


Zoonotic origin and transmission of Middle East respiratory syndrome coronavirus in the UAE

C. R. Paden^{1,2*}  | M. F. B. M. Yusof^{3*} | Z. M. Al Hammadi^{3*} | K. Queen^{1,2*} | Y. Tao¹ | Y. M. Eltahir³ | E. A. Elsayed³ | B. A. Marzoug³ | O. K. A. Bensalah³ | A. I. Khalafalla³ | M. Al Mulla⁴ | A. Khudhair⁴ | K. A. Elkheir⁴ | Z. B. Issa⁴ | K. Pradeep⁴ | F. N. Elsaleh⁴ | H. Imambaccus⁵ | J. Sasse⁵ | S. Weber⁵ | M. Shi⁶ | J. Zhang¹ | Y. Li¹ | H. Pham¹ | L. Kim¹ | A. J. Hall¹ | S. I. Gerber¹ | F. I. Al Hosani^{4#} | S. Tong^{1#} | S. S. M. Al Muhairi^{3#}

¹Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

²Oak Ridge Institute for Science Education, Oak Ridge, TN, USA

³Abu Dhabi Food Control Authority, Abu Dhabi, United Arab Emirates

⁴Health Authority Abu Dhabi, Abu Dhabi, United Arab Emirates

⁵Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates

⁶The University of Sydney, Sydney, NSW, Australia

Correspondence

Suxiang Tong, CDC, Atlanta, GA, USA.
Email: sot1@cdc.gov

Funding information

National Center for Immunization and Respiratory Diseases

Summary

Since the emergence of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, there have been a number of clusters of human-to-human transmission. These cases of human-to-human transmission involve close contact and have occurred primarily in healthcare settings, and they are suspected to result from repeated zoonotic introductions. In this study, we sequenced whole MERS-CoV genomes directly from respiratory samples collected from 23 confirmed MERS cases in the United Arab Emirates (UAE). These samples included cases from three nosocomial and three household clusters. The sequences were analysed for changes and relatedness with regard to the collected epidemiological data and other available MERS-CoV genomic data. Sequence analysis supports the epidemiological data within the clusters, and further, suggests that these clusters emerged independently. To understand how and when these clusters emerged, respiratory samples were taken from dromedary camels, a known host of MERS-CoV, in the same geographic regions as the human clusters. Middle East respiratory syndrome coronavirus genomes from six virus-positive animals were sequenced, and these genomes were nearly identical to those found in human patients from corresponding regions. These data demonstrate a genetic link for each of these clusters to a camel and support the hypothesis that human MERS-CoV diversity results from multiple zoonotic introductions.

KEYWORDS

dromedary camel, epidemiology, genomics, middle east respiratory syndrome, viral pathogens, zoonoses

*These authors contributed equally to this article.

#These authors are co-principal investigators.

1 | INTRODUCTION

Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in the Kingdom of Saudi Arabia (KSA) in September 2012 (Zaki, van Boheemen, Bestebroer, Osterhaus, & Fouchier, 2012). It is a group C betacoronavirus, distantly related to the severe acute respiratory syndrome coronavirus (SARS-CoV) which caused an outbreak of severe respiratory illness in 2002–2003 (Zaki et al., 2012). Middle East respiratory syndrome coronavirus also causes a similar acute respiratory illness, and as of December 2017, 2103 cases have been confirmed in 27 countries with 733 deaths (35% case fatality ratio) (World Health Organization, 2017). Infection has been diagnosed in multiple countries, but all cases have an epidemiologic link to the Middle East.

Like SARS-CoV, MERS-CoV is thought to be of animal origin. Investigations of bats and other animals have found near identical sequences, including MERS-CoV in camels (Briese et al., 2014; Corman et al., 2014; Hemida et al., 2014; Raj et al., 2014; Reusken, Farag, et al., 2014; Reusken, Messadi, et al., 2014). Middle East respiratory syndrome coronavirus can replicate efficiently in the upper respiratory tract of dromedary camels (referred to hereafter as “camels”) (Adney et al., 2014). Further studies identified that the MERS-CoV receptor, dipeptidyl peptidase 4 (DPP4), is relatively conserved among mammals, suggesting a likelihood of cross-species transmission (Raj et al., 2013). However, only camels and alpacas have been found seropositive (Reusken, Ababneh, et al., 2013; Reusken et al., 2016), and the virus has only been isolated from camels (Hemida et al., 2014; Raj et al., 2014; Reusken, Ababneh, et al., 2013). The evidence suggests that MERS-CoV may jump between other mammals and camels, and that camels play a role as an intermediate host that transmits MERS-CoV directly to humans (Al Hammadi et al., 2015; Anthony et al., 2017; Azhar et al., 2014; Drosten, Kellam, & Memish, 2014; Memish et al., 2014; Nowotny & Kolodziejek, 2014; Sabir et al., 2016).

Based on phylogenetic analysis of MERS-CoV genomes, it appears that there have been multiple, independent zoonotic introductions of MERS-CoV into the human population, resulting in the observed human MERS-CoV diversity (Cotten et al., 2013, 2014). Nosocomial transmission has accounted for several clusters associated with multiple hospitals in the KSA (Cotten et al., 2013, 2014; Memish, Al-Tawfiq, & Assiri, 2013). Human-to-human transmission appears to require extended close contact with an infected individual. Consequently, most of the clusters have occurred in families and healthcare workers. Tertiary transmission was observed with the 2015 outbreak of MERS-CoV in South Korea (Cho et al., 2016; Park et al., 2015).

The first half of 2014 saw a large increase in the number of MERS-CoV cases, including clusters in hospitals and household settings in the United Arab Emirates (UAE). These larger outbreaks raise the question of whether these outbreak-associated strains have enhanced transmissibility (Drosten, Muth, et al., 2014). SARS-CoV acquired characteristic genetic changes as the virus was sampled from humans during the early epidemic, suggesting that these mutations may play a role in either replication fitness or transmissibility (Bolles, Donaldson, & Baric, 2011; Chinese Sars Molecular Epidemiology

Impacts

- Middle East respiratory syndrome coronavirus (MERS-CoV) is an important human pathogen with a high mortality rate that emerged from a zoonotic reservoir and has been transmitted between humans.
- This study shows the close genetic relationship between the MERS-CoV virus genome sequences transmitted within outbreak clusters in the United Arab Emirates, which will aid in future epidemiological studies.
- Camels are an important reservoir of MERS-CoV; in this study, MERS-CoVs sampled from UAE camels are sequenced and they demonstrate that the outbreak viruses emerged repeatedly from the animal reservoir.

Consortium, 2004; Song et al., 2005; Wong, Li, Moore, Choe, & Farzan, 2004). Genome sequencing of MERS-CoV is critical in understanding molecular determinants of pathogenesis and in understanding transmission patterns.

As part of the response to the increased numbers of MERS-CoV cases and clusters in and around Abu Dhabi, respiratory samples were collected from patients with MERS and contacts along with extensive epidemiological data (Hunter et al., 2016). In this study, to understand how genomics can help resolve questions of transmission, full MERS-CoV genomes from 19 of the 2013–2014 MERS clinical samples were sequenced and analysed, along with four additional partial sequences (spike and nucleocapsid genes). These cases include patients from three hospital-associated clusters, three household-associated clusters and three sporadic cases from the UAE (Al Hosani et al., 2016; Hunter et al., 2016). Additionally, due to the risk factors associated with contact with dromedary camels, respiratory samples were collected from camels at farms near where the human cases originated. Full MERS-CoV genomes were sequenced from six of the camel samples to better understand the role of animals in these outbreaks and in the recent evolution of the virus.

