della polimerasi
- <u>96.1% (tutto il genoma)</u>
- <u>92.9% nt in the S protein (97.4 aa)</u>
- RBM is totally different (1/6 aa)


Eddie Holmes

- field collection
- Bat SARS-rCoV RmYN02
- *93.3% (tutto il genoma)*
- But extremely similar in most genomic regions (3a, E, 6, 7a, N and 10) including the polimerase (97.2%)
- But…very low in the S protein (71.8)
- Sito clivaggio PAA !!!!!!!!!!!!!
- RBM is totally different (1/6 aa)

SARS-CoV-1 and SARS-CoV-2

Le origini..

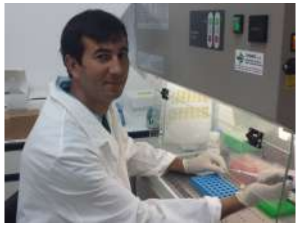
Per capire SARS-CoV-2 bisogna guardare al SARS-CoV-1

- **Similarità e differenze**
- **Animal exposure**
- **SARS-CoV and Abs in masked palm civets and animal handlers**
- **Probably trasmitted to palm civets from other animals**

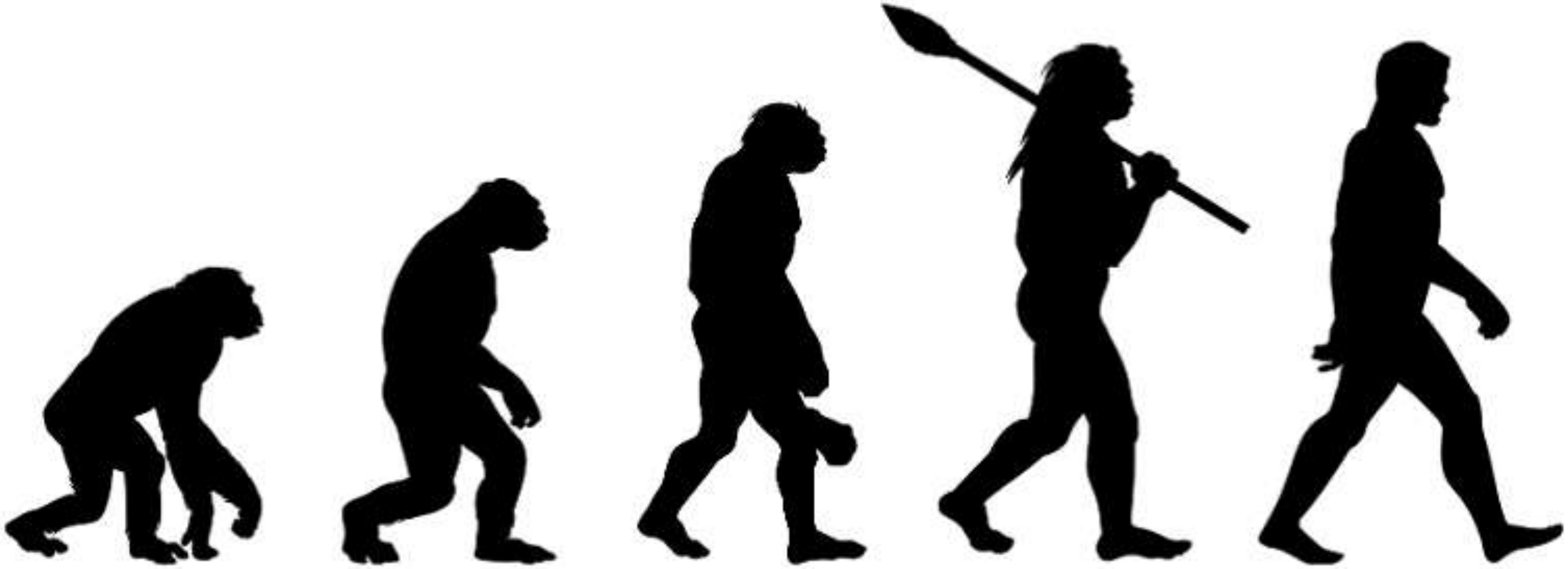
….. la genesi sembra essere la stessa…..

HALLMARK

Coronaviruses: recombination events, cross species jumps, insertions/deletions



Nicola Decaro



PLOS PATHOGENS

RESEARCH ARTICLE

Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus

Ben Hu[1]☯, Lei-Ping Zeng[1]☯, Xing-Lou Yang[1]☯, Xing-Yi Ge[1], Wei Zhang[1], Bei Li[1], Jia-Zheng Xie[1], Xu-Rui Shen[1], Yun-Zhi Zhang[2,3], Ning Wang[1], Dong-Sheng Luo[1], Xiao-Shuang Zheng[1], Mei-Niang Wang[1], Peter Daszak[4], Lin-Fa Wang[5], Jie Cui[1] *, Zheng-Li Shi[1] *

1 CAS Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases of Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China, **2** Yunnan Institute of Endemic Diseases Control and Prevention, Dali, China, **3** Dali University, Dali, China, **4** EcoHealth Alliance, New York, New York, United States of America, **5** Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

☯ These authors contributed equally to this work.
* jiecui@wh.iov.cn (JC); zlshi@wh.iov.cn (Z-LS)

Check for updates

- Highly diverse SARS-rCoVs in one cave
- CoVs strains in this cave contain all genetic elements needed to form SARS-CoV
- …direct progenitor of SARS CoV-1 originated by recombination within bats and then transmitted to civets or other mammals

..e SARS-CoV-2 ??

