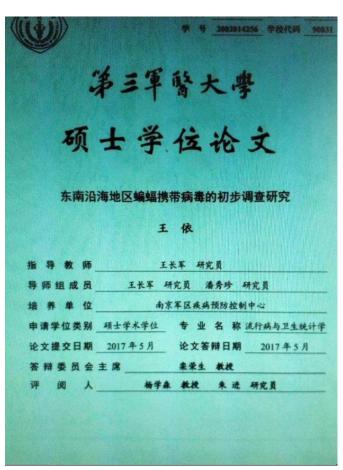
Brief summary of evidence of lab-made 2019 nCoV

From 2017-2019, a series academic papers about a novel zoonotic coronavirus isolated from bats in Zhoushan, Zhejiang (in short, ZS bat-CoV) were published in English (1) and Chinese

- (1. post-graduate thesis: Preliminary study about bat-virus in South-east coastland http://le.cnki.net/kmobile/Master/detail/SYJT_PHAM/1017235765.nh)
- (2. Molecular identification and analyse of bat-coronavirus in Zhoushan area http://kns.cnki.net/kcms/detail/detail.aspx?filename=JSCY201901004&dbcode=CJFQ &dbname=CJFDTEMP&v=).

The main investigators were from Chinese military institution (The Third Military Medical University and Military medical institute of Nanjing Command) (*Pic. 1-3*).





Pic. 2

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物分类学 > 摘要

舟山地区蝙蝠轮状病毒分子鉴定和分析

《寄生虫与医学昆虫学报》2019年 第1期 | 何婷 朱长强 艾乐乐 胡丹 吕瑞辰 谭伟龙 王长军 钱晖 江苏大学医学院 江苏镇江212000 东部战区疾病预防控制中心 江苏南京210000 中国人民解放军疾病预防控制中心 北京100071



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摘 要:为监测东南沿海舟山地区蝙蝠携带轮状病毒的流行情况,探索 其流行机制,2015-2018年,在浙江省舟山地区采集蝙蝠,以蝙蝠肠组织为 模板,设计针对轮状病毒VP3序列的特异引物进行RT-PCR检测。结果显 示,2015年采集的65只蝙蝠,轮状病毒阳性率为6.15%(4/65),2016年采集的 48只蝙蝠,阳性率为2.08%(1/48),2017年采集的70只蝙蝠,阳性率为2.86%(2/70),而2018年采集的101只蝙蝠,未检测到轮状病毒序列。针对2017年 小菲菊头蝠Rhinolophus hipposideros体内发现的一株轮状病毒进行基因 组扩增,获取部分基因组序列并进行生物信息学分析,发现VP7基因序列 与蝙蝠源轮状病毒相似度最高,同源性为96%;VP3基因序列与人源轮状 病毒同源性为90%;VP6基因序列与猫同源性为95%。根据上述结果,我们 推测舟山蝙蝠来源轮状病毒是一株重组病毒株,具有潜在跨种传播给人 类的可能性。本研究系首次在东南沿海舟山地区蝙蝠体内检测到轮状病 毒,对该地区轮状病毒的监测和预警具有一定的公共卫生意义。

