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Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia

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Outbreaks of Middle East respiratory syndrome (MERS) raise questions about the prevalence and evolution of the MERS coronavirus (CoV) in its animal reservoir. Our surveillance in Saudi Arabia in 2014 and 2015 showed that viruses of the MERS-CoV species and a human CoV 229E–related lineage co-circulated at high prevalence, with frequent co-infections in the upper respiratory tract of dromedary camels. Including a betacoronavirus 1 species, we found that dromedary camels share three CoV species with humans. Several MERS-CoV lineages were present in camels, including a recombinant lineage that has been dominant since December 2014 and that subsequently led to the human outbreaks in 2015. Camels therefore serve as an important reservoir for the maintenance and diversification of the MERS-CoVs and are the source of human infections with this virus.

Major outbreaks of Middle East respiratory syndrome (MERS) have been repeatedly reported in the Arabian Peninsula since 2012 and recently in South Korea (*1*–*3*), renewing concerns about potential changes in the mode of MERS coronavirus (CoV) transmission. Although increasing evidence suggests that dromedary camels are the most likely source of human infections (*4*–*14*), the prevalence and evolution of the MERS-CoV in this animal and the route of virus transmission to humans are not well defined, and little is known of other CoV species that may circulate in camels and how they might influence CoV ecology.

We conducted surveillance for CoVs in dromedary camels in Saudi Arabia, the country most affected by MERS, from May 2014 to April 2015. Initially, paired nasal and rectal swabs were collected from camels at slaughterhouses, farms, and wholesale markets in Jeddah and Riyadh. Because rectal swabs were negative for MERS-CoVs (tables S1 and S2), only nasal swabs were subsequently collected at these sites and in Taif (*15*). Of the 1309 camels tested, 25.3% were positive for CoV, as established by reverse transcription polymerase chain reaction (RT-PCR) and confirmed by Sanger sequencing. The majority of the CoV-positive camels came from wholesale markets (tables S1 and S2), where indigenous camels mixed with camels imported from Sudan and Somalia. Local camels had significantly higher positive rates for MERS-CoVs and other CoVs than did imported camels (Pearson's χ^2 test, $P < 0.05$; tables S1 and S2).

Three CoV species were detected in dromedary camels: MERS-CoV (betacoronavirus, group C); betacoronavirus 1 (betacoronavirus, group A); and human CoV 229E (alphacoronavirus) (fig. S1). Viruses from the latter two species are designated as camel β1-HKU23-CoVs and camelid α-CoVs, respectively. Although CoVs were detected almost year-round in these animals, a relatively higher prevalence of both MERS-CoV and camelid α-CoV was observed from December 2014 to April 2015 (tables S1 and S2). Juvenile camels (0.5 to 1 year old) had the highest levels of respiratory infections with both the MERS-CoV and camelid α-CoV, followed by calves under 6 months, both at about twice the rate observed in camels aged 1 to 2 years (table S2). Younger camels seem to play a more important epidemiological role in maintaining both viruses, which is consistent with previous findings (*10*, *11*, *16*, *17*).

The overall positive rates for MERS-CoV and camelid α-CoV from nasal swabs were 12.1 and 19.8%, respectively (tables S1 and S2). However, only 3 of 304 camel rectal swabs were CoV-positive for either camelid α- or camel β1-HKU23-CoVs (tables S1 and S2). Thus, a major mode of virus shedding of the MERS- and camelid α-CoVs is from the respiratory tract of dromedary camels. Over half of MERS-CoV–positive nasal swabs (56.6%) were also positive for camelid α-CoVs, indicating frequent co-infections of these viruses (tables S1 and S2). Nasal swabs from two animals contained all three species of CoVs detected in our survey. The high prevalence of these viruses suggests that they are enzootic in dromedary camels.

To examine the genetic diversity and evolution of the camel CoVs, metagenomic sequencing was carried out using the original swab materials that were positive in the initial RT-PCR screening. A total of 93 full-length viral genomes (67 MERS-CoVs, 25 camelid α-CoVs, and one camel β1-HKU23-CoV) were obtained from 79 nasal swab samples. Thirty-eight of these samples presented co-infections of MERS-CoV with one or both of the two other CoV species, but only 14 samples yielded two complete genomes.

