A One Health Approach to Studying the Differences in the Evolutionary Dynamics of MERS and SARS Coronaviruses

Xu Zhang

South China Agricultural University

Jamal S. M. Sabir

King Abdulaziz University

Xuejuan Shen

South China Agricultural University

Zhiqing Pu

South China Agricultural University

Nahid H. Hajrah

King Abdulaziz University

Mohamed M. M. Ahmed

King Abdulaziz University

Tingting Luo

South China Agricultural University

Meshaal J. Sabir

King Abdulaziz University

Onaizan Godian Al-Zogabi

Al-Bukairiah General Hospital

Junbin Pan

South China Agricultural University

David M. Irwin

University of Toronto

Yongyi Shen (✉ shenyy@scau.edu.cn)

South China Agricultural University

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Abstract

Background

SARS-CoV and MERS-CoV are two coronaviruses that received great attention due to their high pathogenicity and mortality rates in human populations. While SARS was controlled, MERS continues to be a global public health concern. To examine differences in the epidemic patterns of these two viruses, we collected all available sequences to compare the different evolutionary characteristics of SARS-CoV and MERS-CoV. Notably, almost all of the human infection cases occurred in the Middle East, and cases that occurred outside of the Middle East involved travelers from this region, while African infections have so far not been reported. It is not clear that genetic differences between Africans and Arabs lead to differences in susceptibility.

Results

In this study, we compared their evolutionary dynamics to provide a One Health perspective of their different results of disease control. The phylogenetic network of SARS-CoVs showed that human isolates gathered into a “super-spreader” cluster, and were distinct from civet isolates. In contrast, dromedary camel- and human-isolated MERS-CoVs were clustered together. Thus, most clades of MERS-CoV can infect humans, and MERS-CoVs seem easier to spill over from animal-to-human interface. Although MERS-CoVs are endemic to dromedary camels in both the Middle East and Africa, all human infections are linked to the Middle East. The nucleotide sequences of the MERS-CoV receptor gene–dipeptidyl peptidase 4 (DPP4) from 30 Egyptians, 36 Sudanese, and 34 Saudi Arabians showed little difference.

Conclusions

Our study reveals the reason why MERS-CoV is not easily controlled. Analysis of genetic differences between Africans and Arabs suggest that human population differences in DPP4 might not be the reason for their different MERS prevalence, raising the possibility that other reasons, such as poorer disease surveillance in Africa, might explain these observations.

Background

Coronaviruses (CoVs), a large family of single-stranded RNA viruses, cause respiratory, hepatic, gastrointestinal, and neurologic diseases of varying severity in a wide range of animal species, including humans [1]. Although some CoVs have been known for decades, the potential threat of these viruses to global health security was not fully realized until the outbreaks of Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) [2–5]. In 2017, a novel HKU2-related bat coronavirus, swine acute diarrhea syndrome coronavirus (SADS-CoV), caused the death of 24,693 piglets [6, 7]. Especially, the outbreak of a new SARS-like Coronavirus (SARS-CoV-2) in Wuhan at the end of 2019 [8, 9] further raises concern about these coronaviruses.

SARS-CoV first emerged in November 2002 in Guangdong province of Southern China and then rapidly spread to 29 countries and regions, infecting over 8,000 individuals with a death toll of nearly 800 [2]. The ultimate reservoir host for SARS-CoVs appears to be horseshoe bats (*Rhinolophus* sp.) [10], where the palm civet (*Paguma larvata*), sold for food at markets in southern China, act as an important intermediate amplifying host [11, 12]. Fortunately, since the control of this SARS outbreak, it has not occurred again since 2005.

Ten years after SARS, MERS emerged in 2012 and continued to spread over the next few years [13]. While dromedary camels (*Camelus dromedarius*) are thought to be the intermediate host for MERS-CoV, bats are widely considered to be the ultimate source of this virus [14–16]. SARS-CoV and MERS-CoV share several important common features, such as, they both have an intermediate host, bats are their natural reservoir. While SARS was controlled, MERS continues to be a global public health concern. To examine differences in the epidemic patterns of these two viruses, here in this study, we collected all available sequences to compare the different evolutionary characteristics of SARS-CoV and MERS-CoV.

The MERS-CoV is endemic in dromedary camels in the Middle East and Africa. However, all of the human infection cases occurred in the Middle East, and cases that occurred outside of the Middle East involved travelers from this region [16], while African infections have so far not been reported. This raises the question whether genetic differences between Africans and Arabians lead to differences in their susceptibility? Since the cell surface receptor that mediates cell entry for the MERS-CoV is dipeptidyl peptidase 4 (DPP4) [17], for this study we further sequenced and compared DPP4 alleles from 30 Egyptians, 36 Sudanese, and 34 Saudi Arabians.