【分 类】 <u>【生物科学】 > 动物学 > 动物分类学(系统动物学) > 哺乳</u> 纲 > 真兽亚纲 > 翼手目

【关键词】 蝙蝠 轮状病毒 进化分析

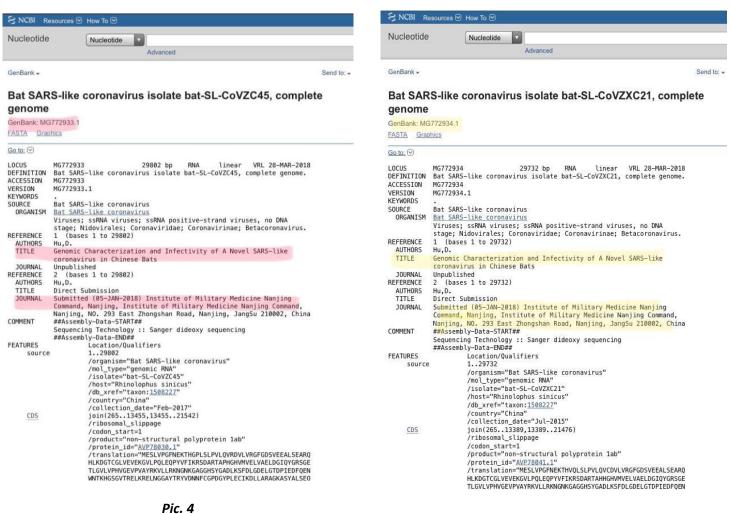
【出处】 《寄生虫与医学昆虫学报》2019年第1期22-29页 共8页

【收录】 中文科技期刊数据库

Pic. 3

It means the novel ZS bat-CoV was owned by Chinese military labs only. In the papers above, they have clarified that ZS bat-CoV has potential cross-species

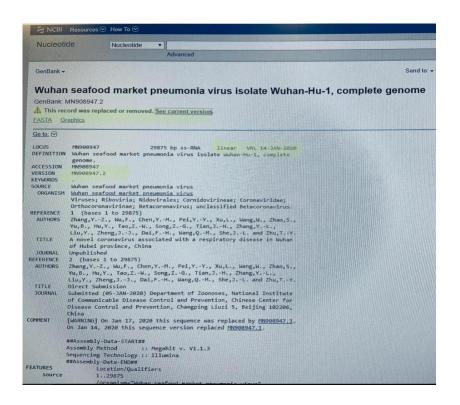
transmission, including human, also emphasized that it is significant for public health. The full genome sequence of certain strains of ZS bat-CoV were released in Genbank of NIH (accession numbers MG772844 through MG772934), provided by Nanjing



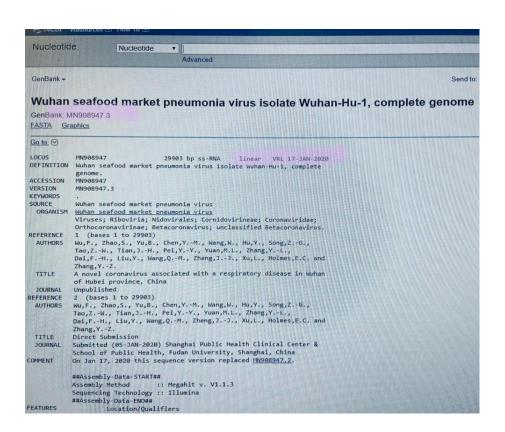
Pic. 5

command (Pic. 4-5).

11 Jan 2020, China CDC released the full genome sequence of 2019 nCoV, and it was upload to GenBank of NIH on 12 Jan (Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, accession number MN908947.1). 14 Jan, MN908947.1 was replaced by China CDC without announced (accession number MN908947.2). 17 Jan, MN908947.2 was replaced by them for unknown reasons (accession number MN908947.3) (*pic.* 6-7).

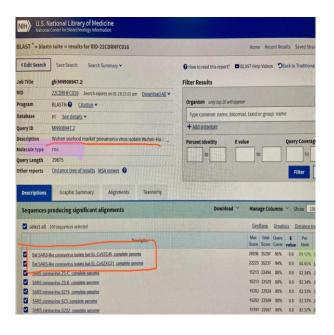


Pic. 6

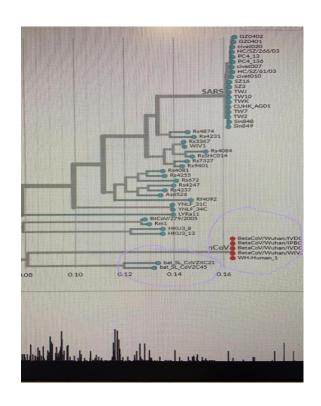


Pic. 7

The blast results based on full genome of MN908947.2 and MN908947.3 shown that ZS bat-CoV is the most closed known coronavirus to 2019 nCoV via online blast tool-NIH (per Identity is 88.65-89.12%, MG772933.1 and MG772934.1) (*pic.* 8). According to analysis of virus evolution tree, ZS bat-CoV is the most closed relative to 2019 nCoV (https://nextstrain.org/groups/blab/sars-like-cov) (*pic.* 9). To note, when blast MN908947.1 at that time, there were some error in the sequence which affected the results (no picture recorded).



