Update on SARS research and other possibly zoonotic coronaviruses[☆]

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ABSTRACT

The global outbreak of severe acute respiratory syndrome (SARS) in 2003 led to an intense and effective global response that stopped the spread of the disease by July 2003. There was also an intensive and very productive research effort to identify the aetiological agent, characterise the clinical and epidemiological features of the disease, understand the pathogenesis of the disease and the molecular biology of the virus, and design antiviral drugs and vaccines to treat and prevent the disease. In parallel with the SARS research effort there have been continuous improvements in our ability to detect and characterise other novel viruses. The SARS outbreak illustrates the importance of such detection tools in the response to public health threats. Studies since the SARS outbreak suggest that many novel viruses exist in animals and some, but probably not many, will present a risk to humans.

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On 11 February 2003 China reported to the World Health Organisation (WHO) that 305 cases of atypical pneumonia of unknown aetiology had been identified in Guangdong Province since 16 November 2002, and that five people had died. On 21 February 2003 a physician from Guangdong Province, ill with atypical pneumonia, visited Hong Kong and stayed overnight in Hotel M. The agent that caused his illness, severe acute respiratory syndrome coronavirus (SARS CoV), was transmitted to at least 10 persons, who subsequently initiated outbreaks in Hong Kong, Singapore, Viet Nam, and Canada [1]. Thus a global outbreak was initiated.

The outbreak triggered a successful global response to control the spread of the disease, and a very productive research effort. One outcome of the SARS and parallel research programmes has been the development of increasingly powerful molecular tools to identify and characterise novel, including zoonotic, pathogens. The response to SARS and the associated research activities demonstrated the public health benefits of these detection tools and the challenges associated with their use.

The severity of the illness associated with SARS CoV infection, and its rapid global spread, led to the intensive response effort. Most of those severely infected with SARS CoV had lower respiratory tract illness, an infiltrate on chest radiograph and were hospitalised. A high percentage of those hospitalised required intensive care, and many died. The overall mortality rate was ~10% but reached nearly 50% in elderly persons. To the surprise of some, SARS

transmission was stopped by the end of June, just over 4 months after the initiation of global spread from Hotel M in Hong Kong.

There have subsequently been three instances of laboratory-acquired infection, one in Singapore [2], one in Taiwan [3] and one in China [4] in 2003 and 2004, and one reintroduction from animals in Guangdong Province, China in December 2003 and January 2004 [5]. None of these occurrences had sufficient secondary human-to-human transmission to generate a threat of a recurrent global outbreak. The laboratory-acquired infection in China was, however, associated with limited community transmission, which highlighted the potential risk to the community of a breach in biosafety procedures in laboratories working on SARS CoV.

The rapid control of SARS CoV during the global outbreak of 2003, and the limited spread during subsequent reintroductions, likely result both from the characteristics of the virus and the effectiveness of control efforts. Features of SARS CoV infection that facilitated its control include: (1) a very high prevalence of serious illness, making it easier to identify cases and know where transmission was occurring and (2) a low risk of transmission before the patient was seriously ill and likely to be hospitalised, diagnosed and placed in isolation.

Case identification and isolation followed by contact identification and management were responsible for limiting the spread of and ultimately stopping the outbreak. Case identification and isolation were usually straightforward if the risk of exposure was recognised, but contact identification and management were sometimes more problematic. Recognition of all potential contacts and implementation of measures to rapidly identify and isolate those that had become infected prevented further spread. Missing potential contacts, on the other hand, sometimes led to unrecognised disease and substantial transmission, as illustrated by one instance in Canada [6] and one in Taiwan [7]. In both instances the extent of

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Table 1

Types of antiviral drugs developed for severe acute respiratory syndrome coronavirus.

Mode of action	Drug
Virus entry blockers	Anti-S protein monoclonal antibodies Peptides that bind to the heptad repeat on the S (spike) protein Peptides that bind to other regions of S and block oligomerisation, etc.
Virus replication blockers	3C-like protease inhibitors Other viral protease inhibitors, e.g. papain-like cysteine protease nsp1–16 Viral polymerase inhibitors Nelfinavir, lopinavir/ritonavir, ribavirin, RNAi, glycyrrhizin, niclosamide
Immune modulators	Type 1 interferons Lopinavir/ritonavir

Adapted from Groneberg et al. [15] and Tong et al. [16,17].

nosocomial transmission was underappreciated, and contacts that were not identified and became infected spread the virus to others.

