

二、英文原文 (PDF 格式截面件)

R. sinicus should be reservoir of SL-CoV but not SARS-CoV: critical comments on Ge et al.'s paper in Nature

XU De-Zhong^{1,2}, Bernard CK CHOI³, ZHANG Lei³, SU Hai-Xia³, ZHANG Jing-Xia³, XU Rui³, GAO Jie³

¹Clinical Epidemiology Unit, Fourth Military Medical University, Clinical Epidemiology Network in China, International Clinical Epidemiology Network, Xi'an 710032, China; ²Department of Epidemiology, Fourth Military Medical University, Xi'an 710032, China; ³Dalla Lana School of Public Health, University of Toronto, Department of Epidemiology and Community Medicine, University of Ottawa, Ontario M5T 3M7, Canada)

The study of the origin of SARS coronavirus (SARS CoV) and SARS-like coronavirus (SL-CoV) is very important for the prevention of novel human infections with these viruses. Recently, Ge et al. suggest that Chinese horseshoe bats are natural reservoirs of SARS-CoV^[1]. But this conclusion is incorrect. It should be "Chinese horseshoe bats are natural reservoirs of SL-CoV"^[2-7]. The paper also has several methodological problems, leading to errors in the conclusions.

First, no epidemiological report has confirmed that people developed SARS through contact with *R. sinicus* with SL-CoV. Just as the efficacy of a drug should be evaluated by the pharmacological effects not only in laboratory research but also in randomized blinded trials among patients, the reservoir of an infectious disease should not be determined only by laboratory studies but also by epidemiological evidences. In short, the results of epidemiological studies are the gold standard for the determination of reservoir. Many papers confirmed that in addition to patients, civets are a source of infection^[8-11]. But civets cannot be a reservoir, because as a reservoir, besides being infection source, must also be able to maintain a continuous spreading of infection among animals and humans.

Second, the conclusion of Ge et al. is not consistent

with the epidemiological evidences. There has been no case of SARS since June, 2004^[2,8-12], as predicted by Holmes^[12-13]. If "Chinese horseshoe bats are natural reservoirs of SARS-CoV" is true, SARS-CoV cannot disappear for 10 years^[2].

Third, the conclusion "Chinese horseshoe bats are natural reservoirs of SL-CoV" has been established by many studies^[2-7]. *R. sinicus* carry SL-CoV but not SARS-CoV^[4-5].

Fourth, it is inappropriate to compare ACE2 receptors between SL-CoV Rs3367 and RsSHC014 and human/civet SARS-CoVs. In Ge et al.'s paper, the two strains are compared with the SARS-CoVs in terms of "the key amino acid residues (AA)". The key AAs, especially 479 and 487, play an important role in the infectivity and transmissibility of human SARS-CoV. The transmissibility of SARS-CoV in the 2002-2003 epidemic was approximately 1000-fold higher than that of 2003-2004 Guangzhou outbreak because of the variation in AA479 and 487^[12,14]. The strains vary a great deal in AA479 and 487 among bats, humans and civets (Table 1). More importantly, Ge et al. did not include human/civet strains in the Guangzhou outbreak, which are different from not only Rs3367 and RsSHC014 but also 2002-2003 human SARS-CoVs^[2,4,6,8,10-11]. Thus,

Author: Xu Dezhong, Master, Director in Clinical Epidemiology Unit, Professor in Department of Epidemiology, Research interest: Epidemiology of infectious diseases. Tel:(029)84774955 Email:xudezhong@163.com

25

Negative

Vol.5 No.2 2014

there is no significant difference between two strains and previous SL-CoVs.

Fifth, it is important to correct for time confounding, but Ge et al.'s paper failed to do so. Confounding is the most important factor compromising the reliability in many studies. Based upon their finding, just 9 years after the SARS epidemic, that Chinese horseshoe bats carry SL-CoV "which has typical coronavirus morphology, 99.9% sequence identity to Rs3367", they made the conclusion "Chinese horseshoe bats are natural reservoirs of SARS-CoV"^[1]. However, during and in the several years after the SARS epidemic, no Rs3367 or RsSHC014 strains were found^[2-7]. Therefore, Ge et al. cannot rule out the possibility that two strains were naturally evolved in *R. sinicus* during recent years.

Sixth, Ge et al.'s paper mentioned "the recent demonstration of MERS-CoV in a Saudi Arabian bat". However, the *T. perforatus* with MERS-CoV was collected in the area of index case occurrence in Bisha in October, 2012^[19].

Seventh, the important comparison on specific 29-nucleotide is missing. The 29-nt (CCAATACAT TACTATTCCGGACTGGTTTAT, nt. 27866-27894 in Bt-SL-CoV or CCTACTGGTTACCAACCTGAATG GAATAT, nt.27869-27897 in human/civet SARS CoV) is related to pathogenicity, transmissibility and cross-species transmission of SARS CoV. Because 29-nt of Rs3367 and RsSHC014 is identical with SL-CoV, but different from SARS-CoV (Table 1), these two strains should be classified as SL-CoVs.