2 | MATERIALS AND METHODS

2.1 | MERS-CoV human case clusters and sample collection

A total of 65 patients with MERS-CoV were identified during our investigation in the UAE from July 2013 through May 2014. Of 65 patients, there were six known clusters of human-to-human MERS-CoV transmission and other sporadic cases verified by extensive epidemiological investigation (Al Hosani et al., 2016; Hunter et al., 2016). The available respiratory samples from 23 patients analysed at the US Centers for Disease Control and Prevention (CDC), and potential camel contacts are placed in context in Figures 1 and 2 and Tables 1 and 2. These samples are from three healthcare-associated clusters (HCA I, II and III), three household clusters

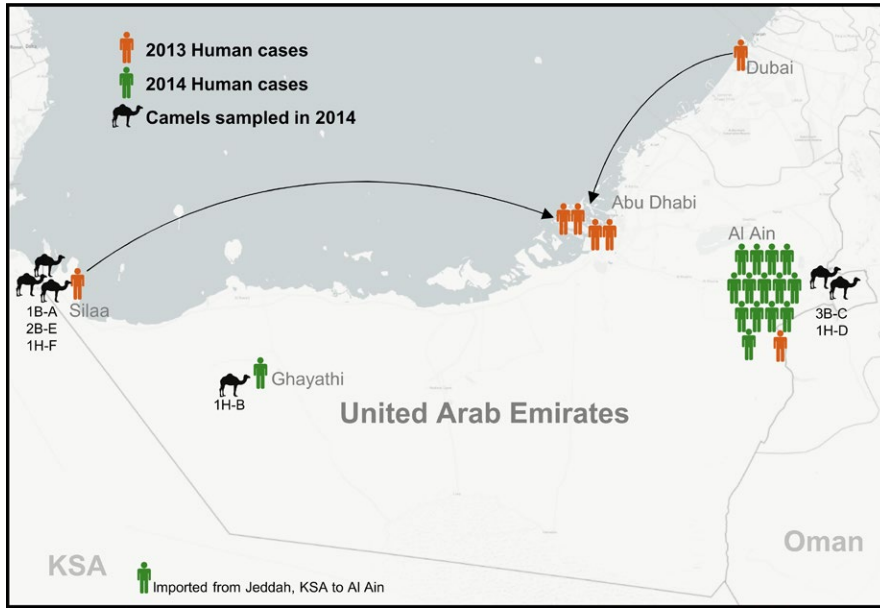


FIGURE 1 Geographic distribution of human and camel cases in the UAE, 2013–2014. Map of the United Arab Emirates showing approximate location of each sequenced human MERS case, as well as the location of the Middle East respiratory syndrome coronavirus (MERS-CoV)-positive camels sampled in this study. Each marker represents an individual case sequenced in this study. Arrows represent the importation of cases to the indicated location. [Colour figure can be viewed at wileyonlinelibrary.com]

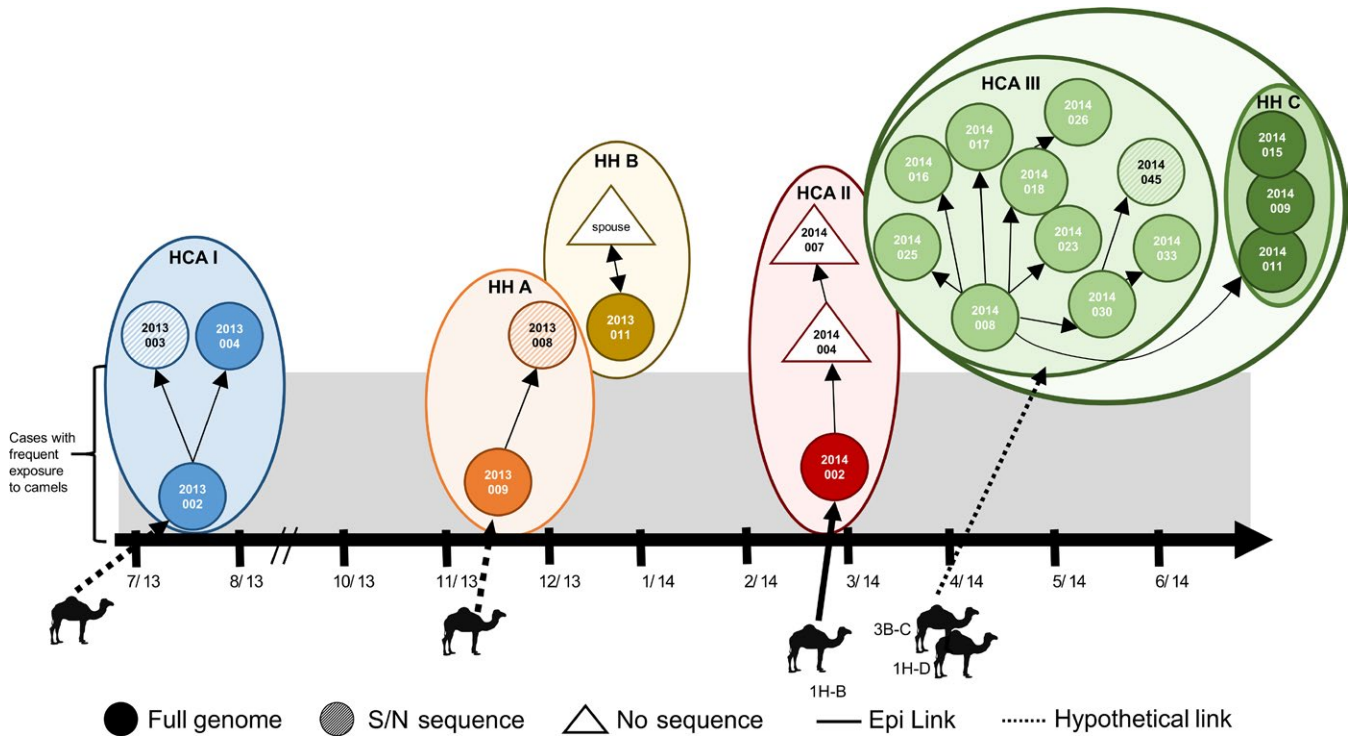


FIGURE 2 Summary of cases sequenced from the UAE 2013–2014 clusters. Each Middle East respiratory syndrome coronavirus (MERS-CoV) genome sequenced from the UAE clusters is shown as a circle. Filled circles represent those human samples where full MERS-CoV genome sequence was obtained, stripe-filled circles represent where only S and N gene sequences were obtained. Triangles represent associated MERS cases where no sequence was obtained. Cases are plotted along a timeline corresponding with the index case infection date, and those associated with frequent exposure to camels are in the shaded region. Camels that were directly implicated in a cluster are connected by a solid arrow. Human cases where the sequence indicates that there is significant similarity to a camel are connected by a dashed arrow. [Colour figure can be viewed at wileyonlinelibrary.com]

(HH A, B, C) and two sporadic cases (Table 1, Figure 1). Activities involved in this investigation were reviewed by CDC and by the Health Authority of Abu Dhabi and were determined to be an urgent public health response that did not constitute human subjects research.

