H Chen 陳鴻霖
Y Guan 管 軼
X Fan 樊曉輝

Animal reservoirs for SARS-like coronavirus in southern China

Key Messages

1. SARS-coronavirus (SARS-CoV) was of zoonotic origin. Wild animals in live-animal markets in Guangdong province were the most likely intermediate hosts and sources of the outbreak in humans. The natural reservoir and emergence pathway of this virus remain largely unknown.
2. Bats appear to be the natural host for coronaviruses, and play a pivotal role in their ecology and evolution.
3. Live-animal markets may have provided an ecosystem that facilitated interspecies transmission of SARS-CoV in Guangdong province.
4. Long-term surveillance of zoonotic pathogens in both humans and animals is important for the prevention of emerging infectious diseases.

Hong Kong Med J 2011;17(Suppl 6):S36-40

Department of Microbiology, LKS Faculty of Medicine, The University of Hong Kong
H Chen, Y Guan
Department of Microbiology and Immunology, Guangxi Medical University, Nanning, Guangxi ,China
X Fan

RFCID project number: 03040782

Principal applicant and corresponding author:
Dr Honglin Chen
Department of Microbiology, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, China
Tel: (852) 2819 9941
Fax: (852) 2855 1241
Email: hlchen@hkucc.hku.hk

Introduction

Severe acute respiratory syndrome (SARS) first occurred in Guangdong province, and subsequently spread to many other countries. A novel SARS coronavirus (SARS-CoV) was the aetiological agent responsible for the outbreak. An investigation of Shenzhen wild-animal markets during the outbreak revealed that SARS-CoV was of zoonotic origin. The outbreak was under control by the summer of 2003, but re-emergence of human infections occurred in December 2003. These cases were again caused by direct transmission from wild animals to humans in the Guangzhou area. Culling of all wild animals in live-animal markets in the Guangzhou area in January 2004 likely averted the second outbreak of SARS in China. We aimed to identify the animal reservoirs for SARS-like CoV and susceptible species that could mediate SARS-CoV transmission to humans.

Methods

This study was conducted from 1 January 2006 to 31 March 2008. Samples collected during the 2003 and 2004 SARS outbreaks were retrospectively analysed. Himalayan palm civets, raccoon, dogs, and other animals in live-animal markets in Guangdong province were the most likely intermediate hosts and sources of the outbreak, but not the natural hosts of SARS-CoV. The natural reservoir and emergence pathway of SARS-CoV remain largely unknown. Molecular epidemiological studies of coronavirus were conducted in wild populations of bats, and also in other wild mammalian animals in live-animal markets in Guangdong during the SARS outbreak (2003/2004) and in Guangxi between 2004 and 2006.[1-3]

Results

Systematic virological surveillances of coronaviruses in bats and other wild animal species were conducted between 2004 and 2007. Approximately 1700 bats were sampled in their natural habitats in 15 provinces of China. In addition, 4420 other mammalian animals belonging to 24 species were sampled in live-animal markets in Guangdong and Guangxi provinces. Bats from 11 of the 15 sampled Chinese provinces tested positive for coronavirus, with an overall detection rate of 4.7% (Table 1). Sequence analysis of the viral genome demonstrated that bats harbour genetically diverse coronaviruses, including some closely related to SARS-like CoV (Fig 1). These findings suggest that bats may be the natural host for coronaviruses and play a pivotal role in coronavirus ecology and evolution.

In live-animal market surveillance, coronaviruses were detected in 81 animals belonging to 10 different mammalian species (Table 2). Phylogenetic analysis of RdRp, Helicase, Spike, Envelope, Matrix, and Nucleocapsid protein genes from these viruses showed that they clustered with the previously known group 1 coronaviruses, suggesting that these coronaviruses are genetically closely related (Fig 2). Further decoding of genome sequences revealed that these viruses share very high amino acid similarity, in some cases even when detected from different host species. This may indicate that these animals were newly infected from a common source inside the live-animal market.