- **SARS-rCoVs in pangolins (Guanxi and Guandong)**
- **Identical RBM (6/6 aa)**

So far…..

- **Bat SARS-rCoV as backbone (RmYN02 like virus)**
- **Homologous recombination with other SARS rCoV..(RaTG13 like virus)**
- **..of bats and pangolins providing the S, the RBD and the cleavage site**

ACE2

S1-NTD

S1-CTD…RBD

318

510

S1

S2

aa. 479/487

479-487

SARS-CoV-1

- **HUMAN SARS-CoV 2002/3 (virulent phase) 479 (N); 487 (T). High affinity for ACE2**
- **CIVET SARS-CoV 2003 479 (K or R); 487 (S). K and R similar biochemical properties- Low affinity for human ACE2 but high to civet ACE2**
- **HUMAN SARS-CoV 2004 (much less virulent phase) 479 (K o R); 487 S**

K/R-479 and S-487 function as the entry barrier for animal-to-human transmission of the SARS-CoV

……The maintenance of this entry barrier requires the conservation of both K-479 and S-487. Once one substitution at positions 479 or 487 occurred in animal SARS-CoV, the virus could greatly increase its affinity toward the human receptor ACE2 and even cross the species barrier to cause animal-to-human transmission…….

……that the S487T amino acid switch may reflect the virus adaptation to human host and that the presence of the SN479/T487 protein is essential for causing the pandemic, i.e. the successful transition from animal-to-human transmission to human-to-human transmission……

- STUDI POSSIBILI PERCHE’ avevano SARS-CoV-1 da uomo e zibetti!

- Per SARS-CoV-2 ancora non è così

IS THE ADAPTATION TO
OTHER MAMMALS BEFORE BEING ABLE TO INFECT HUMANS ESSENTIAL?

We use cookies to personalise content and ads, to provide social media features and to analyse our traffic. We also share information about your use of our site with our social media, advertising and analytics partners in accordance with our Privacy Policy You can manage your preferences in 'Manage Cookies'.

Springer Nature is making Coronavirus research free. View research | View latest news | Sign up for updates

Letter | Published: 30 October 2013

Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor

Xing-Yi Ge, Jia-Lu Li, Xing-Lou Yang, Aleksei A. Chmura, Guangjian Zhu, Jonathan H. Epstein, Jonna K. Mazet, Ben Hu, Wei Zhang, Cheng Peng, Yu-Ji Zhang, Chu-Ming Luo, Bing Tan, Ning Wang, Yan Zhu, Gary Crameri, Shu-Yi Zhang, Lin-Fa Wang, Peter Daszak ✉ & Zheng-Li Shi ✉