2.2 | Camel sample collection

Nasopharyngeal swabs from dromedary camels were collected in the UAE (Figure 1, Table 2) as approved by the CDC Institutional Animal Care and Use Committee. Three samples (1B-A, 2B-E and 1H-F) were

TABLE 1 Summary of 23 Middle East respiratory syndrome coronavirus clinical samples sequenced and submitted to GenBank

Cluster	Case	Type	Sample collection date	Sequence Type	GenBank accession	Distance to index (nucleotide)
Healthcare-associated clusters (HCA) I (Abu Dhabi)	2013_002	Index	2013 Jul 10	Genome	KY581684	-
	2013_003	Secondary	2013 Jul 12	S/N	S: KY673146; N: KY673143	0 ^a
	2013_004	Secondary	2013 Jul 12	Genome	KY581685	1
Other (Oman)	2013_007	Sporadic	2013 Oct 12	S/N	S: KP236092 N: KP236093	-
HH A (Abu Dhabi)	2013_008	Index	2013 Nov 24	S/N	N: KY673144	-
	2013_009	Secondary	2013 Nov 25	Genome	KP209312	0 ^a
HH B (Dubai/Abu Dhabi)	2013_011	Secondary	2013 Dec 23	Genome	KY581687	-
HCA II (Western Region)	2014_002	Index	2014 Mar 16	Genome	KP209310	-
HH C (Al Ain)	2014_008 ^b	Index	2014 Apr 09	Genome	KP209306	-
	2014_009	Secondary	2014 Apr 10	Genome	KY581686	2
	2014_011	Secondary	2014 Apr 10	Genome	KY581688	2
	2014_015	Secondary	2014 Apr 10	Genome	KY581689	2
HCA III (Al Ain)	2014_008 ^b	Index	2014 Apr 09	Genome	KP209306	-
	2014_016	Secondary	2014 Apr 12	Genome	KP209308	3
	2014_017	Secondary	2014 Apr 12	Genome	KY581690	2
	2014_018	Secondary	2014 Apr 12	Genome	KP209307	2
	2014_023	Secondary	2014 Apr 14	Genome	KY581691	0
	2014_025	Secondary	2014 Apr 15	Genome	KY581692	1
	2014_026	Tertiary	2014 Apr 15	Genome	KP209313	1
	2014_030	Secondary	2014 Apr 16	Genome	KP209309	0
	2014_033	Tertiary	2014 Apr 20	Genome	KP209311	0
2014_045	Tertiary	2014 Apr 25	S/N	S: KY673147; N: KY673145	0 ^a	
Other (Jeddah, Kingdom of Saudi Arabia)	2014_032	Sporadic	2014 Apr 20	Genome	KY581693	-
Other	2014_XXX	Unknown	Unknown	Genome	KY581694	-

^aSequences compared are not full genomes.

^b2014_008 is the index patient for both HH C and HCA III.

TABLE 2 Summary of Middle East respiratory syndrome coronavirus genomes sequenced from UAE camels

Region	Sample ID	Sample date	Closest human case	Distance from human (nucleotide)	GenBank accession
Kingdom of Saudi Arabia Border	1B-A	2014 May 28	2014_XXX	8	KY581695
	2B-E ^a	2014 May 28			KY581699
	1H-F ^a	2014 May 28			KY581698
Al Ain	3B-C ^b	2014 Feb 17	2014_008, 011, 030, 033	7	KY581700
	1H-D ^b	2014 Feb 17			KY581697
Western Region	1H-B	2014 Mar 11	2014_002	3	KY581696

^aThese sequences are identical to each other.

^bThese sequences are identical to each other.

collected in May 2014 at the border with Saudi Arabia where there were no known directly linked human cases. Data on age, sex or clinical signs were not available. Samples 3B-C and 1H-D were collected

in February 2014 from two-one-year-old male camel located within 500 metres from another farm linked with a human case in Al Ain area. Sample 1H-B was collected in March 2014 from a 2-month-old male

camel which presented with mucopurulent discharge. This male camel belonged to a farm located in the Western Region, which was linked to human infection reported 10 Mar 2014.

2.3 | Sanger sequencing and deep sequencing analysis

Middle East respiratory syndrome coronavirus genomes were amplified by 32 pairs of nested, genome-spanning RT-PCRs, in 50 μ l reactions or in nano-volume reactions using the Fluidigm Access Array (van Boheemen et al., 2012; Hunter et al., 2016). When sample quality or quantity was too low, the spike and/or nucleocapsid (S/N) genes were sequenced using alternative nested PCR primers. Sanger data were analysed using Sequencher 5.0. For high-throughput sequencing, amplicon pools from each sample were sheared from 800–1,200 bp to 400–500 bp and were used to generate barcoded libraries with the NEBNext Ultra DNA library prep kit (NEB, Ipswich, MA). Sequencing was performed using an Illumina MiSeq instrument, multiplexing 5–10 samples per 2×250 bp MiSeq run.

Next-generation sequencing data were analysed using a custom workflow in CLC Genomics Workbench 8.5 (Qiagen, Hilden, Germany). Adapters, and an additional 26 bp, were trimmed from each end to remove any residual PCR primer sequence. Remaining reads were trimmed from the 3' end using a CLC cumulative quality score of 0.05. Trimmed reads were aligned to a reference, and a consensus sequence was called based on regions that had 10 \times or greater coverage.

Variants from the reference sequence comprising at least 5% of reads were identified by the quality-based variant detection algorithm in CLC Genomics Workbench, using a neighbourhood radius of 5, minimum neighbourhood quality score of 25 and a minimum central quality score of 29.

2.4 | Phylogenetic and molecular dating analysis

The final consensus genome sequences after Sanger and Illumina sequencing were aligned with the available complete or near complete MERS-CoV genomes in GenBank using MUSCLE (Edgar, 2004). Similarly, Spike gene and protein sequences were aligned. Phylogenetic trees were then inferred using the maximum likelihood (ML) method available in PHYLIP version 3.0 (Guindon et al., 2010) using a general time-reversible (GTR) model with a discrete gamma-distributed rate variation among sites (Γ_4) and a SPR tree-swapping algorithm. To construct a time-scaled tree, we first identified and removed all the recombinant sequences using RDPv4 (Martin, Murrell, Golden, Khoosal, & Muhire, 2015), and the remaining full genome alignment was then analysed in BEAST v1.8.3 (Drummond, Suchard, Xie, & Rambaut, 2012), using HKY + Gamma4 substitution model and an uncorrelated lognormal relaxed molecular clock. Genome and S protein alignment and Single nucleotide polymorphism (SNP) visualization were performed using Harvest (Treangen, Ondov, Koren, & Phillippy, 2014).

3 | RESULTS

3.1 | Sequence analysis of six clusters of human-to-human transmission in the UAE

Respiratory samples were collected from confirmed MERS-positive individuals from three HCA, three household clusters and three sporadic cases from the UAE in 2013 and 2014 (Figures 1 and 2). Using genome-walking Sanger sequencing and/or Illumina amplicon sequencing, we were able to obtain full genome consensus sequences (30,123 bases) from a total of 19 available patient specimens. We obtained S and N gene sequences for an additional four samples that failed full genome sequencing (Figure 2, Table 1). Alignment of these sequences with the other known MERS-CoV sequences showed >99% genetic identity. Single nucleotide polymorphism analysis of the aligned full genome sequences from this study against the HCoV-EMC/2012 sequence showed a range of 98 to 113 nucleotide (nt) variations scattered along the genome (Figure 3a). Comparison of the spike genes to the HCoV-EMC/2012 strain showed a total of 31 SNPs (Figure 3b), causing nine amino acid (AA) changes, but none of these mutations appear to be distinct to these clusters. All sequences generated in this study were deposited in GenBank (Table 1).