Results

The evolutionary characteristics of SARS-CoVs

Of the SARS-CoV gene examined, the S gene in bats has the highest genetic diversity. The nucleotide diversity of all genes (ORF1ab, S, E, M, and N) from human and carnivore isolates are significantly lower than those of the bat isolates (Figure 1A). Except for the E and M genes, the genetic diversity of the remaining genes (ORF1ab, S, and N) isolated from carnivores are higher than those from human isolates.

To test for neutrality in the evolution of the SARS-CoV sequences, we conducted Tajima's D and Fu & Li's D* tests. Significant negative Tajima's D and Fu & Li's D* values are indications of deviation from neutrality that suggest selective sweeps and/or population expansions. Values for Tajima's D and Fu & Li's D* calculated from human isolated S, M, and N genes were significantly less than zero. The dN/dS values for S genes from carnivores and the human M genes were greater than 1. Site model tests using PAML [21] revealed that the human and carnivore S genes had undergone positive selection, with a series of positive selected amino acid sites detected (Table 1).

The median-joining network based on the S gene of SARS-CoVs showed that the human-isolated SARS-CoV sequences were isolated mainly in 2003 and were particularly concentrated (Figure 2). 55 of 174 human SARS-CoV sequences in the analysis were related to the central haplotype (the biggest blue cycle in Figure 2) and 109 human SARS-CoV sequences were clustered with this haplotype. Those strains that cause super transmission (HZS2-C, HZS2-D, Sin2500, Sin2677, Sin2748, CUHK-Su10, CUHK-AG01, CUHK-AG03, CUHK-AG03) were all in this haplotype. Therefore, most human infection cases were due to this cluster. Bat-isolated SARS-CoVs showed much more divergence than the human isolates, while the carnivore SARS-CoV sequences are very close to the human isolates.

The evolutionary characteristics of MERS-CoV

Similar to SARS-CoVs, the nucleotide diversity of all genes from the human and dromedary camel isolates are significantly lower than those from the bat isolates (Figure 1B). The nucleotide diversity of the human isolate E genes is much lower than that from the dromedary camel isolates, while the other genes had similar nucleotide diversity in these two groups. The E gene is the shortest gene of MERS-CoV and is only 249 bp. Therefore, any mutations may lead to the sharp increase of nucleotide diversity. It is possible that the significant difference of nucleotide diversity of E gene between human- and dromedary camel isolates may not be reliable. Values for Fu & Li's D* calculated for all genes from the human and dromedary camel isolates were significantly less than zero. The dN/dS value for the E gene from dromedary camel was greater than 1. Site models revealed that the S gene from human and carnivore isolated MERS-CoV underwent positive selection, with a series of positive selected amino acid sites detected (Table 1).

A median-joining network was constructed using the 690 MERS-CoV S gene sequences (Figure 3). A total of 313 haplotypes were identified in these sequences. As shown in Figure 3, the dromedary camel MERS-CoVs from the Middle East was divided into multiple clusters, most of which were shared by dromedary camels and humans. The African MERS-CoVs formed a clear single cluster, with no clustering with human MERS-CoV sequences.

DPP4 did not show any difference between Arabs and Africans

DPP4 exon and partial intron sequences, in 14 fragments, were obtained from 30 Egyptians, 36 Sudanese, and 34 Saudi Arabian and were sequenced. We identified 4 exon haplotypes and 6 intron haplotypes in these sequences. The central haplotype for both the exon and intron sequences were shared by the Egyptians, Sudanese, and Saudi Arabians (Figure 4). The genetic diversity of the exons and introns were 0.0~0.3% and 0.0~0.1%, respectively, indicating that there was a very limited difference in the DPP4 sequences between Arabs and Africans.

Discussion

Bats are the reservoir hosts for both SARS-CoV and MERS-CoV, which then use palm civets and dromedary camels as intermediary host before dissemination to humans [23, 24]. Although they both have the same reservoir host, and use intermediary hosts before dissemination to humans, SARS was controlled quickly while MERS continues to have sporadic human infections.