Contacts can be managed in different ways, with the appropriateness of the different strategies depending on the extent and risk of the exposure [8–10]. Less stringent strategies are appropriate for low-risk exposures and more stringent ones for high-risk exposures. Some approaches to contact management are: (1) telling contacts about their possible exposure to SARS CoV and providing guidance on what to do if they develop a SARS-like illness, (2) actively monitoring contacts for illness and placing them in isolation and (3) placing contacts in quarantine.

Also key to stopping global transmission was WHO's timely updates on where SARS cases were occurring, the clinical and epidemiological features of infection, laboratory methods, and strategies to control spread. WHO's ability to coordinate the intensive collaborative global response to SARS was impressive.

The aetiological agent, SARS CoV, was identified within weeks of global spread [11–13] and this discovery was followed by an impressive sequence of research accomplishments. These included the development of diagnostic assays; the characterisation of the clinical, epidemiological and virological features of infection [14]; the identification and initial evaluation of antiviral drugs (Table 1) [15–17]; the development of animal models of SARS CoV infection

[18–22]; the development and evaluation of candidate vaccines (Table 2) [23–26]; the identification of receptors for SARS CoV [27,28]; the development of a reverse genetics system [29] and more. From the discovery of the virus in March 2003, until October 2009, there have been ~3000 articles published on the virus, the clinical and epidemiological features of infection, the social and societal impact of the outbreak and the treatment and prevention of the disease.

One of the early findings after the discovery of the SARS CoV was the near absence of SARS CoV antibodies in those who were not SARS cases [12,13,30]. This finding demonstrated that SARS CoV had not circulated to any significant extent in humans before 2003 and was introduced into humans from animals. Reports of multiple independent early cases from Guangdong Province during the first outbreak in November 2002 and February 2003, and reports of non-linked cases during the second outbreak in December 2003 and January 2004 [5,31], suggested that the animal reservoir for SARS CoV had contact with humans, directly or indirectly, in multiple locations. Initial studies in humans and animals in wild-animal markets in Guangdong Province suggested that animals in these markets might be the source of human infection (Table 3) [32]. However, subsequent studies showed that SARS infection in animals before arrival in the markets was uncommon and these animals were therefore not the original reservoir of the outbreak virus.

Two studies published in 2005 identified bats as the likely reservoir for SARS CoV [33,34]. In these studies, horseshoe bats from China were shown to have a high rate of antibodies that reacted against SARS CoV antigens or a high level of SARS CoV-like RNA in faecal specimens (Table 4). The sequences of the amplified RNA were closely related but not identical to SARS CoV, with ~8% differences from SARS CoV in multiple genes. This level of difference was too great for the RNA to be from the parent to the outbreak virus. However, the presence of multiple SARS CoV-like viruses, the inability to detect SARS CoV-like viruses in other species of wild-living animals and the detection of a wide range of other coronaviruses in bats suggests that they are a rich source of coronaviruses and the most likely animal reservoir for the SARS outbreak virus [35–37].

Analysis of the RNA sequences of outbreak isolates over time suggests that once introduced into humans the virus adapted. Sequential isolates from early but not later in the outbreak showed

Table 2

Vaccines for severe acute respiratory syndrome.

Vaccine type	Animal studies	Induction of neutralizing antibodies and/or protection	Human trials
Inactivated virus	Mice	+	+
Subunit or expressed protein	Mice	+	–
Viral or bacterial expression vectors (S or N protein)	Mice, ferrets, primates	+	–
DNA vaccine (S, N, M protein)	Mice, primates	+	+
Live attenuated virus	Hamsters	+	–

Adapted from Enjuanes et al. [23], Gillim-Ross et al. [24], Lin et al. [25] and Martin et al. [26].

Table 3

Severe acute respiratory syndrome coronavirus (SARS CoV) in a wild-animal market, Guangdong Province, China, 2003.

Animal	Positive by RT-PCR or isolation		SARS CoV antibody-positive
	Nasal	Rectal	Serum
Civet cat	6/6	5/6	3/4
Raccoon dog	0/1	1/1	1/1
Chinese ferret-badger	0/2	0/2	1/2
Hog-badger	0/3	0/3	0/1
Domestic cat	0/4	0/4	0/3
Chinese hamster, hare, muntjac and beaver	0/8	0/8	0/7

RT-PCR, reverse transcription polymerase chain reaction. Adapted from Guan et al. [32].

Table 4
Severe acute respiratory syndrome-like coronaviruses in bats and other mammals.