Genetic relatedness of MERS-CoV genomes within each cluster supports a close association between proposed transmission partners (Figure 2, Table 1). In HCA cluster I, the index case (2013_002) was a man who owned a camel farm. The index case genome and the genome from one of the contact cases (2013_004) differ by one nucleotide. The index and another contact (2013_003) have identical S genes (genome sequence unavailable). In HH cluster A, case 2013_008 had exposure to camels at a camel market. Case 2013_009 was the spouse of 2013_008, and the S gene sequences recovered from both cases are identical. Interestingly, the genome sequence from patient 2013_009 clusters with a camel sample collected in Dubai (GenBank KP719927). For HH cluster B and HCA cluster II, there was only one specimen available from each cluster, so there is no comparative genomic data within these clusters. However, case 2013_011, although linked to a Dubai case, clustered with the sequences from HH A (2013_009 and 2013_008) and the related Dubai camel sequence (Figure 4).

All cases sequenced from both the HCA cluster III and HH cluster C are recorded to be cases of direct transmission from the index case 2014_008, except patients 2014_026, 2014_033 and 2014_045, who were apparent tertiary transmission cases. All six patients in HCA cluster III with direct contacts to 2014_008 differ by 0–3 nt, compared to the index case 2014_008 (Table 1). According to the contact tracing data, case 2014_030 may have been directly or indirectly infected by 2014_008 during their overlapping stays in the hospital ward, and 2014_030 went on to infect 2014_045 and 2014_033 (Hunter et al., 2016). Complete virus genomes from patients 2014_008, 2014_030 and 2014_033 were sequenced and found to be identical (Figure 3a, Table 1). Both S and N sequences from patient 2014_045 were identical to those from 2014_030 (Figure 3b, Table 1). These data are consistent with the epidemiological data (Figure 2) (Hunter et al., 2016). The genomic data provide clarification regarding the tertiary patient 2014_026. The recorded transmission chain (2014_008 > 2014_018 > 2014_026)

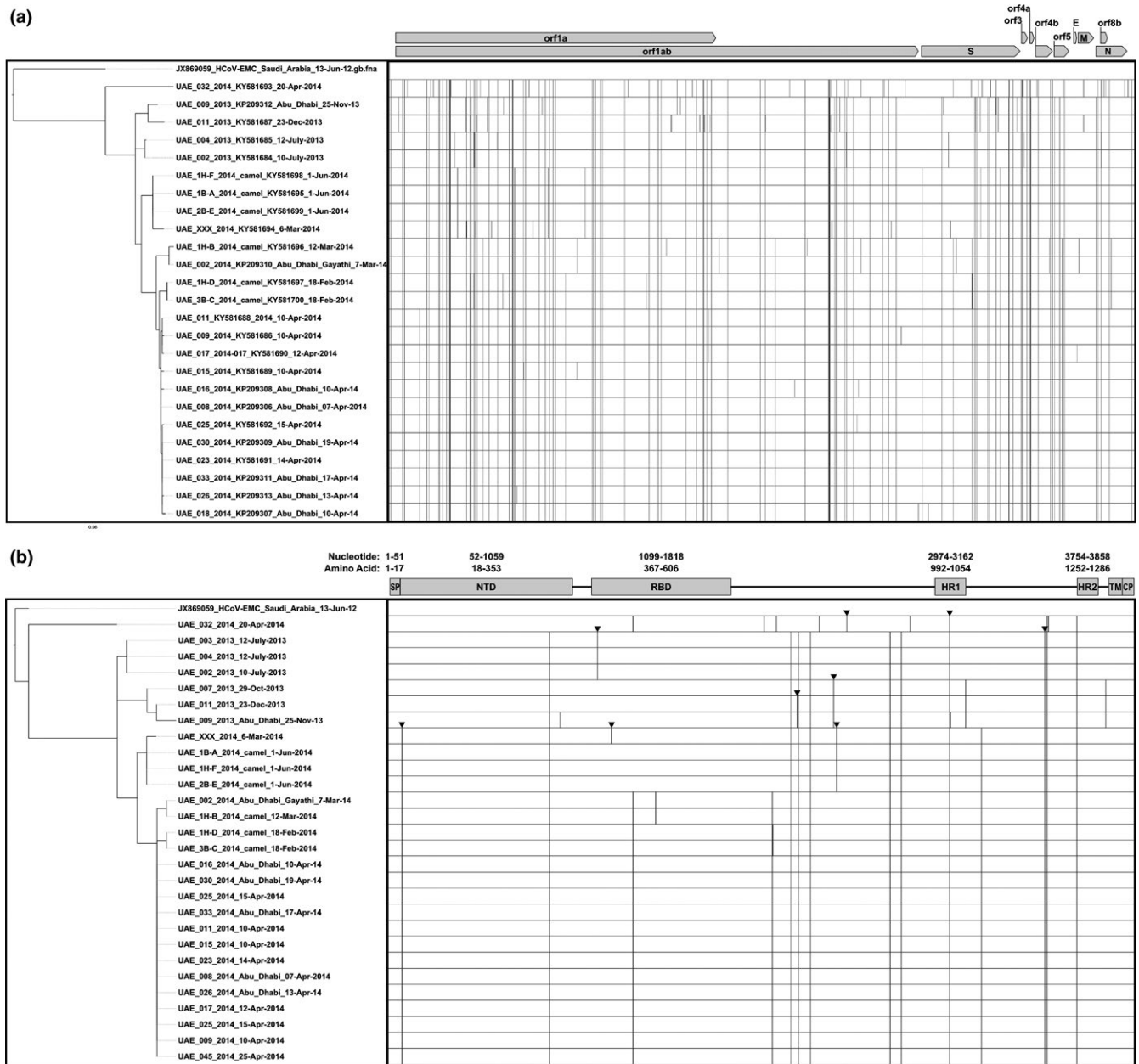


FIGURE 3 Genome alignment and Single nucleotide polymorphism (SNPs) within UAE cases. (a) Full genome sequences or (b) S gene nucleotide sequences from the 2013–2014 UAE Middle East respiratory syndrome coronavirus clusters, sporadic cases and camels were aligned with parsnp, using Human betacoronavirus 2c EMC/2012 (GenBank JX869059) as a reference sequence. Gingr was used to visualize SNPs, compared to the reference genome. Each vertical bar in the graph represents a single SNP. Asterisks in S gene alignment represent amino acid changes

would require three nt changes, including two reversions, while a different, but plausible chain (2014_008 > 2014_023 > 2014_026) only requires one nt change (Table 1). There is insufficient epidemiological data to clarify this discrepancy. Genomes from the other three direct contacts in HH cluster C to 2014_008 differ by one-two nt (Table 1).