Table 1. Coronavirus distribution in different bat species in southern China

Family and species of bat	Common name	No. of samples (No. of coronavirus positive)	Coronavirus group(s)
Rhinolophidae			
Rhinolophus pusillus	Least horseshoe bat	116	-
Rhinolophus malayanus	Malayan horseshoe bat	15	-
Rhinolophus affinis	Intermediate horseshoe bat	79	-
Rhinolophus ferrumequinum	Greater horseshoe bat	41 (4)	1, 4, 5
Rhinolophus thomasi	Thomas's horseshoe bat	14	-
Rhinolophus sinicus	Chinese horseshoe bat	67 (1)	4
Rhinolophus pearsoni	Pearson's horseshoe bat	75 (1)	1
Rhinolophus macrotis	Big-eared horseshoe bat	45 (1)	4
Rhinolophus rex	King horseshoe bat	2	-
Rhinolophus luctus	Woolly horseshoe bat	4	-
Rhinolophus osgoodi	Osgood's horseshoe bat	1	-
Rhinolophus paradoxolophus	Bourret's horseshoe bat	6	-
Rhinolophus rouxi	Rufous horseshoe bat	1	-
Hipposideros armiger	Great leaf-nosed bat	182	-
Hipposideros larvatus	Intermediate leaf-nosed bat	82 (6)	1
Hipposideros pratti	Pratt's leaf-nosed bat	10	-
Hipposideros pomona	Pomona leaf-nosed bat	138 (5)	1
Coelops frithi	East Asian tailless leaf-nosed bat	7	-
Aselliscus stoliczkanus	Stoliczka's Asian trident bat	12	-
Vespertilionidae			
Pipistrellus pipistrellus	Common pipistrelle	27 (6)	5
Pipstrellus abramus	Japanese pipistrelle	41 (14)	5
Pipstrellus sp	-	3	-
Scotophilus kuhlii	Lesser Asiatic yellow house bat	43 (5)	1
Myotis daubentonii	Daubenton's bat	41	-
Myotis mystacinus	Whiskered bat	1	-
Myotis ricketti	Rickett's big-footed bat	56 (14)	1
Myotis chinensis	Large Myotis	3	-
Myotis sp	-	99 (1)	1
Nyctalus aviator	Birdlike noctule	6	-
Nyctalus velutinus	Villus noctule	1	-
Nyctalus noctula	Noctule	17	-
Scotomanes ornatus	Harlequin bat	8	-
Barbastella leucomelas	Eastern barbastelle	1	-
Tylonycteris pachypus	Lesser bamboo bat	14 (2)	5
Ia io	Great evening bat	30 (1)	1
Kerivoula hardwickei	Hardwicke's woolly bat	1	-
Kerivoula sp	-	11	-
Murina leucogaster	Greater tube-nosed bat	5	-
Murina sp	-	4	-
Miniopterus schreibersi	Schreiber's long-fingered bat	135 (17)	1
Miniopterus sp	-	4	-
Pteropodidae			
Cynopterus sphinx	Greater short-nosed fruit bat	11	-
Rousettus leschenaulti	Leschenault's Rousette	31	-
Rousettus sp	-	12	-
Eonycteris spelaea	Lesser dawn bat	3	-
Emballonuridae			
Taphozous melanopogon	Black-bearded tomb bat	181 (1)	-
Megadermatidae			
Megaderma lyra	Greater false vampire bat	2	-
Megaderma spasma	Lesser false vampire bat	4	-
Total	48	1692 (79)	1, 4, 5