Table 1 The comparison of different hosts of SARS-CoV and Bt-SL-CoV, occurrence years of strains by amino acid sites 479 and 487, and characteristic 29-nt based on data in the literatures^[1-4]

Host	Year of occurrence	Amino acid sites		Characteristic 29-nt
		479	487	
Bat (Bt-SL-CoV, Rp3)	Around 1998	S	V	CCAATACATTACTATTCCGGACTGGTTTAT, nt. 27866-27894
Bat (Bt-SL-CoV, Rs3367)	Unknown	N	N	CCAATACATTACTATTCCGGACTGGTTTAT, nt. 27866-27894
Bat(Bt-SL-CoV, RsSHC014)	Unknown	R	A	CCAATACATTACTATTCCGGACTGGTTTAT, nt. 27866-27894
Palm civet (SARS CoV)	2002-2003 epidemic	K, R and N	S	CCTACTGGTTACCAACCTGAATGGAATAT, nt.27869-27897
	2003-2004 Guangzhou outbreak	N and R	S	
Human (SARS CoV)	Very early in 2002-2003 epidemic	N	T	CCTACTGGTTACCAACCTGAATGGAATAT, nt.27869-27897
	2002-2003 epidemic	N	T	deletion
	2003-2004 Guangzhou outbreak	N, K and R	S	CCTACTGGTTACCAACCTGAATGGAATAT, nt.27869-27897

We are afraid that Ge et al.'s conclusion would not only have a negative influence on scientific nature, but also obstruct the research on the true origin of SARS CoV.

[References]

- [1] Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor[J]. Nature, 2013, 503(7477):535-538.

- [2] Xu DZ, Sun HM, Tan YH. SARS CoV no longer exists in nature and population[J]. *Negative*, 2013, 4(1):22-26. (In Chinese)
- [3] Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses [J]. *Science*, 2005, 310(5748): 676-679.
- [4] Yip CW, Hon CC, Shi M, et al. Phylogenetic perspectives on the epidemiology and origins of SARS and SARS-like coronaviruses[J]. *Infect Gene Evol*, 2009, 9(6):1185-1196.
- [5] Tang XC, Zhang JX, Zhang SY, et al. Prevalence and genetic diversity of coronaviruses in bats from China[J]. *J Virol*, 2006, 80(15):7481-7490.
- [6] Janies D, Habib F, Alexandrov B, et al. Evolution of genomes, host shifts and the geographic spread of SARS-CoV and related coronaviruses[J]. *Cladistics*, 2008, 24(2):111-130.
- [7] Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats[J]. *Proc Natl Acad Sci U S A*, 2005, 102(39):14040-14045.
- [8] Song HD, Tu CC, Zhang GW, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human[J]. *Proc Natl Acad Sci U S A*, 2005, 102(7): 2430-2435.
- [9] Xu RH, He JF, Evans MR, et al. Epidemiologic clues to SARS origin in China[J]. *Emerg Infect Dis*, 2004, 10(6):1030-1037.
- [10] Wang M, Yan M, Xu H, et al. SARS-CoV infection in a restaurant from palm civet[J]. *Emerg Infect Dis*, 2005, 11(12):1860-1865.
- [11] Chinese SARS Molecular Epidemiology Consortium. Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China[J]. *Science*, 2004, 303(5664):1666-1669.
- [12] Holmes KV. Structural biology. Adaptation of SARS coronavirus to humans[J]. *Science*, 2005, 309(5742):1822-1823.
- [13] Roberts M. Threat of new SARS outbreak 'low' [N/OL]. (2005-02-20). <http://news.bbc.co.uk/1/1/2/hi/health/4280253.stm>.
- [14] Li W, Zhang C, Sui J, et al. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2[J]. *EMBO J*, 2005, 24(8):1634-1643.
- [15] Memish ZA, Mishra N, Olival KJ, et al. Middle east respiratory syndrome coronavirus in bats, Saudi Arabia[J]. *Emerg Infect Dis*, 2013, 19(11):1819-1823.

(2014-02-17收稿; 2014-03-01修回)

三、中译文

DOI:10.13276/j.issn.1674-8913.2014.02.005

R. sinicus 应为 SL-CoV 的贮存宿主而非 SARS-CoV

——对 Nature 的 Ge XY 等论文之批评意见

徐德忠^{1,2}, Bernard CK CHOI³, 张磊², 苏海霞², 张景霞², 徐锐², 高洁²

(1 国际临床流行病学工作网第四军医大学临床流行病学中心; 2 第四军医大学流行病学教研室; 3 加拿大渥太华大学流行病学与社会医学教研室)

SARS 冠状病毒(SARS CoV)和 SARS 样冠状病毒(SL-CoV)的起源研究,对于预防此类病毒所致的新发传染病非常重要。最近,Ge XY 等提出“中华菊头蝠(Chinese horseshoe bats)为 SARS CoV 的自然贮存宿主(natural reservoir, reservoir)”^[1]。但此结论不正确,应为