3.2 | Minor variant analysis

We performed a minor variant analysis on the available next-generation sequencing (NGS) data to investigate whether or not

MERS-CoV exists as a diverse population in humans, as it does in camels (Briese et al., 2014), and to identify relationships between MERS cases based on minor variant associations. For the analysis, we ran CLC's quality-based variant detection algorithm using 5% as a conservative cut-off. We observed mixed bases distributed throughout the genome in each sample without noting obvious hot spots. This analysis shows that at position 11775 in index patient 2014_008, bases T and C are present at almost an equal number of reads, coding for amino acids as isoleucine (base T) or threonine (base C). However, in the HCA cluster III and HH cluster C, only one nucleotide or the

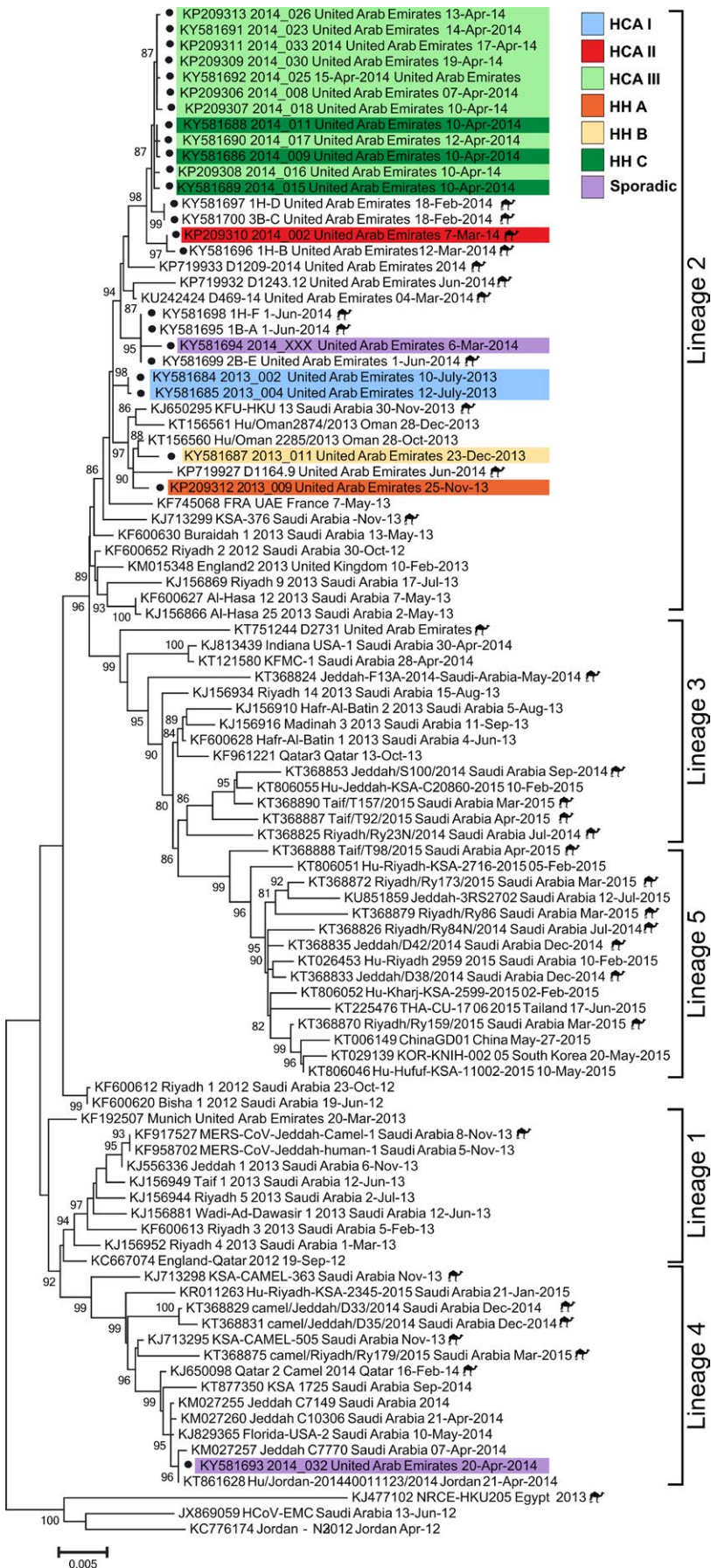


FIGURE 4 PhyML tree analysis of Middle East respiratory syndrome coronavirus (MERS-CoV) genome sequences. Maximum likelihood tree 97 MERS-CoV genomes, generated using PhyML. The trees include sequences from the 2013–2014 UAE clusters as well as camel-derived viruses sequenced in this study and representative sequences from GenBank. The lineages described in Sabir et al. are indicated. The clusters described in the paper are highlighted in coloured boxes. [Colour figure can be viewed at wileyonlinelibrary.com]

other is present in the contact cases. Of the nine direct contact cases sequenced, five harbour the C variant and four harbour the T variant exclusively. Another notable observation was that secondary cases 2014_011 and 2014_017 contain the nonsynonymous mutation A12891C (ORF1ab E4208A). In the index case 2014_008, 30.6% of the bases sequenced at that position are C. This suggests multiple possibilities for founder viruses upon transmission.

3.3 | Phylogenetic analysis of the UAE cases and their relatedness to camel MERS-CoV genomes

To understand how the 2013–2014 UAE viruses relate to other known outbreak and camel strains, camels were sampled from regions of the UAE corresponding to human MERS-CoV cases. We sequenced MERS-CoV genomes recovered from three camels (1B-A, 2B-E and 1H-F) near the KSA border, two camels (3B-C and 1H-D) from a farm near Al Ain and one camel (1H-B) from a farm in the Western Region, also linked with a known human case (Table 2). The sequences recovered from these camels were very similar to the human MERS-CoV sequences in this study, differing by 3–8 nt, compared to the nearest UAE human MERS-CoV sequence (Table 2). Further, we constructed maximum likelihood trees on the full genomes and S genes (Figure 4, Figure S1). The majority of human and camel MERS-CoVs sequenced in this study belongs to lineage 2 as defined by Sabir et al., (2016). Sequences for which we only have the S/N sequence (2013_007, 2013_008, 2013_003 and 2014_045) also cluster within lineage 2 (Figure S1). Healthcare-associated cluster III and HH cluster C form a monophyletic group and are closely related to two camel sequences 3B-C and 1H-D. The rest of the clusters are scattered at different positions within the lineage. Among them, HCA cluster I from 2013 defines an isolated cluster within lineage 2. HH clusters A and B are closely related to each other and to one of the Oman cases from around the same time period (Figure 4). The sequences from HH clusters A and B also cluster with a MERS-CoV genome recovered from a camel in the UAE earlier that year (GenBank KP719927). The sequence from the HCA cluster II index case (2014_002) is closely related to the camel sequence 1H-B, which comes from a farm in the Western Region. They differ by three nt which is consistent with their potential epidemiologic link (the patient was from the Western Region and had contact with camels). While there may be no direct linkage among the different small UAE clusters (HCA I, HH A, HH B and HCA II), HCA cluster III or HH cluster C, the phylogenetic closeness implies a temporal and geographic constraint. The close connection between geographic and phylogenetic relatedness is also apparent in the context of the other known MERS-CoV sequences (Figure 4).

We also sequenced several sporadic cases of MERS-CoV. The first was a UAE case, which was thought to be acquired on travel to Jeddah, KSA (2014_032). This genome clusters with the Jeddah 2014 group in lineage 4 and is genetically distinct from the other UAE cases (Figure 4). The second is from a person from Oman who had extensive contact with farm animals (2013_007). We sequenced the S gene sequence from this virus and found that it clusters near other Omani human

cases from 2013 as well as several camel MERS-CoV genomes from the same time period (Figure S1). Another case (2014_XXX) had no available case information, but notably, its genome sequence is phylogenetically linked to the three camel MERS-CoV sequences (seven nt difference) which were sampled from the KSA/UAE border in this study (1B-A, 2B-E and 1H-F) (Figure 4, Table 2).

To further define when the outbreak MERS-CoVs emerged with respect to other clusters and to the camel viruses, we constructed a time-scaled tree using BEAST (Figure 5). Our estimation of evolution rate was between 6.5×10^{-4} and 9.2×10^{-4} for the entire MERS-related genome dataset (recombinants excluded). Under that rate, the divergence date between UAE clusters and the Al Hasa cluster was most likely before August 2012 (Figure 5). Furthermore, the divergence date of the genome at Node A, the common ancestor of human and camel viruses associated with UAE cluster HCA III and HH C, is estimated to be early January 2014 (Node A). Node C represents a separate divergence of human 2014_XXX and camel CoVs which occurred around the same time. Human and camel case-associated viruses also are estimated to have diverged at Node B in February 2014 (Figure 5). Each of these nodes represents a separate introduction of camel viruses into the human population.