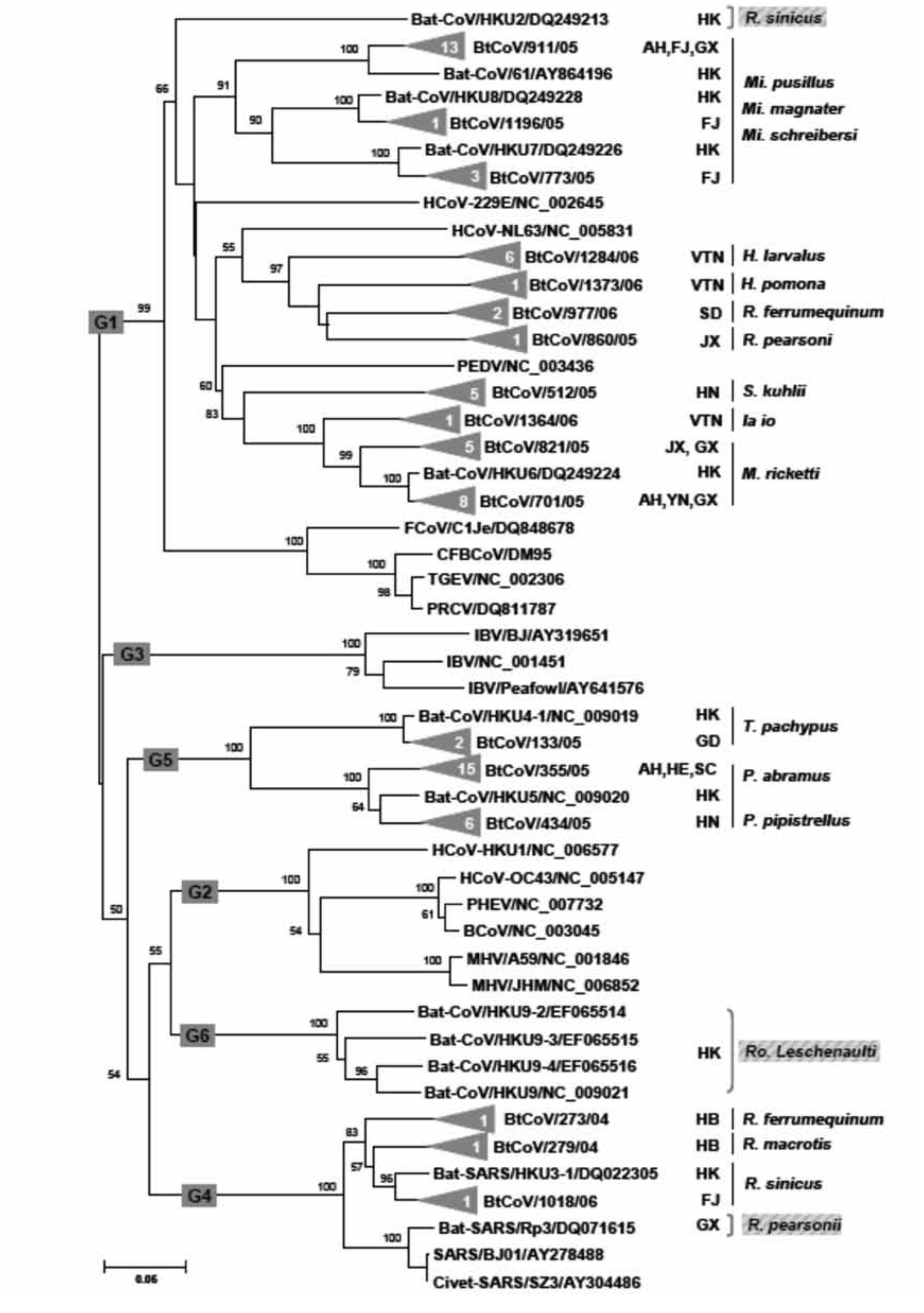

Fig 1. Phylogenetic relationships of coronavirus isolated from bats in China
The tree diagram was based on 440 nucleotides of the RdRp region by the neighbour joining method. Numbers below branch nodes indicate neighbour joining bootstrap values (%), calculated from 1000 bootstrap replicates. Terminal nodes containing bat coronavirus isolated in this study are collapsed and represented by a grey triangle with the number of viruses indicated within. The tree diagram was rooted to Breda virus (AY427798). Scale bar, 0.05 substitution per site. AH denotes Anhui, FJ Fujian, GD Guangdong, GX Guangxi, HA Hainan, HB Hubei, HE Henan, JX Jiangxi, SC Sichuan, SD Shandong, YN Yunnan, HK Hong Kong, and VTN Vietnam