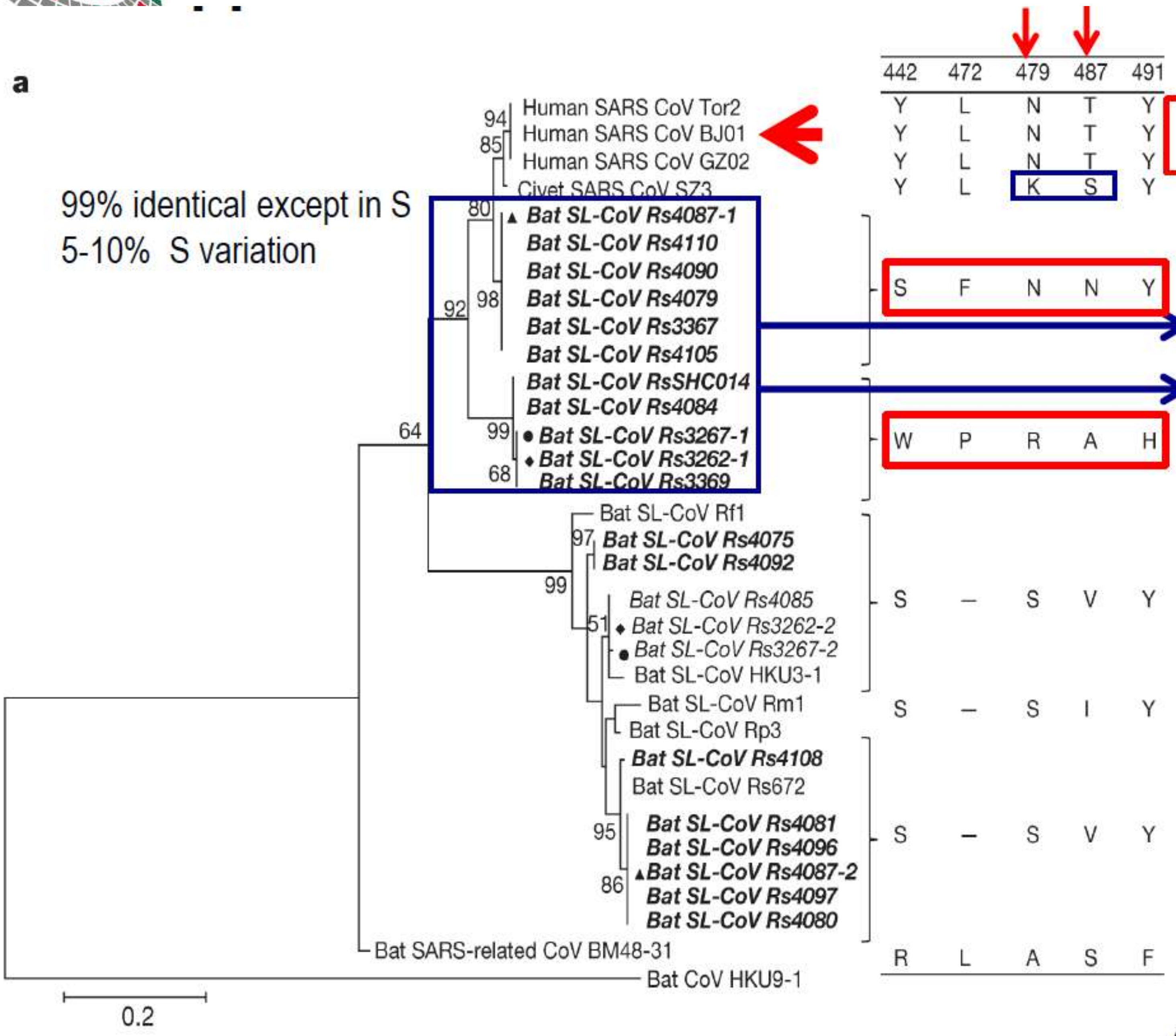
Nature **503**, 535–538(2013) | Cite this article

Editorial Summary

A SARS-like virus in bats

Peter Daszak and colleagues identify two novel coronaviruses from Chinese horseshoe bats that are closely related to severe acute… show more

Direct transmission from bats ?