4 | DISCUSSION

Genomic studies of MERS-CoV with accompanying epidemiology are important and can reveal spatiotemporal transmission chains to support epidemiologic investigations from a zoonotic event with subsequent human-to-human transmission. These studies may also lead to improved understanding of the underlying genotypic mutations that drive phenotypic changes. Larger transmission events, like those in Al Hasa, Jeddah and Korea (Assiri et al., 2013; Oboho et al., 2015; Park et al., 2015) provide opportunities for molecular epidemiological analysis to understand whether changes in the virus genome lead to increased transmission, better fitness or adaptation to treatment. In spring of 2014, there was a steep increase in the number of MERS-CoV cases reported to WHO. This study demonstrates that these outbreaks in the UAE are likely due to independent zoonotic transmission events followed by nosocomial amplification which is consistent with the epidemiological investigations (Al Hosani et al., 2016; Hunter et al., 2016).

In the UAE during 2013–2014, there appear to be six independent introductions of the virus, emerging from the same lineage (lineage 2) (seven, including imported case 2014_032). Unsurprisingly, sequences within each human cluster were very close at a nucleotide level, and in general, did not vary more than three nucleotides within the same transmission chain (Table 1). Further, we did not identify any signature amino acid changes in the spike genes associated with the larger transmission clusters. Thus, there is no compelling genetic evidence for a more transmissible virus, rather, the high rate of transmission was likely due to the close contact of the patients with MERS.

This study provides temporal, geographic and genetic data linking actual human infections (clusters and sporadic cases) to camel MERS-CoV. It is now clear that dromedary camels are a major reservoir of

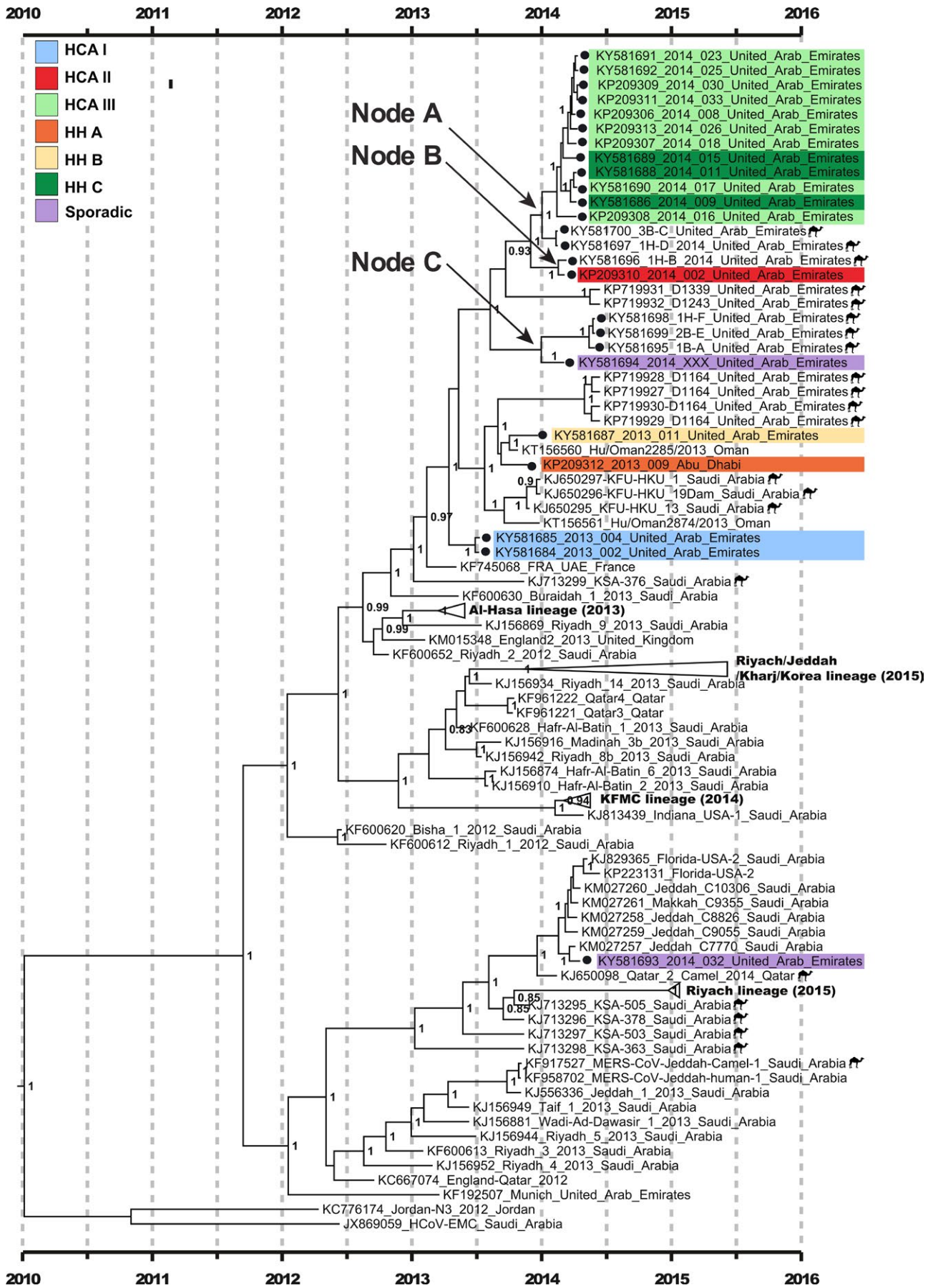


FIGURE 5 BEAST time-scaled tree of Middle East respiratory syndrome coronavirus (MERS-CoV) cases. After filtering recombinant MERS-CoV genomes using RDP, the remaining genomes were analysed using BEAST to understand divergence times between the various human cases and between the human and camel cases. Node A shows a divergence between a camel virus and the 2014_008-associated cluster (Healthcare-associated clusters III and HH C) virus that occurred separately, but concurrent, with the divergence at Node C, between a camel virus and human case 2014_XXX. Likewise, the divergence of the 2014_002 virus from a camel virus happened at Node B, separately and later than the other two examples. The clusters described in the paper are highlighted in coloured boxes. [Colour figure can be viewed at wileyonlinelibrary.com]

MERS-CoV and an important species in transmitting the virus to humans (Alagaili et al., 2014; Azhar et al., 2014; Ferguson & Van Kerkhove, 2014). We know that several of the index cases in this study had close contact with camels from different regions of the UAE (Al Hosani et al., 2016; Hunter et al., 2016). Here, we show genomic evidence linking the MERS-CoV genome from camel 1H-B (Western Region) to case 2014_002, a person who visited the farm where this animal was kept—the genomes were a distance of three nt. Although the camels from KSA/UAE border (1B-A, 2B-E, and 1H-F) were not directly associated with any known human cases, the genome sequence clustered with human case 2014_XXX at a distance of eight nucleotides. This suggests a zoonotic link to case 2014_XXX. In HCA cluster III, although there was no recorded camel contact with the index patient, sequences collected from the Al Ain-area camels 3B-C and 1H-C just before the outbreak (February 2014) show close similarity at the nucleotide level (seven nt) to the human index case 2014_008 (Figure 3, Table 2). Additionally, the 2013_002 case, who lived near the KSA border and had frequent contact with camels, harboured a MERS-CoV sequence closely linked to a camel-derived sequence from KSA already published in GenBank (Muhairi et al., 2016). The fact that the camel from Dubai (KP719927) is closely related to HH A cases suggests that MERS-CoV may have been imported to an Abu Dhabi camel market from Dubai.