Source	Location	Antibodies in sera <i>N</i> (<i>N</i> tested)	PCR-positive faeces <i>N</i> (<i>N</i> tested)
Lau et al. [33]	Hong Kong		
<i>Rhinolophus sinicus</i> bats (59)		31 (37)	23 (59)
Other bats (68)		0	0
Other mammals (60 rodents, 20 monkeys)		0	0
Li et al. [34]	Three provinces of China		
<i>Rhinolophus pearsoni</i> , <i>R. pusillus</i> , <i>R. ferrumequinum</i> , macrotis bats		20 (63)	5 (67)
Other bats		2 (204)	0 (261)

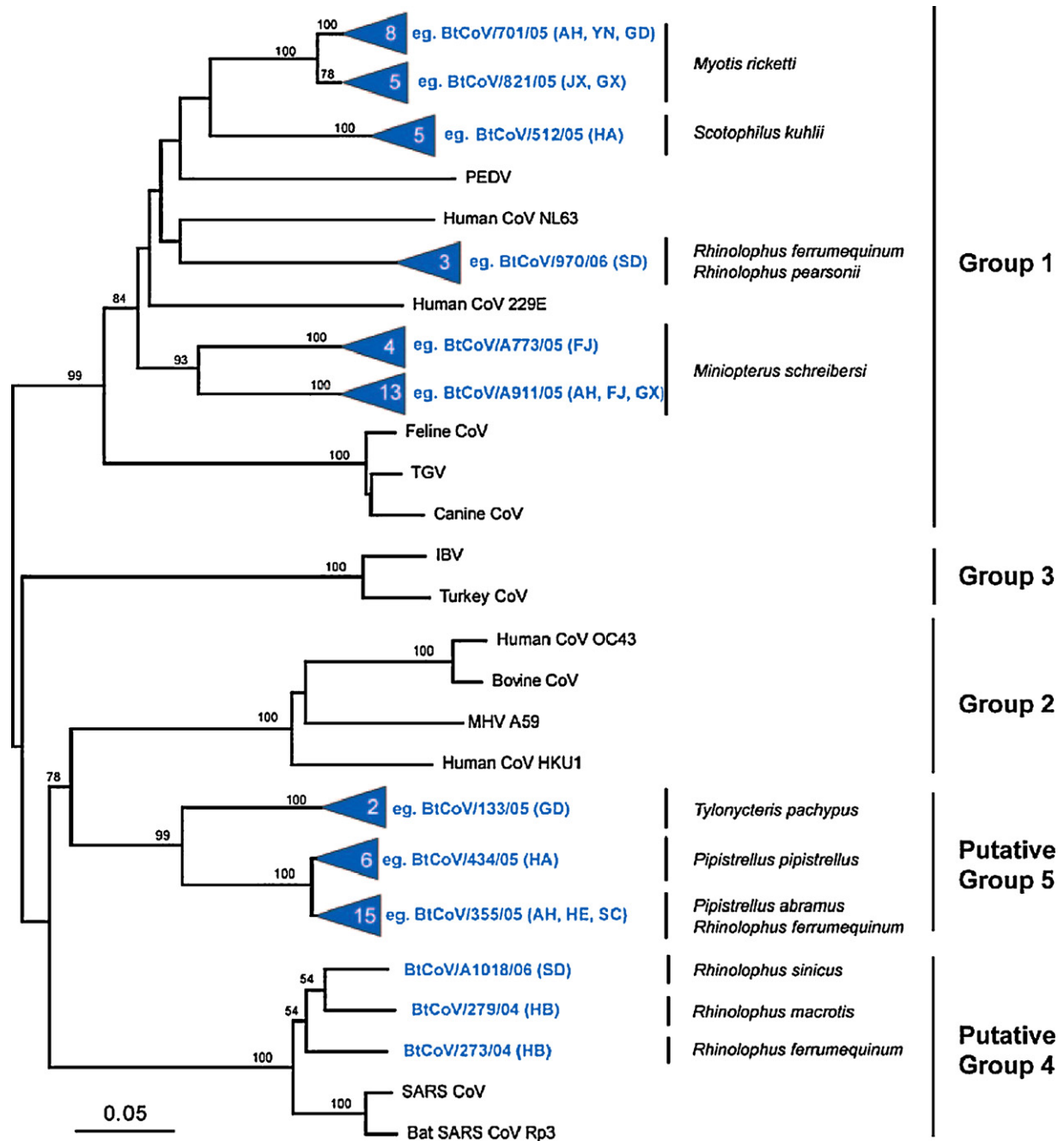


Fig. 1. Phylogenetic relationships of 64 coronaviruses isolated from bats in China. The tree was generated based on 440 nucleotides of the RNA-dependent RNA polymerase region by the neighbour-joining method in the MEGA programme. Numbers above branches indicate neighbour-joining bootstrap values (percent) calculated from 1000 bootstrap replicates. Terminal nodes containing bat coronaviruses isolated in this study are collapsed and represented by a triangle with the number of viruses indicated within. The tree was rooted to Breda virus (AY427798). Scale bar: 0.05 substitution per site. Abbreviated blue text in parentheses indicates provinces from where viruses were isolated. AH, Anhui; FJ, Fujian; GD, Guangdong; GX, Guangxi; HA, Hainan; HB, Hubei; HE, Henan; JX, Jiangxi; SC, Sichuan; SD, Shandong; YN, Yunnan. Adapted from Tang et al. [49]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