99% identical except in S
5-10% S variation

Human

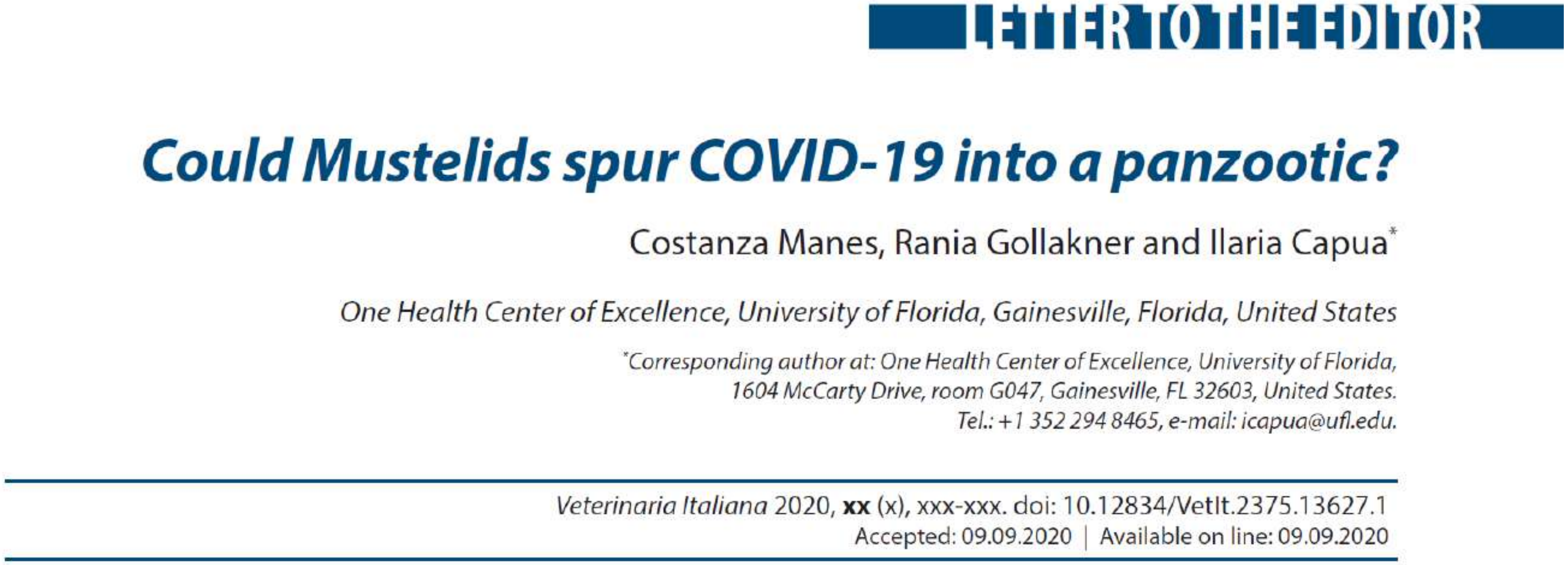
Civet

Human, civet, Bat ACE2 use?

Yes (WIV-1)

No

Ge et al., Nature 2013



LETTER TO THE EDITOR

Could Mustelids spur COVID-19 into a panzootic?

Costanza Manes, Rania Gollakner and Ilaria Capua*

One Health Center of Excellence, University of Florida, Gainesville, Florida, United States

**Corresponding author at: One Health Center of Excellence, University of Florida,*
1604 McCarty Drive, room G047, Gainesville, FL 32603, United States.
Tel.: +1 352 294 8465, e-mail: icapua@ufl.edu.

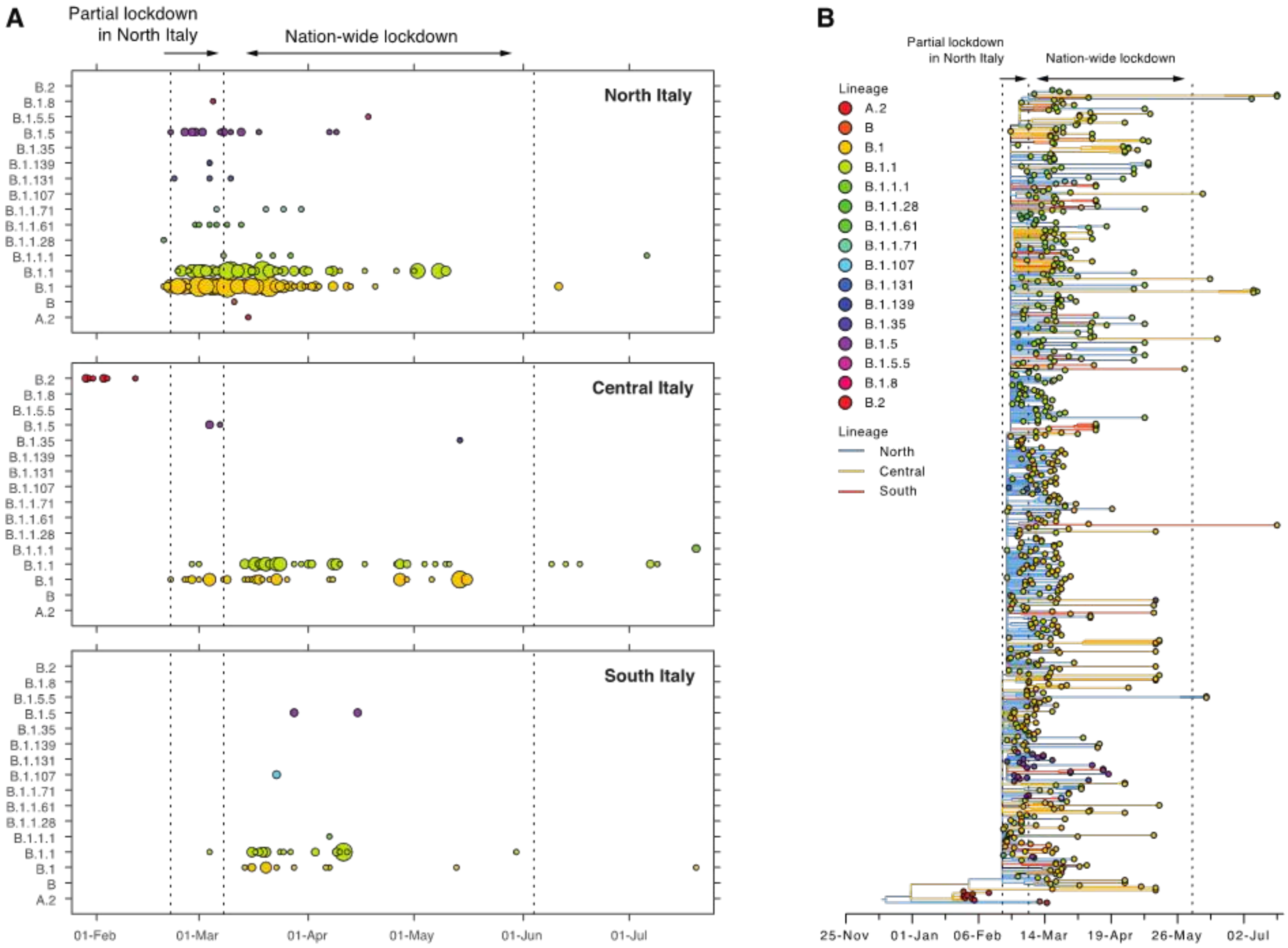
Veterinaria Italiana 2020, **xx** (x), xxx-xxx. doi: 10.12834/VetIt.2375.13627.1
Accepted: 09.09.2020 | Available on line: 09.09.2020