The camel- and human-derived MERS-CoV sequences do not phylogenetically segregate based on host, rather, animal and human viruses are interspersed throughout the trees. This suggests repeated independent introductions or cocirculation between camels and humans, as has been concluded in other cases (Cotten et al., 2013; Drosten, Kellam, et al., 2014; Memish et al., 2014). Taken together with the molecular clock data showing different human/camel virus divergence dates for different human MERS outbreaks, we conclude that the human and camel viruses are not distinct viruses and at least some strains are likely capable of infecting both species. Notably, human-to-human transmission chains have been relatively short, which suggests that MERS-CoV may not be transmitted efficiently from human to human at this time, having a transient and mostly dead-end infection. Further study of MERS-CoV in camel populations may lead to an understanding of the viral genes that are important in tropism and replication fitness in camel versus human hosts.

It is becoming more important to understand the contribution of viral quasi-species and minor variants in the MERS-CoV life cycle. We demonstrated some limited usefulness in linking epidemiological cases, where a minority population converts to the predominant population in the transmission partner. This complicates simple phylogenetic relationships between cases, as there may be appreciable diversity in an infected host to transmit different viruses to

two different contacts. Briesse et al. demonstrated this phenomenon in linking a camel MERS-CoV minor population genome directly to a human MERS case (Briesse et al., 2014). A newer study examining camel MERS-CoV isolates observed that although there were only a handful of differences between strains at the consensus level, there were hundreds of intrahost variants (Borucki et al., 2016). Appreciating this phenomenon will help in more accurately and robustly identifying and dating transmission pairs and in understanding variants associated with fitness.

This study provides genomic information complementing an epidemiological investigation of a MERS-CoV outbreak and provides evidence that the outbreak viruses emerged from camels either directly or in the months leading up to the outbreak. The molecular data generated here support and clarify the contact tracing records and provide contextual information to potential future outbreaks. These data will be important in determining what molecular changes in the virus may lead to increased transmission between humans. Exposure to camels is a risk factor for human MERS-CoV infection, and undoubtedly, new cases will continue to emerge. Surveillance of camels on the Arabian Peninsula and in eastern Africa has shown a high rate of seropositivity as well as some MERS-CoV shedding (Alagaili et al., 2014; Corman et al., 2014; Haagmans et al., 2014; Müller et al., 2014; Raj et al., 2014; Reusken, Haagmans, et al., 2013). There is a camel MERS-CoV vaccine in development (Haagmans et al., 2015; Song et al., 2013), but in the near term, it is critical that individuals exposed directly to camels take precautions to minimize the risk of MERS-CoV transmission. Broader genetic and/or culture-based screening of camel populations is needed to understand the prevalence and distribution of coronaviruses and other viruses which may cause disease in humans.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