higher than expected ratios of non-synonymous to synonymous changes (Ka/Ks) in the S gene [38]. Presumably the strains early in the outbreak had the strongest positive selection pressures and had more advantageous amino acid changes available to them. It is not surprising that the S gene would be involved in adaptation to human infection. The S gene encodes the spike protein that binds to the cellular receptor and facilitates viral infection of the cell, and has been associated with pathogenesis of disease [39].

Changes in the receptor-binding domain of the S gene proved particularly interesting [40]. The S protein from a globally spread virus was found to bind more efficiently to the human angiotensin-converting enzyme 2 (ACE2) molecule than a virus from a civet cat and a virus from the cluster of four cases in December 2003 and January 2004 in Guangdong Province, China. The investigators identified critical amino acids in the receptor-binding domain of the S protein of the globally spread virus that were associated with increased binding efficiency to the human ACE2 molecule. They also identified amino acids in the ACE2 molecule associated with S protein-binding efficiency. The importance of the receptor-binding domain for adaptation to human infection is supported by in vitro studies showing changes in this region in viruses selected for improved replication in human cells [41,42].

As already mentioned, the SARS outbreak led to a wide range of very productive research efforts directed toward understanding the molecular biology of the infection and the pathogenesis of the disease [43,44]. The rapid development of a SARS CoV reverse genetics system has proven very useful [29]. It has not only provided a tool with which to explore the molecular virology of SARS CoV but also a means to develop candidate live-virus vaccines and generate virus strains that could not otherwise be isolated [45–48].

One concern about developing a live SARS CoV vaccine is the ability of strains of coronavirus to recombine with each other and possibly replace attenuated parts of the genome with non-attenuated genome, resulting in a pathogenic virus. Using reverse genetics, a creative approach has been developed that should eliminate the risk of recombination between coronavirus strains [47]. In this system, transcription regulatory sequences (TRSs) upstream of each gene are generated that are distinct from known coronavirus TRSs, and any recombinant virus would have different TRSs within its genome and be non-viable.

Before and since the SARS outbreak there have been continuing improvements in our ability to detect novel pathogens. At CDC the virus was isolated, characterised as a coronavirus by electron microscopy and shown to be a coronavirus distinct from the known human coronaviruses by using three PCR assays: one for 229e, one for OC43 and one for any member of the coronavirus genus [12]. Broadly reactive PCR assays have subsequently been used to detect a wide variety of coronaviruses in bat faecal specimens [35–37]. Sequence studies of the amplified genome have shown a wide range of coronaviruses representing the previously identified group I and II viruses, including a variety of bat SARS CoV-like viruses, none of which, as noted above, is the parent to the outbreak viruses. Some of these novel bat coronaviruses are sufficiently different from other group I and II viruses that they may ultimately be placed into putative new groups IV and V [49] (Fig. 1).

Interestingly, the pattern of bat coronavirus detection suggests that some may be specific to a bat species, some to a given location or region and some present in different species and locations. More recently a number of novel viruses have been detected in bats, including paramyxoviruses [50], polyomaviruses [51], astroviruses [52] and adenoviruses [53]. We have used pan Paramyxoviridae, pan Adenoviridae and pan Polyomaviridae PCR assays to detect novel members of these families in faecal specimens from bats (publication in preparation). Bats appear to be a rich source of novel viruses. It is, however, not clear which, if any, of these novel viruses present a risk to humans or other animals.

Various other molecular tools have been developed and applied to samples from humans, and a number of novel viruses have been detected, including novel coronaviruses [54–56], parvoviruses [57,58], polyomaviruses [59,60], astroviruses [61] and rhinoviruses [62]. These tools will continue to improve and likely identify new pathogens, some associated with disease and others of unknown significance as human pathogens. Identifying diseases that might be associated with a novel pathogen and then establishing a causal relationship between infection and the disease can be as challenging or more challenging than identifying the pathogen.

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