- **Mutations in the S protein (Y453F, I692V, M1229I)**
- **Deletion in the S protein (69-70)**
- **Loss of neutralization**
- **Transmission to humans**

DIAGNOSI e CARATTERIZZAZIONE

INDAGINI EPIDEMIOLOGICHE

IZSAM


A
Partial lockdown in North Italy
Nation-wide lockdown
North Italy
Central Italy
South Italy
B.2
B.1.8
B.1.5.5
B.1.5
B.1.35
B.1.139
B.1.131
B.1.107
B.1.1.71
B.1.1.61
B.1.1.28
B.1.1.1
B.1.1
B.1
B
A.2
01-Feb
01-Mar
01-Apr
01-May
01-Jun
01-Jul
B
Partial lockdown in North Italy
Nation-wide lockdown
Lineage
A.2
B
B.1
B.1.1
B.1.1.1
B.1.1.28
B.1.1.61
B.1.1.71
B.1.107
B.1.131
B.1.139
B.1.35
B.1.5
B.1.5.5
B.1.8
B.2
Lineage
North
Central
South
25-Nov
01-Jan
06-Feb
14-Mar
19-Apr
26-May
02-Jul

SHAME

Outline

- Highlights
- Abstract
- Keywords
- 1. Introduction
- 2. Materials and methods
- 3. Results
- 4. Discussion
- Funding
- Declaration of Competing Interest
- Acknowledgement
- Appendix A. Supplementary data
- References

Show full outline

Veterinary Microbiology
Available online 24 November 2020, 108933
In Press, Journal Pre-proof

SARS-CoV-2 replicates in respiratory *ex vivo* organ cultures of domestic ruminant species

Giovanni Di Teodoro [a], Fabrizia Valleriani [a], Ilaria Puglia [a], Federica Monaco [a], Chiara Di Pancrazio [a], Mirella Luciani [a], Ivanka Krasteva [a], Antonio Petrini [a], Maurilia Marcacci [a, b], Nicola D'Alterio [a], Valentina Curini [a], Mariangela Iorio [a], Giacomo Migliorati [a], Marco Di Domenico [a], Daniela Morelli [a], Paolo Calistri [a], Giovanni Savini [a], Nicola Decaro [b] … Alessio Lorusso [a]