Genetic diversity of all genes from human- and carnivore-isolated SARS-CoVs was much lower than that in the bat-isolated SARS-CoVs (Figure 1A). It seems that only a group of the viruses could be transmitted through carnivores, and then able to infect humans. This suggests that most SARS-CoVs might be highly incompatible with humans and palm civets. Tajima's D and Fu & Li's D* values revealed that population expansion occurred in the human isolates. This is in accord with the rapid expansion of human infections. Although a total of 8,096 human infection cases were reported, they were not scattered in the network, but rather occurred around a "super-spreader" strain. Only the "super-spreader" can spread, further illustrating that most SARS-CoVs were incompatible with human transmission. Although bats were the reservoir host for SARS-CoV [25], carnivore SARS-CoVs were much closer to the human isolates. This indicates that without the intermediary host of carnivores, bat-derived SARS-CoVs likely cannot directly infect humans. Therefore, although SARS-CoVs are still circulating in bats [26], management of carnivores to prevent infection allowed quick control of SARS. However, as bats remain as a reservoirs for coronavirus, cross-species transmission of viruses from bats still poses a threat to human and animal health [6].

Compared with SARS, MERS has been a much longer epidemic. Specific antibodies against MERS-CoV have been detected in the serum of dromedary camels from Africa and Arabian Peninsula collected since 1992 [27, 28], suggesting that MERS-CoV has had a long co-evolutionary time to develop a bat-dromedary camel-human transmission route. The genetic diversity of all genes, except the E gene, were similar from both human- and dromedary camel-isolated MERS-CoV (Figure 1B). In addition, most clades shared both dromedary camel and human isolates (Figure 3), which is consistent with previous studies[29-33], indicating that most clades of MERS-CoV can infect humans. This suggests that MERS-CoV has a better ability to spread from dromedary camels to humans than SARS-CoV. Moreover, since the carnivore-intermediate amplifying host of SARS-CoV was easy to control by closing wild animal markets, the dromedary camel, as an important livestock species in Arab countries playing key roles in transportation, food, fabric (wool), and entertainment, could not be so easily controlled [34]. It is likely impossible to eliminate all dromedary camels from Arab countries. Serological surveys have also showed that there is a high prevalence of MERS-CoV-neutralizing antibodies in dromedary camels [35, 36], therefore, unlike SARS-CoV, the spread of MERS-CoV has a stable natural reservoir and thus, its epidemic may last longer. Eradication of MERS-CoVs from the dromedary camels is the primary condition needed for the control of this disease in the Arabian Peninsula.

To study the genetic basis of the host jumps, a series of adaptive evolutionary analyses were conducted. The S gene from SARS-CoVs in carnivore and human isolates, and the S gene of MERS-CoVs in dromedary camels showed evidence of experiencing positive selection (Table 1). Although SARS-Cov and MERS-Cov use different receptors [37-39], their S proteins form spikes on the surface of CoVs particles which are involved in receptor recognition and are important for viral entry into host cells, thus can alter viral tropism [2, 40]. Adaptive evolution of the S protein should help viruses recognize receptors in the new hosts, a conclusion supported by previous studies [12, 41, 42].

Although MERS-CoV is endemic to dromedaries in Africa [33, 43-48], zoonotic infections have not been reported from this area. The major determinant of viral species-tropism is host cell entry level, which is mediated by the MERS-CoV spike protein binding to dipeptidyl peptidase 4 (DPP4) on host cells [38, 39]. Therefore, we compared sequences of DPP4 alleles from Arabs and North Africans to determine whether difference in the DPP4 sequences might explain differences in susceptibilities. However, no differences were found in the DPP4 alleles found in these two groups of people (Figure 4). The difference in reported susceptibility might be due to the failure to detect cases of human infection with MERS-CoV in Africa due to poor disease surveillance in Africans.

Conclusions

In summary, our study compares the different evolutionary characteristics between SARS-CoV and MERS-CoV. Most SARS-CoVs are incompatible with humans, with most human infections by SARS-CoVs being mainly caused by “super-spreaders”. The intermediate host (civets) for the SARS-CoV epidemic is an exotic animal, which was easy to control to reduce new human infections. In contrast, all clades of MERS-CoVs appear to be able to infect humans. In addition, the intermediate host (dromedary camel) of the MERS-CoV epidemic is an important livestock species for Arab countries. What is more, a high percentage of dromedary camels have been exposed to MERS-CoV, which could potentially lead to human infection. Both of these factors lead to difficulties in disease control. It is important to note that DPP4 protein sequences do not show differences between Arabs and Africans; thus, there is no genetic evidence that supports the proposal that Africans are less likely to be infected by MERS-CoV. These findings indicate that African countries should strengthen their monitoring of MERS-CoV, with an expanded scope of surveillance, especially for those with frequent contact with dromedary camels, to determine the true extent of MERS-CoV infections in Africa.

