Figure 1. The Host Distribution of RNA Viral Families in Vertebrates. The host distribution of RNA viral families in vertebrates is based on the 10th report of the International Committee on Taxonomy of Viruses (ICTV) (http://ictv.global/report). The viruses known previously and identified by Shi *et al*. [3] are labelled by green and orange circles, respectively. Abbreviations: dsRNA viruses, double-stranded RNA viruses; (−)ssRNA viruses, negative-sense single-stranded RNA viruses; (+)ssRNA viruses, positive-sense single-stranded RNA viruses.

DNA viruses to which human hepatitis B virus belongs. Until recently, hepadnaviruses were thought to only infect mammals and birds. Recent paleovirological and meta-transcriptomic analyses reveal their presence in fish, amphibians, and reptiles [9,10]. Further sampling of more species worldwide might reveal the complete picture for the diversity, origin, and evolution of viruses in vertebrates.

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Forum

Bat-Origin Coronaviruses Expand Their Host Range to Pigs

Liang Wang,[1] Shuo Su,[2] Yuhai Bi,[1,3] Gary Wong,[3] and George F. Gao[1,3,4,*]

Infections with bat-origin coronaviruses have caused severe illness in humans by 'host jump'. Recently, novel bat-origin coronaviruses were found in pigs. The large number of mutations on the receptor-binding domain allowed the viruses to infect the new host, posing a potential threat to both agriculture and public health.

Coronavirus Transmission in Wildlife

The host range expansion of coronaviruses (CoVs) from wildlife to humans via genetic recombination and/or mutations on the receptor-binding domain in the spike (S) gene is well established and results in several diseases with high fatality rates, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [1,2]. Despite their bat origins, SARS-CoV and MERS-CoV have infected humans via an intermediate host, rather than through a direct infection from bats. Palm civets have been suggested, but not definitively confirmed, to be an intermediate host for SARS-CoV, and dromedary camels are confirmed to be the intermediate host for MERS-CoV [3]. Currently, the number of known intermediate hosts involved in the transmission of bat-origin coronaviruses to humans is limited. Compared to many other species, pigs are in frequent contact with both humans and other animals such as cats, dogs, horses, and aquatic birds, and theoretically possess a greater chance to promote cross-species viral transmission. For instance, pigs are susceptible to infection with human and avian influenza A viruses (IAVs), such as H1N1 and H3N2, which then reassort to infect humans [4]. Thus, pigs are regarded as mixing vessels for IAVs. However, pigs were not known to be susceptible to bat-origin coronaviruses until recently, when two independent groups reported the detection of novel swine enteric alphacoronaviruses (SeACoVs) distinct from known swine coronaviruses (with one group successfully isolating live virus). The SeACoVs were found to be phylogenetically close to bat coronavirus HKU2 [5,6]. This suggests that bat-origin coronaviruses may have 'jumped' the species barrier to infect pigs.

The Emergence of Bat-Origin Coronaviruses Infecting Pigs

In February 2017, outbreaks of severe watery diarrhea of suckling piglets were reported in commercial pig farms in Guangdong Province, China. The disease had high case fatality rates (CFRs, over 35% for <10-day-old suckling piglets), and none of the animals were positive for known pathogens responsible for porcine diarrhea [6]. Instead, two genomes of novel SeACoV were detected in the ill piglets by two independent groups, and preliminary analysis showed that the SeACoVs possibly originated from bat HKU coronaviruses [5,6]. It is currently unclear whether SeACoVs had been circulating undetected in pigs, if the viruses