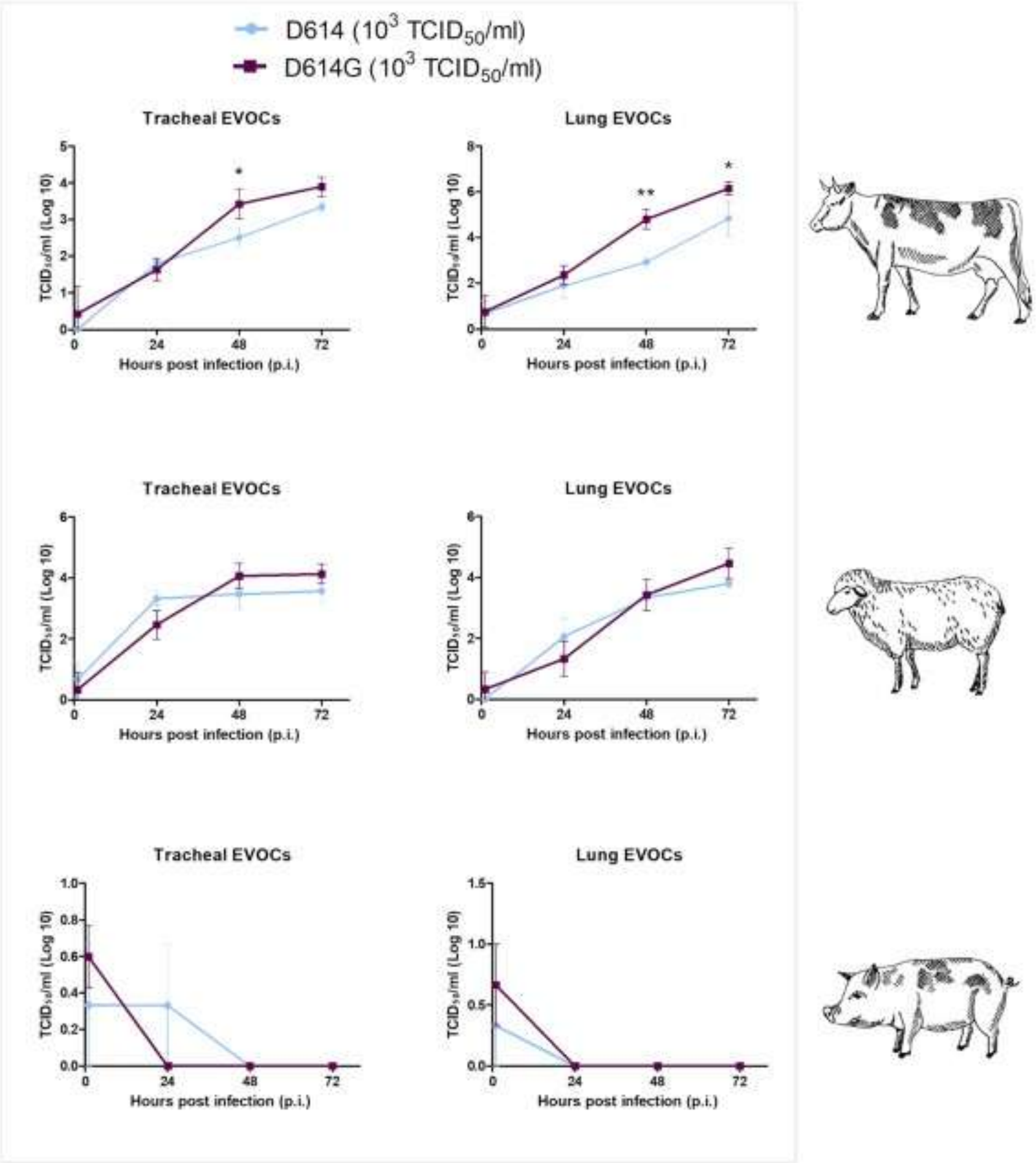
Show more

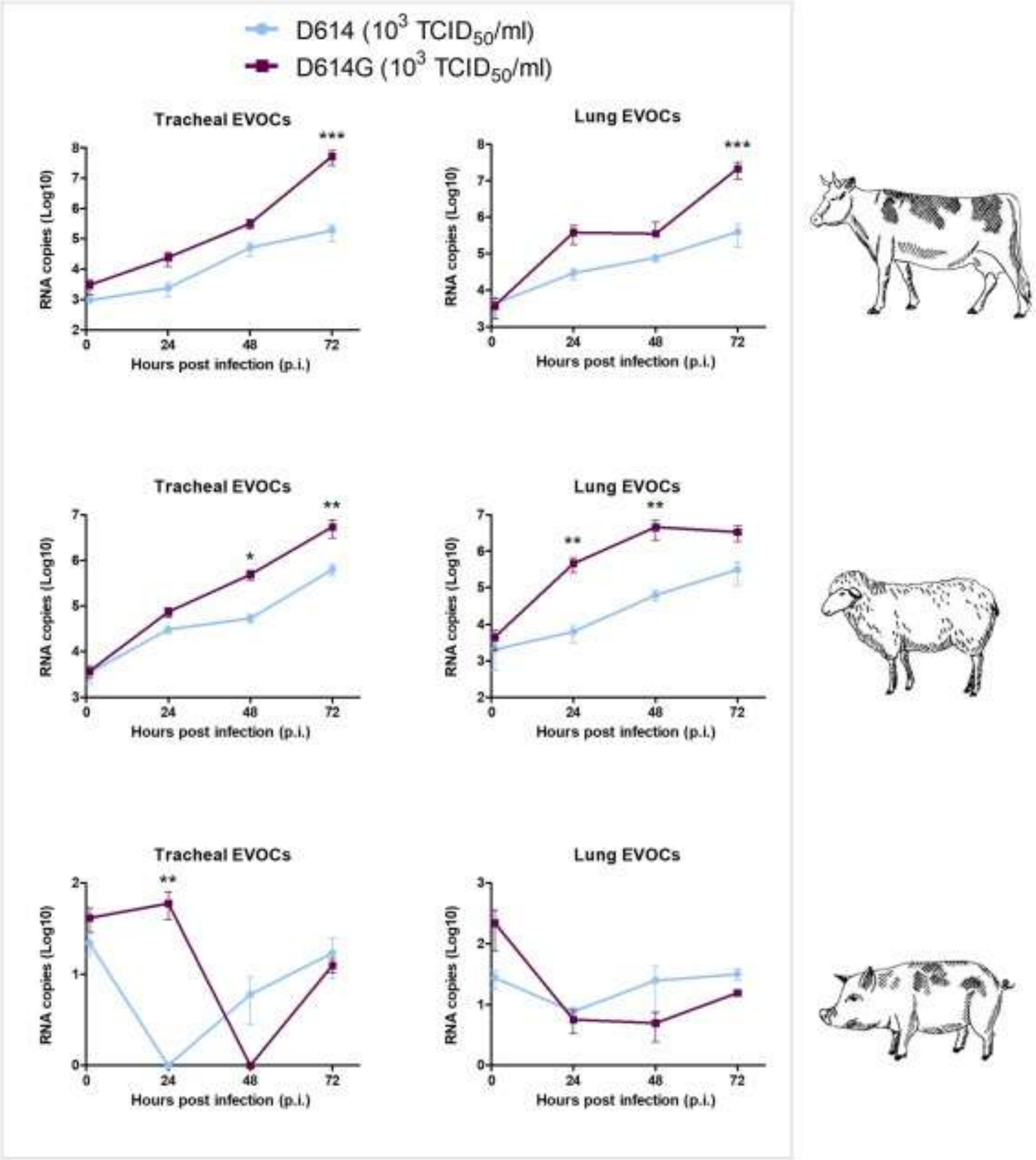