Methods

Source of virus genomes and sequence treatment

All published genomic sequences for MERS-CoV (total 690) and SARS-CoV (total 274) were obtained from GenBank (Supplementary Table 1). SARS-CoV sequences were separated into human, bat, and carnivore groups, based on the host source of the virus. MERS-CoVs were similarly separated into human, dromedary camel, and bat groups. The ORF1ab, S (spike protein), E (envelope protein), M (matrix protein), and N (nucleocapsid protein) gene sequences were extracted from each genome and separately aligned using MAFFT v7.245 [18]. Nucleotide diversity (π) of these genes in each population was calculated using Mega 6.05 [19].

We analyzed the selective pressure acting on the protein-coding S, E, M, and N genes. Nonsynonymous to synonymous substitution ratios (dN/dS) were calculated using Mega 6.05 [19]. To quantify levels of selection and population expansion, Tajima's D and Fu & Li' D* tests were performed using DnaSP 5.10 [20]. Models M8 (beta&w) and M8a (beta&w_s=1) from PAML 4.9 [21] were applied to identify sites that potentially experienced positive selection, where the null model M8a was compared to model M8 that allows positive selection. When likelihood ratio tests for these models were significant ($P < 0.01$), amino acid residues that showed posterior probabilities (PP) >95% under a Bayes Empirical Bayes (BEB) analysis [22] were regarded as being under positive selection.

Median-joint networks for the S (spike) gene sequences from MERS-CoV and SARS-CoV genomes were separately constructed using Network 5.0 (<http://www.fluxus-engineering.com/sharennet.htm>).

DNA extraction, PCR, and sequencing DPP4 gene

Blood samples from 30 Egyptians, 36 Sudanese, and 34 Saudi Arabians were collected. The study was approved by the research ethics committee of King Abdulaziz University. Genomic DNA was extracted by the standard phenol/chloroform method. To amplify the DPP4 gene, 14 pairs of primers were designed (Supplementary Table 2). PCR was performed in a 50

μL volume containing 25 μL of 2×PrimeSTAR™ GC Buffer, 0.25mM dNTPs, 0.2μM each primer, 1.5 U PrimeSTAR™ HS DNA Polymerase (TaKaRa Biosystems, Dalian, China), and 100ng genomic DNA. The PCR amplification profile was 95°C for 5 min, followed by 35 cycles of 98°C for 10s, 53°C for 15s, and 72°C for 1 min, with a final extension for 10 min at 72°C. PCR products were visualized on 1.0% agarose gels, purified on spin columns (Watson Biotechnologies Inc., Shanghai, China), and directly sequenced for both strands using a BigDye™ Terminator Cycle Sequence Kit (ABI Applied Biosystems 3730, USA) according to the manufacturer's manual. DNA sequences were edited using DNASTar software (DNASTAR Inc., Madison, WI, USA), with the newly determined sequences deposited into GenBank (Accession numbers: MK670823-MK670922).

Abbreviations

MERS: Middle East Respiratory Syndrome

SARS: Severe Acute Respiratory Syndrome

CoVs: Coronaviruses

DPP4: dipeptidyl peptidase 4

Declarations

Ethics approval and consent to participate

The verbal informed consent have been obtained from all participants. The Research Ethics Committees of the King Abdulaziz University [References No:239-14] approved this procedure.

Consent for publication

Not applicable.

Availability of data and materials

The data used in this study can be obtained in NCBI.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YS designed the experiments; XZ and XS performed the research; JSMS, NHH, MMMA, MJS, and OGA conducted surveillance; ZP and TL did the phylogenetic networks; JP provided a technical help; DMI edited the paper; YS, and XZ wrote the paper. All authors have read and approved the manuscript, and ensure that this is the case.

Acknowledgements

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Tables

Table 1: Genetic diversity, neutrality, and selective pressure analyses of the S, E, M, and N genes from SARS-CoV and MERS-CoV.

	Gene	Nucleotide diversity (π)	Neutrality analyses		Selective pressure analyses				M8 vs. M8a	
			Tajima's D	Fu and Li's D*	dN	dS	dN/dS		<i>P</i> value	Positive selected sites (PP>0.95)
SARS	Human S	0.00110	-2.41035**	-5.42954**	0.001	0.00119	0.84		<0.001	2 F,5 L,12 S,14 S,20 T,49 S,75 T,77 G,78 N,138 F,139 A,144 M,147 Q,239 S,244 I,311 G,344 K,345 I,350 A,353 S,360 F,384 A,425 T,436 Y,437 N,442 Y,463 D,472 L,480 D,487 T,501 F,577 S,600 D,605 D,608 T,609 A,613 D,623 S,652 I,665 L,666 L,684 D,743 T,765 A,778 Y,794 P,856 T,860 V,861 S,863 T,882 M,969 V,1001 R,1008 A,1083 S,1107 N,1132 E,1148 L,1163 K,1169 N,1179 L,1183 Q,1208 A,1223 C,1247 L