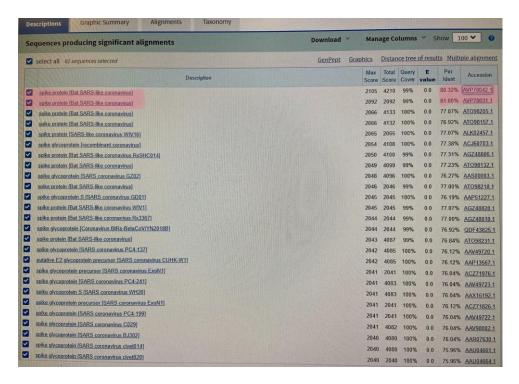
Pic. 8



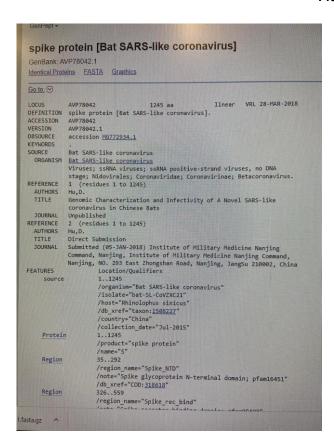
Pic.9

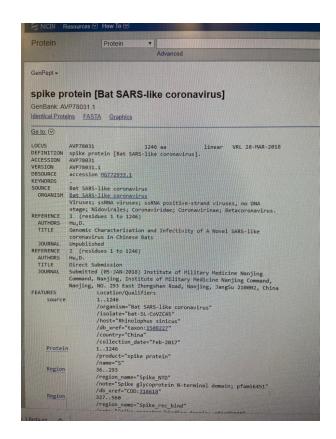
Further blast results for the most critical proteins and segments between 2019 nCoV and all the other known coronavirus in Genbank, still show the most significant similarity to ZS bat-CoV, which is much higher than compared with any others. The similarity is Spike protein 80.32-81.00% (S protein) (*pic. 10-12*) and Nucleocapsid protein 94.03% (*pic. 13-15*) to two strains of ZS bat-CoV (MG772933.1 and MG772934.1), while Envelop protein 100% (E protein) (*pic. 16-18*), ORF8 segment

94.21% (*pic. 19-20*), and Membrane glycoprotein 98.65% (*pic. 21-22*) to MG772933.1, as well as RdRp gene 95.75% (*pic. 23-24*) to MG772934.1.

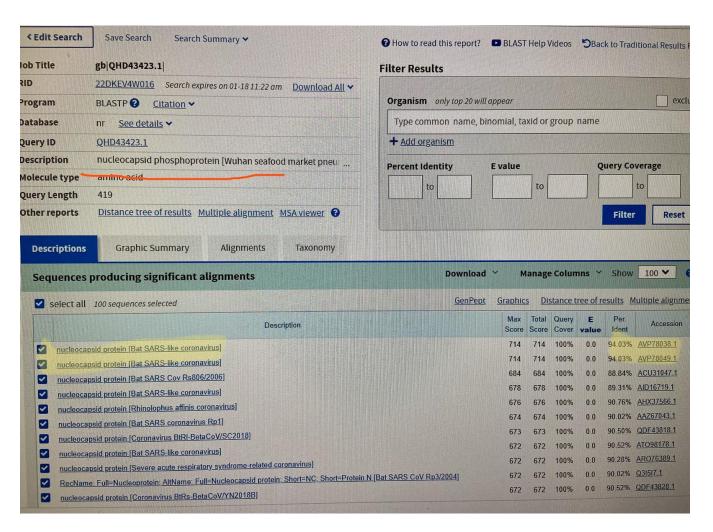


Pic. 10

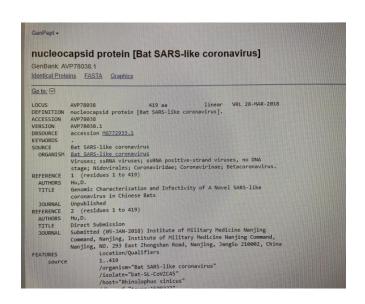




Pic. 11 Pic. 12



Pic. 13



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nucleocapsid protein [Bat SARS-like coronavirus]

GenBank AVP78049:1

Identical Proteins FASTA Graphics

Go to ♥

LOCUS AVP78049 419 as linear VRL 28-MAR-2018

DEFINITION nucleocapsid protein [Bat SARS-like coronavirus].