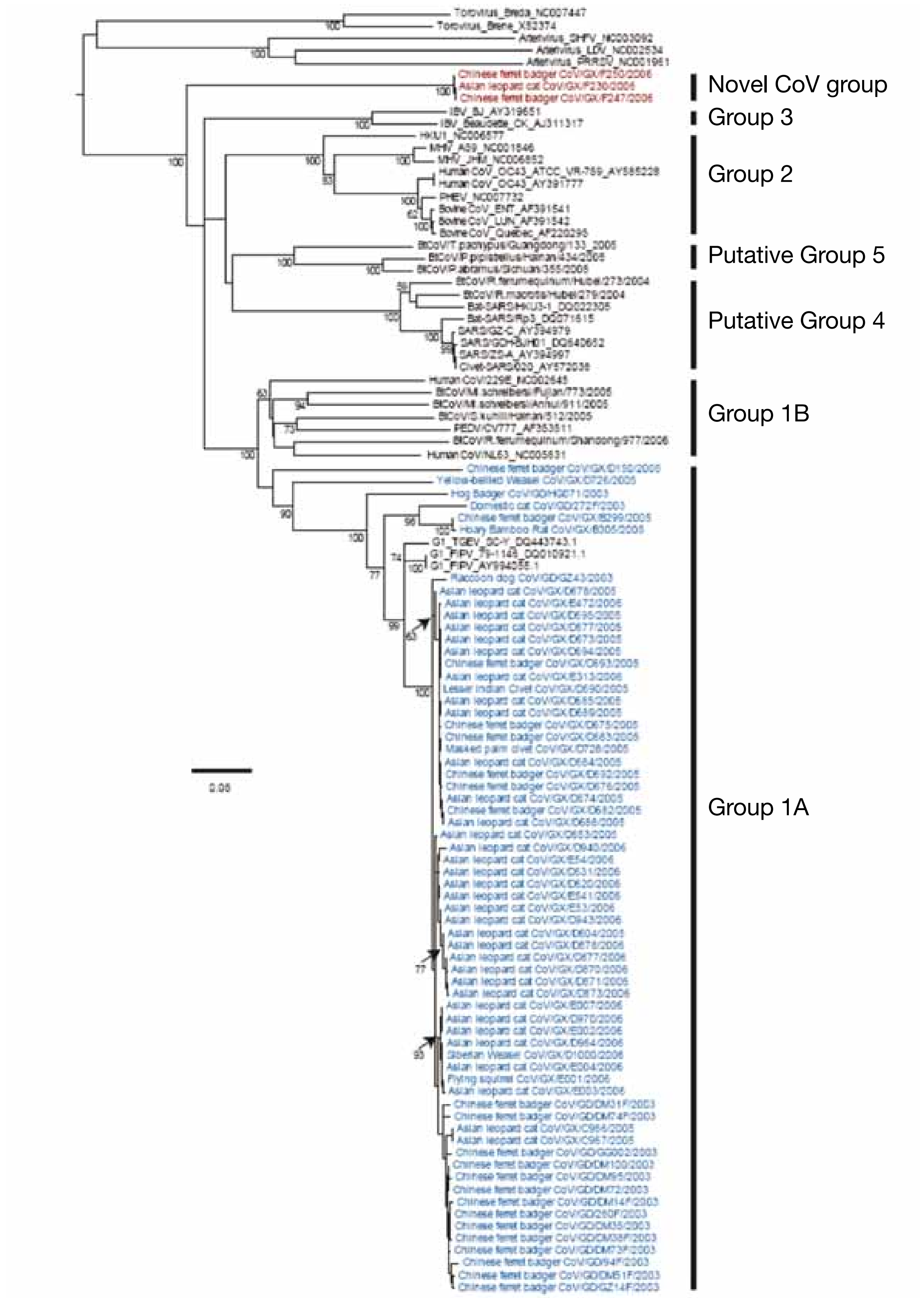

Fig 2. Phylogenetic relationships of coronavirus isolated from wild animals in live-animal markets in China
The tree diagram was based on 440 nucleotides of the RdRp region by the neighbour joining method. Numbers below branch nodes indicate neighbour joining bootstrap values (%), calculated from 1000 bootstrap replicates. The tree diagram was rooted to Gill-associated Okavirus (AF227196)

Table 2. Coronaviruses detected in wild animals in live-animal markets

Animal species	No. of samples (No. of coronavirus positive)	
	2003/2004	2005/2006
Masked palm civet *(Paguma larvata)*	154	1439 (1)
Hog badger *(Arctonyx collaris)*	49	0
Chinese ferret badger *(Melogale moschata)*	120 (23)	966 (10)
Asian leopard cat *(Prionailurus bengalensis)*	5	1453 (35)
Raccoon dog *(Nyctereutes procyonoides)*	27 (6)	8
Hoary bamboo rat *(Rhizomys pruinosus)*	3	108 (1)
Yellow bellied weasel *(Mustela kathiah hodgson)*	0	33 (1)
Lesser Indian civet *(Viverricula indica)*	1	61 (1)
Siberian weasel *(Mustela sibirica)*	0	81 (1)
Flying squirrel *(Petaurista sp)*	0	150 (1)
Sabel *(Martes zibellina)*	0	4
Asiatic brush tailed porcupine *(Atherurus macrourus)*	0	6
Asian small clawed otter *(Aonyx cinerea)*	0	8
Crab eating mongoose *(Herpestes urva)*	0	25
Rhesus macaque *(Macaca mulatta)*	19	74
Wild boar *(Sus scrofa)*	1	3
Nutria *(Myocaster coypus)*	5	0
Domestic cat *(Felis catus)*	11 (1)	0
Chinese hare *(Lepus sinensis)*	26	-
Barking deer *(Muntiacus muntjak)*	2	1
Black goat	4	0
Beaver *(Castor fiber)*	6	0
Horse *(Equus)*	10	0