Bat S	0.18097	-0.32861	0.36802	0.162	0.322	0.50310	0.997	-
Carnivora S	0.00225	-1.43353	-1.56984	0.00248	0.00159	1.5597	<0.001	77 D,91 A,108 N,113 S,139 A,147 Q,194 L,227 K,239 L,243 D,244 T,261 K,294 E,336 S,344 R,360 S,440 Y,460 F,462 P,472 L,479 K,480 D,558 F,577 S,607 P,609 A,613 D,632 Q,644 T,647 E,650 I,665 S,701 L,703 S,743 A,754 V,765 A,778 D,821 E,839 G,844 P,894 A,900 E,1052 S,1080 N,1163 E,1219 M
Human E	0.00141	-1.85459*	-1.24525	0.001	0.003	0.33333	0.128	-
Bat E	0.02887	-1.35625	-0.73142	0.012	0.088	0.13636	0.075	-
Carnivora E	-	-	-	0	0	-	0.992	-
Human M	0.00152	-1.95635*	-2.78659*	0.002	0.001	2	0.114	-
Bat M	0.06488	-1.25051	-1.06230	0.019	0.272	0.06985	0.998	-
Carnivora M	0.00073	-1.34917	-1.12152	0	0.002	0	0.952	-
Human N	0.00050	-2.26582**	-4.28718**	0	0.001	0	0.372	-
Bat N	0.05267	-1.77307	-2.54335*	0.02	0.214	0.09345	0.357	-
Carnivora N	0.00307	-0.76062	0.04240	0.002	0.005	0.4	0.977	-

MERS	Human S	0.00301	-2.23565**	-8.11949**	0.00081	0.00993	0.08157	0.38	26 V, 459 S, 465 S, 612 S, 723 F, 1193 A, 1224 G
	Camel S	0.00387	-1.89972*	-5.5249**	0.00142	0.01155	0.12294	<0.001	
	Bat S	0.29177	-0.14059	0.43849	0.26994	0.35995	0.74994	1	
	Human E	0.00030	-2.00740*	-5.18345**	0.00016	0.00077	0.20779	0.99	
	Camel E	0.00185	-1.23028	-2.78622*	0.00225	0.00065	3.46154	-	
	Bat E	0.13870	0.09547	0.33394	0.06394	0.38241	0.16720	1	
	Human M	0.00316	-1.52979	-4.75400**	0.00243	0.00550	0.44182	0.078	
	Camel M	0.00337	-1.50885	-3.72290**	0.00221	0.00707	0.31259	0.42	
	Bat M	0.19280	0.03247	0.63456	0.07335	0.56393	0.13007	0.99	
	Human N	0.00248	-2.17290**	-4.66093**	0.00189	0.00436	0.43349	0.15	
	Camel N	0.00363	-1.98168*	-4.7562**	0.00139	0.01072	0.12966	0.88	
	Bat N	0.18046	-0.15684	0.41694	0.09412	0.45385	0.20738	0.95	

Note: * $P \leq 0.05$; ** $0.05 > P > 0.001$; *** $P \leq 0.001$

Figures

Figure 1

Nucleotide diversity of SARS-CoV and MERS-CoV genes. ORF1ab, S, E, M, and N genes from SARS-CoV and MERS-CoV genomes isolated from primary bat, intermediate carnivore (SARS-CoV) or camel (MERS-CoV), and human hosts. (A) SARS-CoVs. (B) MERS-CoVs.

Figure 2

Median-joint networks of the S (spike) gene sequences from SARS-CoV genomes. (A) Phylogenetic network marked for host information (human, bat, carnivore or other animals). (B) Phylogenetic network displaying epidemic year.

Figure 3

Median-joint networks of the S (spike) gene sequences from MERS-CoV genomes. (A) Phylogenetic network displaying geographical distribution of virus isolation (Middle East, South Korea, Africa, other countries) and host information (human, camel, or bat). (B) Phylogenetic network displaying epidemic year.

Figure 4

Median-joint networks of exon and intron regions of DPP4 gene. DPP-4 exon and partial intron sequence was isolated from 14 PCR amplifications. (A) Intron network. (B) Exon network.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.xlsx](#)
- [SupplementaryTable2.XLSX](#)