β1-HKU23-CoVs have been detected in camels in Dubai (*18*), and the camelid α-CoVs are closely related to a virus isolated from alpacas in California in 2007 (fig. S1) (*19*, *20*). The camelid α-CoVs clustered with the human CoV 229E (fig. S1), a causal agent of common colds in humans. The high prevalence of asymptomatic infections with camelid α-CoVs in Saudi Arabian camels emphasizes the important role this species plays in CoV ecology.

Recombination has been reported in the MERS-CoV species (*21*, *22*). Phylogenetic analysis of the MERS-CoV full-genome sequences obtained in this study (n = 67), together with those available in public databases (n = 106), revealed recombination signatures that defined five major phylogenetically stable lineages, all of which contained human and camel MERS-CoV sequences (Fig. 1 and figs. S2 and S3). A few viruses that showed inconsistent topologies in subgenomic trees, suggesting that they have a more varied history of recombination, were not classified within the five main lineages (fig. S2). MERS-CoVs from Saudi Arabian camels were found within each of the five lineages; the viruses sequenced in this study fell into lineages 3, 4, and 5, with the exception of some minor recombinants (Figs. 1 and 2 and figs. S2 and S3). Thus, the evolution of MERS-CoVs within camels has led to diverse lineages that have all caused human infections, indicating that there is a low barrier for interspecies transmission.

MERS-CoVs obtained between July and December 2014 mainly fell into lineages 3 and 5, whereas those from 2015 were principally from lineage 5 (Fig. 2 and figs. S2 and S3). Four viruses sampled during December 2014, which showed evidence of a small recombinant region, and a virus from March 2015 belonged to lineage 4 (Fig. 2 and figs. S2 to S4). Viruses from lineage 5, which are associated with the Korean outbreak and the recent human infections in Riyadh (Fig. 1) (*3*), were first identified in our surveillance in July 2014 and have been predominant in Saudi Arabian camels since November 2014. However, all of the human viruses of this lineage were reported from February 2015 onward. The MERS-CoV variants associated with the recent outbreak of human infections in South Korea [e.g., ChinaGD01-v1/2015 and KOR/KNIH/002-05/2015 (*23, 24*)] show the highest similarity (99.96 to 99.98%, full genome) to a camel virus (Camel/Riyadh/Ry159/2015) sampled in March 2015 (Fig. 1 and figs. S2 and S3).

A statistically significant signal for phylogenetic incongruence in lineage 5 defined two recombinant sources of the MERS-CoV genome: (i) positions 1 to 16,173 and 24,191 to end, and (ii) 16,174 to 24,190 (Fig. 1). The phylogeny indicates that lineage 5 viruses evolved from a recombinant virus that acquired the 5' part of ORF1ab and the 3' part of the S (spike) gene from lineage 4 and the remaining genomic regions from lineage 3 (Fig. 1C). In both subgenomic phylogenies, lineage 5 viruses were closely related to lineage 3 and 4 viruses from Saudi Arabian camels, suggesting that they hosted this recombination event. Ten synonymous nucleotide changes and a Thr6381Ala amino acid substitution in the nsp14-exonuclease of the ORF1ab polyprotein, relative to lineage 3, were due to the recombination in lineage 5 (Fig. 1A). The possible function of these substitutions requires further investigation. A molecular clock dating analysis indicates that the recombination event probably occurred between December 2013 and June 2014 (fig. S5). Nine other putative MERS-CoV recombinant strains (fig. S4) were seemingly generated by sporadic events and have not persisted in the population, or may represent mixed infections of MERS-CoV strains from different lineages. Although frequent co-infections of MERS- and camelid α-CoVs were observed (tables S1 and S2), no evidence of recombination among them was identified.