ACCESSION AVP78049:1

DESOURCE accession MG72934.1

KEYMORDS

SOURCE Bat SARS-like coronavirus

ORGANISH Bat SARS-like coronavirus

ORGANISH Bat SARS-like coronavirus

ORGANISH Bat SARS-like coronavirus

ORGANISH Bat SARS-like coronavirus

AUTHORS

Hu,D.

TITLE Genomic Characterization and Infectivity of A Novel SARS-like coronavirus in chinese Bats

JOURNAL Unpublished

REFERENCE 2 (residues 1 to 419)

AUTHORS

Hu,D.

TITLE Direct Submission

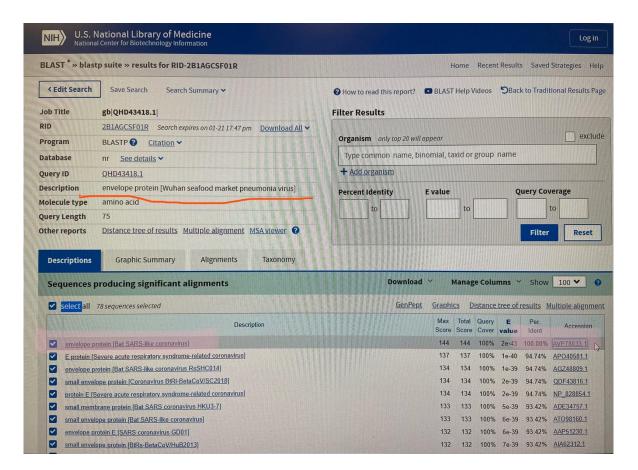
JOURNAL Submitted (65-JAN-2018) Institute of Military Medicine Nanjing Command, Nanjing, Institute of Military Medicine Nanjing Command, Nanjing, No. 299 East Ethongshan Road, Nanjing, JangSu 210902, China Location/Qualifiers

1.419

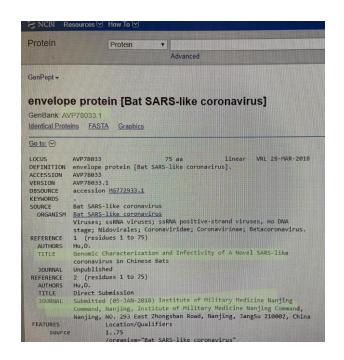
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//do.mession="Sars-like corona
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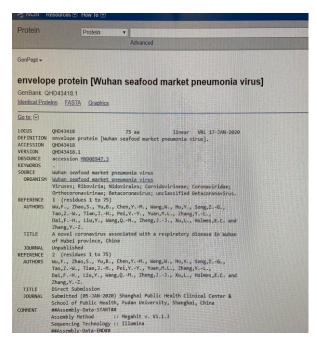
Pic. 14 Pic. 15

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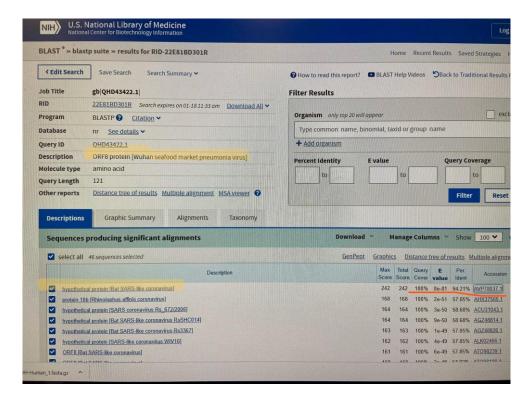