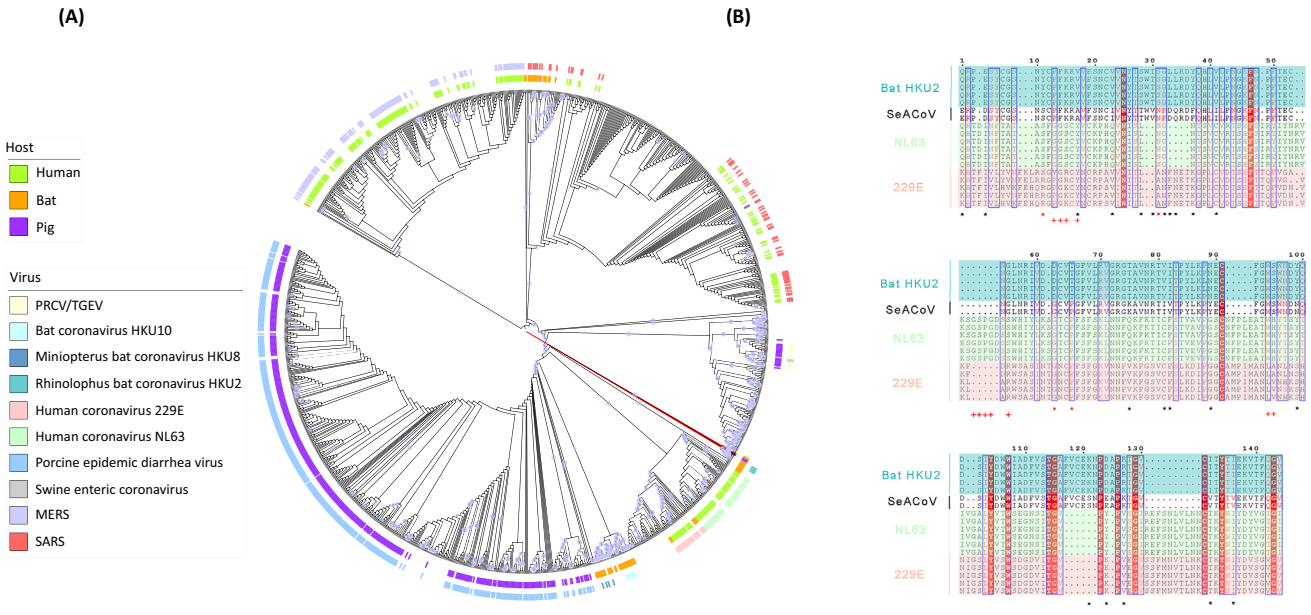

Figure 1. Phylogenetic Overview of Swine Enteric Alphacoronavirus (SeACoV). (A) The phylogenetic relationship among SeACoV, other alphacoronaviruses, SARS-CoV, and MERS-CoV. Conserved genomic regions were used for phylogenetic reconstruction by using the maximum likelihood method under the GTR + G model. The extended majority rules (autoMRE) bootstrapping convergence criterion was applied here to determine the most suitable number of replicates. Bootstrapping convergence was considered to be reached if over 99% permutations have low Weighted Robinson-Foulds distances (<3%). The phylogenetic tree was visualized using SARS-CoV and MERS-CoV as the outgroup. Only bootstrap values >90% were visualized as a purple circle in the middle of the branch. The size of the circle is proportional to the bootstrap value. (B) Alignment of the receptor-binding domain in the S gene. Residues in direct contact with the human receptor for NL63 were indicated by a '+' sign. Twenty-five substitutions between bat and pig were indicated by a star. If a substitution in pigs occurred in 229E or NL63, it was marked by a red star. Sequences from bat HKU, 299E, and NL63 were indicated by a different color, consistent with those from the phylogenetic tree.