Four CoV species circulate widely in humans, and two others have caused severe sporadic infections with limited human-to-human transmission (*1*, *25*). The wide species range of CoVs and their propensity to cross species boundaries suggest that more will emerge in the future. Since the first report of MERS in 2012 (*1*, *2*), the causative virus has been transmitted to over 25 countries, mostly by international travelers that have been to the Middle East (*3*). Even though a high prevalence of MERS-CoVs has been detected in this work and in previous studies of dromedary camels (*4–14*), limited quarantine and biosecurity measures are in place to reduce the exposure of humans to the virus, and more cases must be expected in the future. The recent outbreak of MERS in Korea (*3*) shows that MERS-CoVs have the ability to cause large outbreaks in environments that are different from the Middle East. Although changes in human population density, climate conditions, and social factors may contribute to the spread of MERS-CoVs in other regions, the prevention of transmission at the animal/human interface is likely to be the most efficient measure to contain the threat from this virus.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/cgi/content/full/science.aac8608/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 and S2
References (*26*–*47*)

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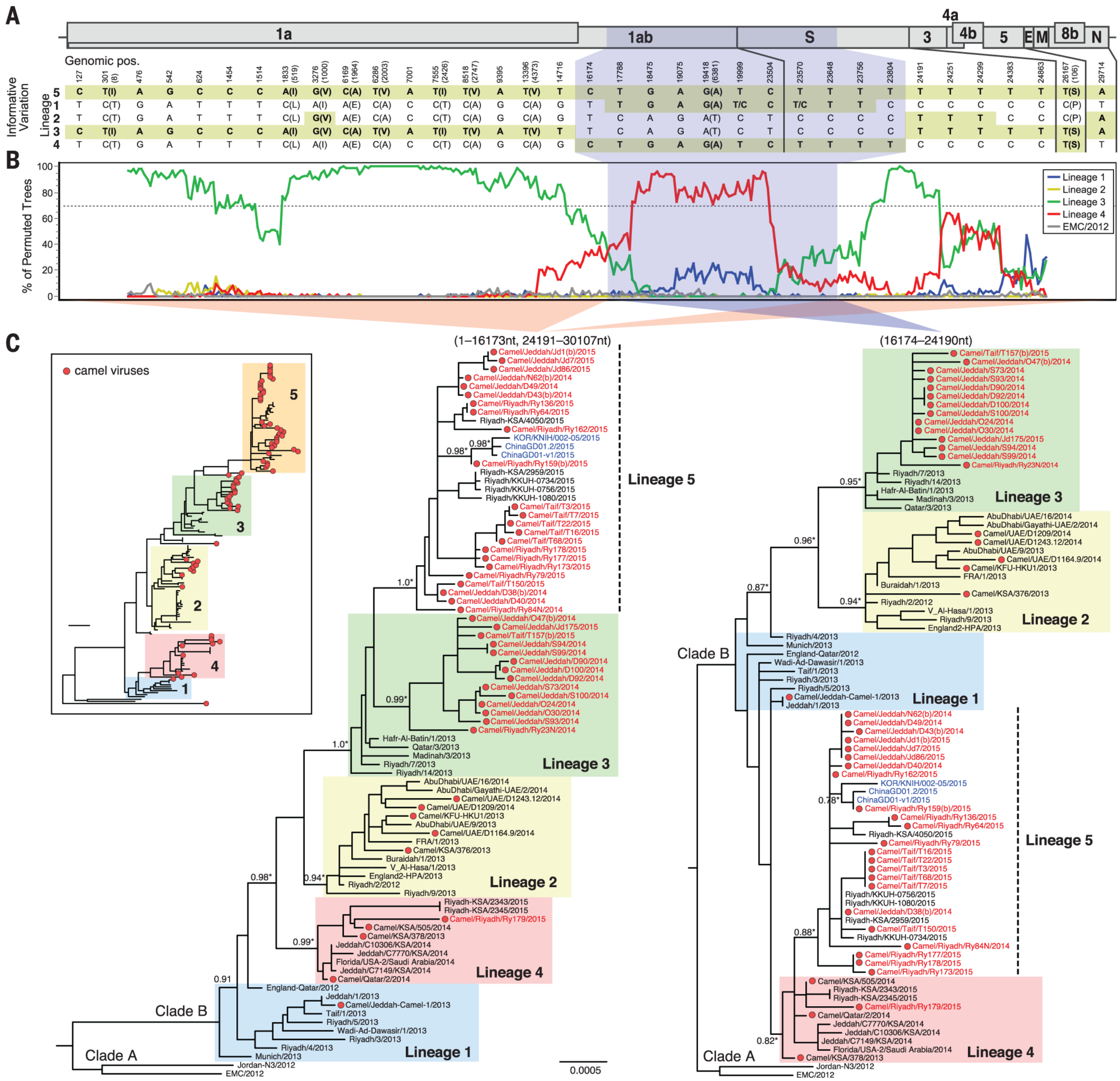