Pic. 16

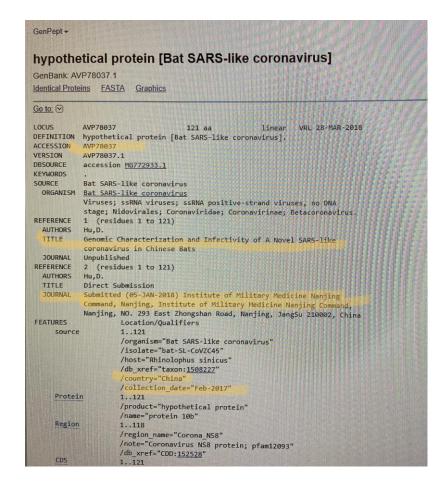




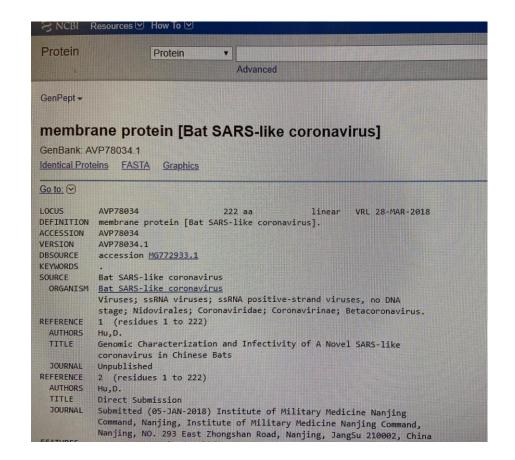
Pic. 17 Pic. 18



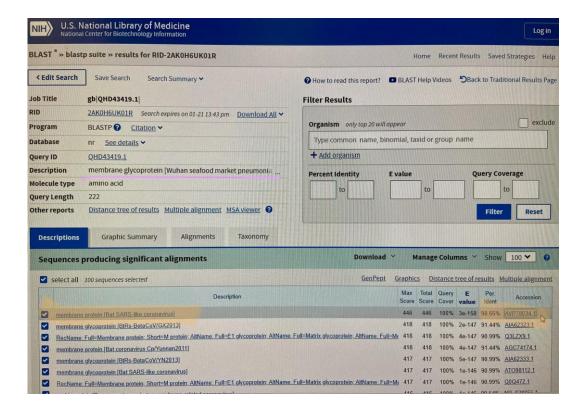
Pic. 19



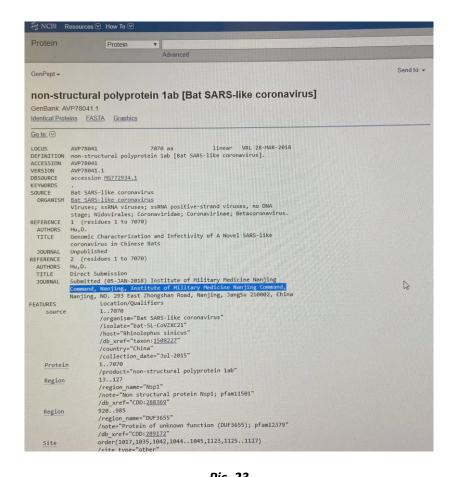
Pic. 20



Pic. 21



Pic. 22



Pic. 23

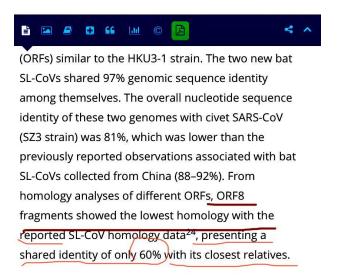
						0
	Max score	Total score	Query cover	E value	Ident	Accession
	1045	1045	97%	0.0	95.75%	AVP78041.1
	887	887	97%	0.0	80.31%	AGC74164.1
	886	886	97%	0.0	80.31%	AIA62309.1
der protein; C	884	884	97%	0.0	79.92%	P0C6F5.1
der protein; C	884	884	97%	0.0	80.58%	P0C6F8.1

Pic. 24

As mentioned in Nanjing military paper(1), from homology analyses of different

ORFs, ORF8 fragments in ZS bat-CoV showed the lowest homology, presenting only 60% identity with its closest relatives (**while 94.21% to 2019 nCoV**) (*pic. 25*). As well, analysis of the RNA-dependent RNA polymerase (RdRp) gene showed that the genomic sequences of bat CoV samples obtained from different parts of the world shared 80–90% identity among themselves and exhibited 87–92% identity with the SARS-CoVs extracted from human or civet sources (**while 95.75% to 2019 nCoV**).