Share
Cite

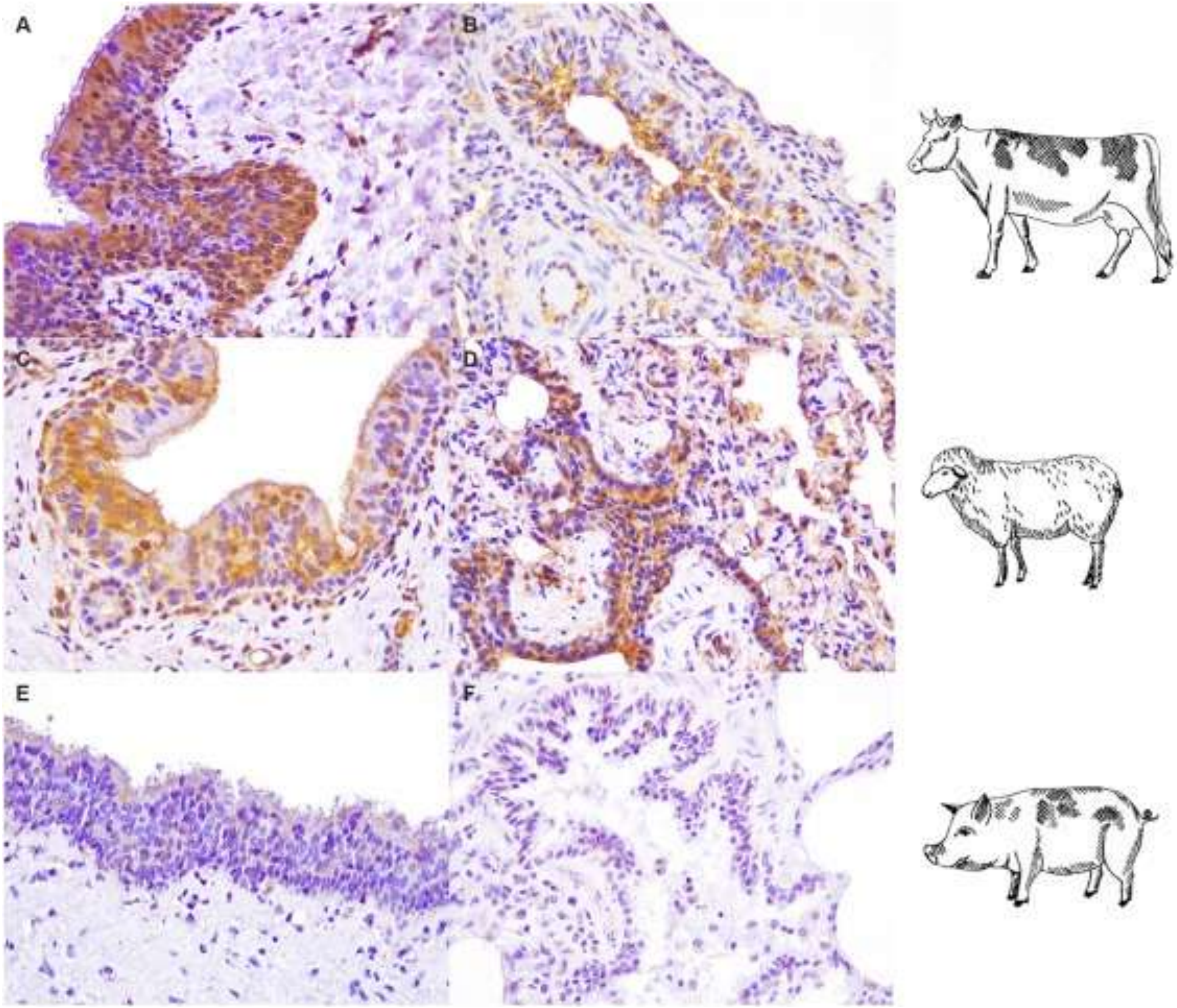
Recommended articles

No articles found.