Fig. 1. Genomic recombination in MERS-CoVs. Only the variable sites (variants shared by more than two sequences; see the supplementary materials) were used for (A) and (B). (A) A rescaled structure of the MERS-CoV genome (top) with consensus nucleotides, and any corresponding amino acid substitutions, that are phylogenetically informative in defining the lineages (bottom) (*15*). Nucleotides common with lineage 5 are highlighted (nucleotide substitution C26167T results in amino acid substitution P106S in ORF4b). The likely exchanged region is shaded blue. (B) Bootscanning recombination analysis based on the variable genomic sites. The dashed line indicates 70% bootstrap support. (C) Maximum-likelihood phylogenetic trees inferred for the outer (left) and inner (right) nonrecombinant regions, indicating that lineage 5 is a recombinant of lineages 3 and 4. A subset of sequences from each lineage was used. Camel viruses are indicated by red circles; those sequenced in this study are shown in red text. Shimodaira-Hasegawa–like branch test values and Bayesian inference clade probabilities >0.9 (indicated by asterisks) are shown at selected lineages. Branch lengths reflect the number of nucleotide substitutions per site, and the trees were rooted by Camel/Egypt/NRCE-HKU205/2013. The inset tree was inferred using all available MERS-CoV genomic sequences ($n = 164$; fig. S2).

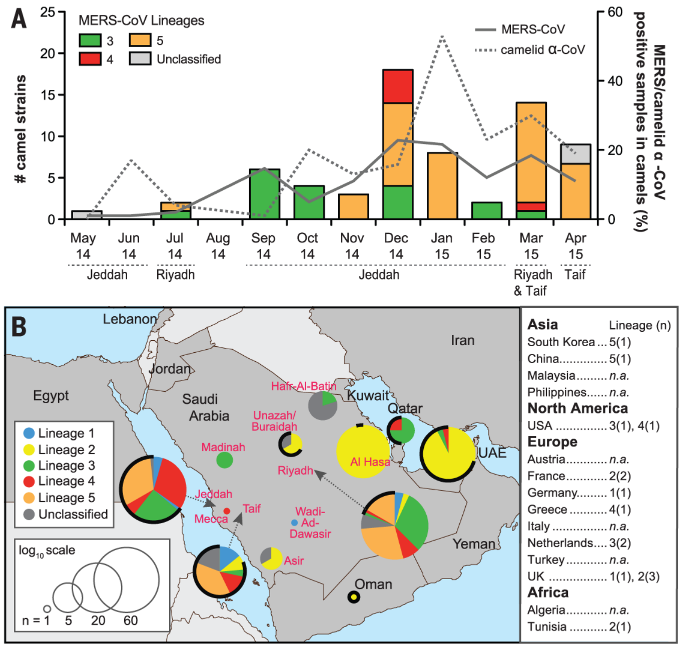

Fig. 2. Lineage distribution of MERS-CoV. Genetic lineages within MERS-CoVs were determined by phylogenetic analysis (fig. S3). (**A**) The bar chart shows the number of camel MERS-CoV sequences obtained, by lineage and month of sampling. Monthly percentages of samples positive for MERS or camelid α-CoVs (determined by RT-PCR) are indicated by solid and dashed lines, respectively (right axis). Sampling sites are indicated below the sampling months. (**B**) Lineage distribution of all available MERS-CoV complete or partial genome sequences from countries that have reported MERS-CoV infections (n.a., sequence not available). For Saudi Arabia, counts are shown by city. In the pie charts, colors represent the lineages, and thick black edges indicate camel sequences.