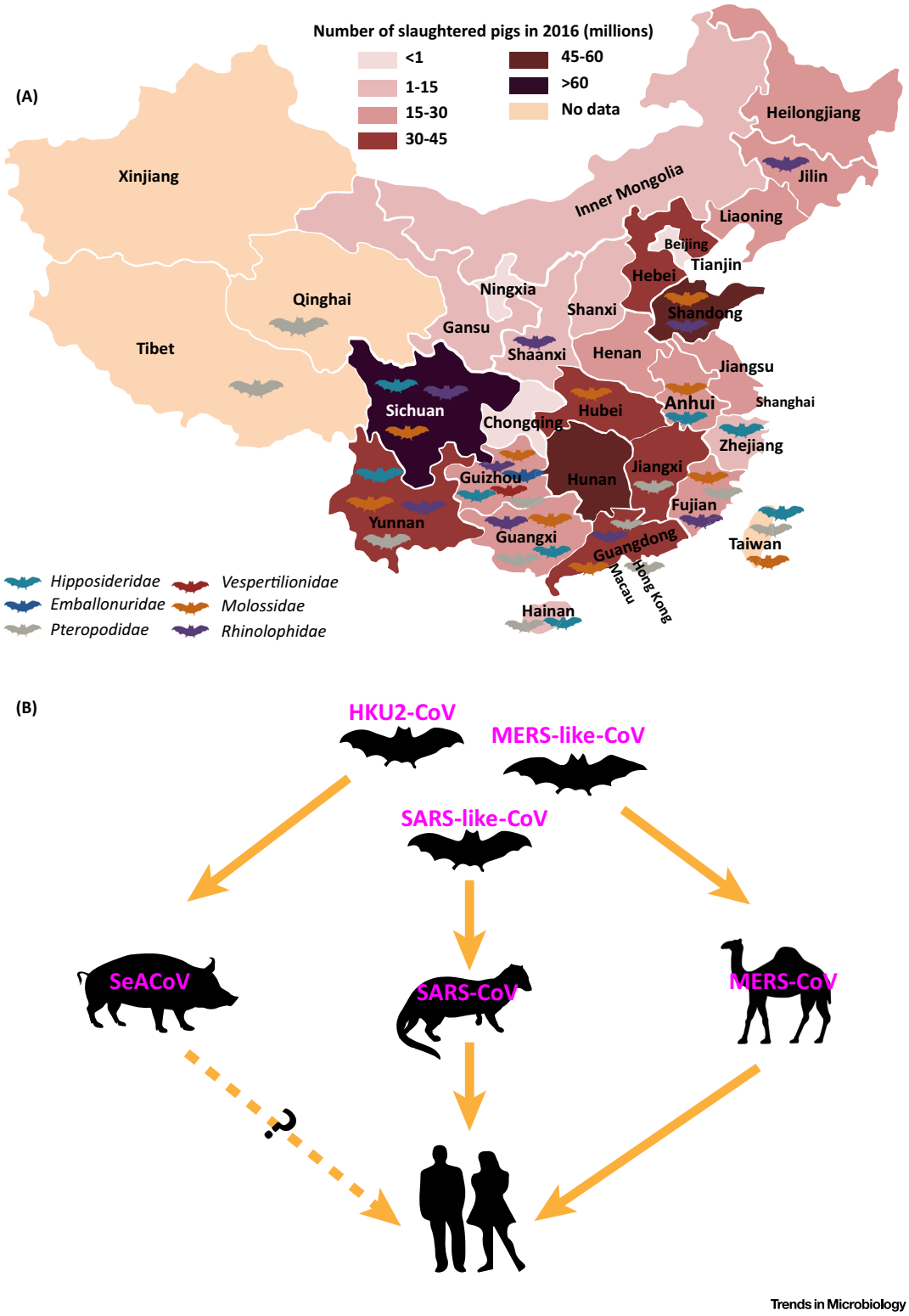

Figure 2. Distribution of Pigs and Bats in China (Map without the Islands in the South China Sea) and Cross-Species Transmission of Bat-Origin Coronaviruses. (A) Pig slaughterhouse densities and the species distribution of bats in China. (B) Suspected routes of cross-species transmission of bat-origin coronaviruses. The dashed line indicates potential, but unknown, transmission from pig to human.