Eurasian badger *(Meles meles)*	14	0
Common pangolin *(Manis pentadactyla)*	6	0
Dog	2	0
Fox	3	0
Chinese pygmy dormouse *(Typhlomys cinereus)*	13	0
Chinese porcupine *(Hystrix hodgsoni)*	8	0
Swamp deer *(Cervus duvauceli)*	9	0
Total	498 (30)	4420 (51)

Conclusions

Bats appear to be the natural reservoir for coronaviruses (which may serve as precursors for other CoVs, including SARS-CoV) that affect humans and animals. However, the immediate precursor of SARS-CoV remains unknown. Further investigation of coronaviruses in a wider range of bat species and over broader geographical regions is necessary. Many species of wild mammals are susceptible to coronavirus infection. Live-animal markets might have facilitated interspecies transmission of SARS-CoV in Guangdong province during the SARS outbreak. Live-animal markets could pose a significant potential risk for emergence of infectious diseases.

Acknowledgement

This study was supported by the Research Fund for the Control of Infectious Diseases (#03040782), Food and Health Bureau, Hong Kong SAR Government.

References

1. Vijaykrishna D, Smith GJ, Zhang JX, Peiris JS, Chen H, Guan Y. Evolutionary insights into the ecology of coronaviruses. J Virol 2007;81:4012-20.
2. Dong BQ, Liu W, Fan XH, et al. Detection of a novel and highly divergent coronavirus from Asian leopard cats and Chinese ferret badgers in Southern China. J Virol 2007;81:6920-6.
3. Tang XC, Zhang JX, Zhang SY, et al. Prevalence and genetic diversity of coronaviruses in bats from China. J Virol 2006;80:7481-90.