Citing articles (0)


D614 (10^3 TCID$_{50}$/ml)
D614G (10^3 TCID$_{50}$/ml)
Tracheal EVOCs
Lung EVOCs
TCID$_{50}$/ml (Log 10)
Hours post infection (p.i.)
0
24
48
72
0
1
2
3
4
5
*
0
2
4
6
8
**
*
Tracheal EVOCs
Lung EVOCs
0
2
4
6
Tracheal EVOCs
Lung EVOCs
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.5
1.0
1.5


D614 (10^3 $TCID_{50}$/ml)
D614G (10^3 $TCID_{50}$/ml)
Tracheal EVOCs
***
RNA copies (Log10)
2
3
4
5
6
7
8
0
24
48
72
Hours post infection (p.i.)
Lung EVOCs
***
RNA copies (Log10)
3
4
5
6
7
8
0
24
48
72
Hours post infection (p.i.)
Tracheal EVOCs
*
**
RNA copies (Log10)
3
4
5
6
7
0
24
48
72
Hours post infection (p.i.)
Lung EVOCs
**
**
RNA copies (Log10)
2
3
4
5
6
7
0
24
48
72
Hours post infection (p.i.)
Tracheal EVOCs
**
RNA copies (Log10)
0
1
2
0
24
48
72
Hours post infection (p.i.)
Lung EVOCs
RNA copies (Log10)
0
1
2
3
0
24
48
72
Hours post infection (p.i.)

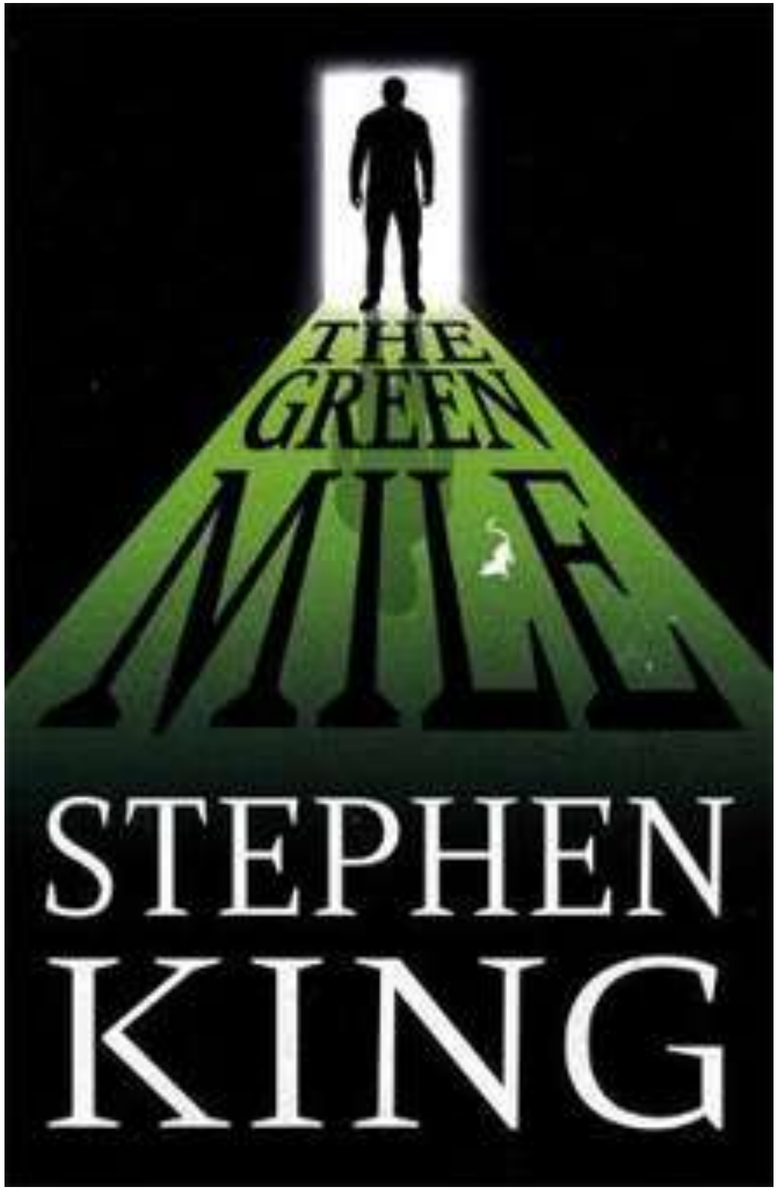

A
B
C
D
E
F

Attività di ricerca IZSAM

- Sorveglianza

- NGS
- Database
- Studi di patogenesi (rg based)
- Immunità e infiammazione
- Nuovi coronavirus

Pandemia e ruolo della medicina veterinaria….



Thank you for your attention