Compared with high mutant S protein, E protein is more conserved (2). However, undergoing a natural evolution, the possibility of 100% identical E protein between the cross-species 2019 nCoV and ZS bat-CoV is almost impossible. It was simlply confirmed by using another online tool-Cluster Omega, which shows that the E protein of one strain of ZS bat-CoV (AVP78033.1, belong to MG772933.1) is more identical (100%) to 2019 nCoV, rather than another ZS bat-CoV (AVP78044.1,





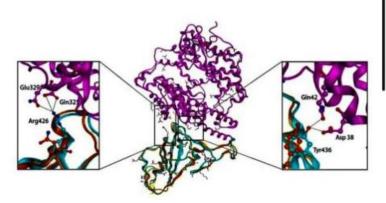
Pic. 25 Pic. 26

belong to MG772934.1) (pic. 26).

21 Jan, the first paper studied 2019 nCoV is published in a journal under Chinese Academy of Sciences, from Beijing Institute of Pharmacology and Toxicology (belonging to military) and Chinese Academy of Sciences (3). In that paper, they mentioned that the natural host of 2019 nCoV may be certain bat (not directly mentioned ZS bat-CoV). In their official Chinese introduction for this paper, they stressed the key point of their finding as: "To be surprised", compared with SARS CoV, 4 of 5 amino acid changed in the receptor binding domain (RBD) of S protein in 2019 nCoV, but still maintains the core structure to support strong interaction with human ACE2 molecules "in a very perfect way". It means 2019 nCoV could infect human respiratory epithelial cells in the same way as what SARS CoV did (pic. 27).



为了分析清楚这个问题,文章作者利用分子结构模拟的计算方法,对武汉冠状病毒。一蛋白和人ACE2蛋白进行了结构对接研究,获得了令人惊讶的结果。虽然武汉冠状病毒S-蛋白中与ACE2蛋白结合的5个关键氨基酸有4个发生了变化,但变化后的氨基酸,却整体性上非常完美的维持了SARS病毒S-蛋白与ACE2蛋白互作的原结构构象。尽管武汉新型冠状病毒的新结构与ACE2蛋白互作能力,由于丢失的少数氢键有所下降(相比SARS病毒S-蛋白与ACE2的作用有下降),但仍然达到很强的结合自由能(-50.6kcal/met)。这一结果说明武汉冠状病毒是通过S-蛋白与人ACE2互作的分子机制,来感染人的呼吸道上皮细胞、研究成果预测了武汉冠状病毒有很强的对人感染能力,为科学防控,制定防控策略和开发检测/干预技术手段奠定了科学理论基础。



pic.27

To noted, 23 Jan, another paper with similar content was upload to BioRxiv, which is from famous bat and SARS CoV investigator Zheng-li Shi's team (4).

There are also a lot of official news and poor-quality academic articles from end of last Dec show that no evidence about wild animals in Huanan seafood market as intermediate host for 2019 nCoV (which can be explained in a detail way later). Hence, one hypothesis of lab-made 2019 nCoV is recombined with SARS RBD of S

protein (to human ACE2 gene), based on ZS bat-CoV (esp. MG772933.1), going through in vitro and in vivo adaptation and amplification in a limited range in the lab, generated an ideal strain (2019 nCoV) with effective RBD, while the other comparable conserved sequence did not change much, or even without any change (E protein). Since stock virus kept in culture media at -80 °C, slowly thaw it on ice could help the virus released in the environment better.

According to Mr. Miles Guo, thymosin is out of stock in commercial market in China. Thymosin is potential therapeutic in the treatment of severe SARS and MERS (5).

PS. More evidence from official media and government response along the time in 2019 nCoV crisis is not written in this brief report.

- 1. Hu D, Zhu C, Ai L, He T, Wang Y, Ye F, et al. Genomic characterization and infectivity of a novel SARS-like coronavirus in Chinese bats. Emerg Microbes Infect. 2018;7(1):154.
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