had originated from cross-species transmission, or if the SeACoVs were the result of viral recombination. To understand the molecular origin and evolution of SeACoVs, we performed a detailed phylogenetic analysis at the genomic level by using all known alphacoronaviruses and bat-origin coronaviruses which are known to cause severe diseases, such as SARS and MERS. A total of 224, 312, and 778 complete genomes from MERS-CoV, SARS-CoV, and alphacoronaviruses, respectively, were used. Phylogeny was reconstructed using conserved regions in genomes by the maximum likelihood method with 300 replicates (Figure 1A). Consistent with previous studies, phylogenetic analysis shows that SeACoVs were closely related to the *Rhinolophus* bat coronavirus HKU2 isolated in southern China (Figure 1A). SeACoV was found to share a common ancestor with human coronavirus 229E/NL63, but these viruses are distant from other known swine alphacoronaviruses, indicating their different origins. Further analysis on the S gene, which determines virus attachment, host cell entry, and 'host jump' of coronaviruses [7], showed that domain 0 in the S1 subunit has structural similarity to that of NL63. The rest of the domain on the S1 subunit was similar to that of murine hepatitis coronavirus (betaCov) [6]. There were 11 residues of the receptor-binding domain (RBD, also called C-terminal domain, CTD) of SeACoV directly in contact with its receptor (angiotensin-converting enzyme 2) that were mutated or deleted [7] (Figure 1B). Seven mutations (a deletion of four amino acids, and three substitutions) among the 11 sites in SeACoV were also found in 229E/NL63, indicating similarities in the receptor-binding mechanism between SeACoV and 229E/NL63. Therefore, SeACoV may be able to infect humans and should be closely monitored. Infection of SeACoV in Vero cells (a primate cell line) will provide experimental evidence to support this possibility [6].

Bat-Origin Coronaviruses in Pigs May Transmit to Humans

Another requirement for the host range expansion of viruses is physical contact between different host species. The novel SeACoVs were both detected in Guangdong Province, whereas closely related bat coronaviruses were also isolated from Guangdong or Hong Kong (Figure 1A). In Guangdong Province, the high density of pig slaughterhouses and the wide distribution of bat species (Figure 2A) promote the possibility of viral cross-species transmission. Additionally, bats have a wide geographical distribution in southern China, with extensive species diversity, unique behaviors (characteristic flight patterns, diet, roosting, and mobility) [8], and constant interactions with both pigs and humans (Figure 2A).

Could Pigs Be Mixing Vessels for Coronaviruses?

Pigs are well established as mixing vessels and as intermediate hosts for IAVs, and coronaviruses have already been shown to possess potential for recombination in animals [9]. Given that pigs are in frequent contact with human and multiple wildlife species, and that pork is one of the most commonly consumed meats in non-Muslim countries, it is important to assess whether pigs could be mixing vessels for the emergence of novel coronaviruses with high agricultural impact and risks to public health. It has already been reported that pigs are susceptible to infection with human SARS-CoV [10] and MERS-CoV [11]. Additionally, the CD26 receptor sequence alignment of pigs and humans shows 94.5% similarity, which is sufficient for potential cross-species transmission [7]. In southern China, the unique climate, the high density of domestic as well as wild pigs, and extensive bat distribution, together with bats carrying large numbers of recombinant novel coronaviruses [12], could lead to the emergence of more novel coronaviruses in the future.

Concluding Remarks

The isolation of SeACoV from ill piglets expands our knowledge of the host range of bat-origin coronaviruses, and potentially poses a threat to public health. Despite considerable progress in characterizing cross-species transmission for coronaviruses, several areas need to be addressed, including: (i) whether other unknown coronaviruses are circulating in pigs; (ii) whether pigs are mixing vessels for coronaviruses; (iii) whether SeACoV infects humans and causes severe disease; and (iv) whether SeACoV vaccines should also be developed to control the spread of this virus in pigs. In-depth epidemiological investigation and comprehensive analysis of these novel coronaviruses should be performed to answer these urgent questions.

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