ORCID

C. R. Paden  <http://orcid.org/0000-0001-8701-8948>

REFERENCES

- Adney, D. R., van Doremalen, N., Brown, V. R., Bushmaker, T., Scott, D., de Wit, E., ... Munster, V. J. (2014). Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. *Emerging Infectious Diseases*, 20(12), 1999–2005. <https://doi.org/10.3201/eid2012.141280>
- Al Hammadi, Z. M., Chu, D. K., Eltahir, Y. M., Al Hosani, F., Al Mulla, M., Tarnini, W., ... Poon, L. L. (2015). Asymptomatic MERS-CoV infection in humans possibly linked to infected dromedaries imported from Oman to United Arab Emirates, May 2015. *Emerging Infectious Diseases*, 21(12), 2197–2200. <https://doi.org/10.3201/eid2112.151132>
- Al Hosani, F. I., Pringle, K., Al Mulla, M., Kim, L., Pham, H., Alami, N. N., ... Gerber, S. I. (2016). Response to emergence of Middle East respiratory syndrome coronavirus, Abu Dhabi, United Arab Emirates, 2013–2014. *Emerging Infectious Diseases*, 22(7), 1162–1168. <https://doi.org/10.3201/eid2207.160040>
- Alagaili, A. N., Briese, T., Mishra, N., Kapoor, V., Sameroff, S. C., Burbelo, P. D., ... Lipkin, W. I. (2014). Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. *mBio*, 5(2), e00884–00814. <https://doi.org/10.1128/mbio.00884-14>
- Anthony, S. J., Gilardi, K., Menachery, V. D., Goldstein, T., Ssebidde, B., Mbabazi, R., ... Mazet, J. A. (2017). Further Evidence for Bats as the Evolutionary Source of Middle East Respiratory Syndrome Coronavirus. *mBio*, 8(2), pii: e00373–17. <https://doi.org/10.1128/mbio.00373-17>
- Assiri, A., McGeer, A., Perl, T. M., Price, C. S., Al Rabeeah, A. A., Cummings, D. A., ... Team, K. M.-C. I. (2013). Hospital outbreak of Middle East respiratory syndrome coronavirus. *New England Journal of Medicine*, 369(5), 407–416. <https://doi.org/10.1056/NEJMoa1306742>
- Azhar, E. I., El-Kafrawy, S. A., Farraj, S. A., Hassan, A. M., Al-Saeed, M. S., Hashem, A. M., & Madani, T. A. (2014). Evidence for camel-to-human transmission of MERS coronavirus. *New England Journal of Medicine*, 370(26), 2499–2505. <https://doi.org/10.1056/NEJMoa1401505>
- van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T. M., Raj, V. S., Zaki, A. M., ... Fouchier, R. A. (2012). Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *mBio*, 3(6), e00473–00412.
- Bolles, M., Donaldson, E., & Baric, R. (2011). SARS-CoV and emergent coronaviruses: Viral determinants of interspecies transmission. *Current Opinion in Virology*, 1(6), 624–634. <https://doi.org/10.1016/j.coviro.2011.10.012>
- Borucki, M. K., Lao, V., Hwang, M., Gardner, S., Adney, D., Munster, V., ... Allen, J. E. (2016). Correction: Middle East respiratory syndrome coronavirus intra-host populations are characterized by numerous high frequency variants. *PLoS ONE*, 11(4), e0154424. <https://doi.org/10.1371/journal.pone.0154424>
- Briese, T., Mishra, N., Jain, K., Zalmout, I. S., Jabado, O. J., Karesh, W. B., ... Lipkin, W. I. (2014). Middle East respiratory syndrome coronavirus quaspecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. *mBio*, 5(3), e01146–01114. <https://doi.org/10.1128/mbio.01146-14>
- Chinese Sars Molecular Epidemiology Consortium (2004). Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science*, 303(5664), 1666–1669. <https://doi.org/10.1126/science.1092002>
- Cho, S. Y., Kang, J. M., Ha, Y. E., Park, G. E., Lee, J. Y., Ko, J. H., ... Kim, Y. J. (2016). MERS-CoV outbreak following a single patient exposure in an emergency room in South Korea: An epidemiological outbreak study. *Lancet*, 388(10048), 994–1001. [https://doi.org/10.1016/S0140-6736\(16\)30623-7](https://doi.org/10.1016/S0140-6736(16)30623-7)
- Corman, V. M., Jores, J., Meyer, B., Younan, M., Liljander, A., Said, M. Y., ... Muller, M. A. (2014). Antibodies against MERS coronavirus in dromedary camels, Kenya, 1992–2013. *Emerging Infectious Diseases*, 20(8), 1319–1322. <https://doi.org/10.3201/eid2008.140596>
- Cotten, M., Watson, S. J., Kellam, P., Al-Rabeeah, A. A., Makhdoom, H. Q., Assiri, A., ... Memish, Z. A. (2013). Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: A descriptive genomic study. *Lancet*, 382(9909), 1993–2002. [https://doi.org/10.1016/S0140-6736\(13\)61887-5](https://doi.org/10.1016/S0140-6736(13)61887-5)
- Cotten, M., Watson, S. J., Zumla, A. I., Makhdoom, H. Q., Palser, A. L., Ong, S. H., ... Memish, Z. A. (2014). Spread, circulation, and evolution of the Middle East respiratory syndrome coronavirus. *mBio*, 5(1), e01062–13. <https://doi.org/10.1128/mbio.01062-13>
- Drosten, C., Kellam, P., & Memish, Z. A. (2014). Evidence for camel-to-human transmission of MERS coronavirus. *New England Journal of Medicine*, 371(14), 1359–1360. <https://doi.org/10.1056/NEJMc1409847#SA1>
- Drosten, C., Muth, D., Corman, V. M., Hussain, R., Al Masri, M., HajOmar, W., ... Memish, Z. A. (2014). An observational, laboratory-based study of outbreaks of Middle East respiratory syndrome coronavirus in Jeddah and Riyadh, Kingdom of Saudi Arabia, 2014. *Clinical Infectious Diseases*, 60(3), 369–377. <https://doi.org/10.1093/cid/ciu812>
- Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29(8), 1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Edgar, R. C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 113. <https://doi.org/10.1186/1471-2105-5-113>
- Ferguson, N. M., & Van Kerkhove, M. D. (2014). Identification of MERS-CoV in dromedary camels. *The Lancet. Infectious Diseases*, 14(2), 93–94. [https://doi.org/10.1016/S1473-3099\(13\)70691-1](https://doi.org/10.1016/S1473-3099(13)70691-1)
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, 59(3), 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Haagmans, B. L., Al Dhahiry, S. H., Reusken, C. B., Raj, V. S., Galiano, M., Myers, R., ... Ghobashy, H. (2014). Middle East respiratory syndrome coronavirus in dromedary camels: An outbreak investigation. *The Lancet. Infectious Diseases*, 14(2), 140–145. [https://doi.org/10.1016/S1473-3099\(13\)70690-x](https://doi.org/10.1016/S1473-3099(13)70690-x)
- Haagmans, B. L., van den Brand, J. M. A., Raj, V. S., Volz, A., Wohlsein, P., Smits, S. L., ... Osterhaus, A. (2015). An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. *Science*, 351(6268), 77–81. <https://doi.org/10.1126/science.aad1283>
- Hemida, M. G., Chu, D. K., Poon, L. L., Perera, R. A., Alhammadi, M. A., Ng, H. Y., ... Peiris, M. (2014). MERS coronavirus in dromedary camel herd, Saudi Arabia. *Emerging Infectious Diseases*, 20(7), 1231–1234. <https://doi.org/10.3201/eid2007.140571>
- Hunter, J. C., Nguyen, D., Aden, B., Al Bandar, Z., Al Dhaheri, W., Elkheir, K. A., ... Al Kaabi, N. (2016). Transmission of Middle East respiratory syndrome coronavirus infections in healthcare settings, Abu Dhabi. *Emerging Infectious Diseases*, 22(4), 647–656. <https://doi.org/10.3201/eid2204.151615>
- Martin, D. P., Murrell, B., Golden, M., Khoosal, A., & Muhire, B. (2015). RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evolution*, 1(1), vev003. <https://doi.org/10.1093/ve/vev003>
- Memish, Z. A., Al-Tawfiq, J. A., & Assiri, A. (2013). Hospital-associated Middle East respiratory syndrome coronavirus infections. *New England Journal of Medicine*, 369(18), 1761–1762. <https://doi.org/10.1056/NEJMc1311004>
- Memish, Z. A., Cotten, M., Meyer, B., Watson, S. J., Alshafiq, A. J., Al Rabeeah, A. A., ... Drosten, C. (2014). Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. *Emerging Infectious Diseases*, 20(6), 1012–1015. <https://doi.org/10.3201/eid2006.140402>
- Muhairi, S. A., Hosani, F. A., Eltahir, Y. M., Mulla, M. A., Yusuf, M. F., Serhan, W. S., ... Abdelazim, A. S. (2016). Epidemiological investigation of Middle East respiratory syndrome coronavirus in dromedary camel

- farms linked with human infection in Abu Dhabi Emirate, United Arab Emirates. *Virus Genes*, 52(6), 848–854. <https://doi.org/10.1007/s11262-016-1367-1>
- Müller, M. A., Corman, V. M., Jores, J., Meyer, B., Younan, M., Liljander, A., ... Drosten, C. (2014). MERS coronavirus neutralizing antibodies in camels, Eastern Africa, 1983–1997. *Emerging Infectious Diseases*, 20(12), 2093–2095. <https://doi.org/10.3201/eid2012.141026>
- Nowotny, N., & Kolodziejek, J. (2014). Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013. *Eurosurveillance Weekly*, 19(16), 20781.
- Oboho, I. K., Tomczyk, S. M., Al-Asmari, A. M., Banjar, A. A., Al-Mugti, H., Aloraini, M. S., ... Madani, T. A. (2015). 2014 MERS-CoV outbreak in Jeddah—a link to health care facilities. *New England Journal of Medicine*, 372(9), 846–854. <https://doi.org/10.1056/NEJMoa1408636>
- Park, H. Y., Lee, E. J., Ryu, Y. W., Kim, Y., Kim, H., Lee, H., & Yi, S. J. (2015). Epidemiological investigation of MERS-CoV spread in a single hospital in South Korea, May to June 2015. *Eurosurveillance Weekly*, 20(25), 1–6.
- Raj, V. S., Farag, E. A., Reusken, C. B., Lamers, M. M., Pas, S. D., Voermans, J., ... Haagmans, B. L. (2014). Isolation of MERS coronavirus from a dromedary camel, Qatar, 2014. *Emerging Infectious Diseases*, 20(8), 1339–1342. <https://doi.org/10.3201/eid2008.140663>
- Raj, V. S., Mou, H., Smits, S. L., Dekkers, D. H., Muller, M. A., Dijkman, R., ... Haagmans, B. L. (2013). Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature*, 495(7440), 251–254. <https://doi.org/10.1038/nature12005>
- Reusken, C. B., Ababneh, M., Raj, V. S., Meyer, B., Eljarah, A., Abutarbush, S., ... Koopmans, M. P. (2013). Middle East respiratory syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013. *Eurosurveillance Weekly*, 18(50), 20662. <https://doi.org/10.2807/1560-7917.es2013.18.50.20662>
- Reusken, C. B., Chrispijn Schilp, V., De Bruin, E., Kohl, R. H., Farag, E. A., Haagmans, B. L., ... Koopmans, M. P. (2016). MERS-CoV infection of alpaca in a region where MERS-CoV is endemic. *Emerging Infectious Diseases*, 22(6), 1129. <https://doi.org/10.3201/eid2206.152113>
- Reusken, C. B., Farag, E. A., Jonges, M., Godeke, G. J., El-Sayed, A. M., Pas, S. D., ... Koopmans, M. P. (2014). Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. *Eurosurveillance Weekly*, 19(23), pii: 20829.
- Reusken, C. B., Haagmans, B. L., Müller, M. A., Gutierrez, C., Godeke, G. J., Meyer, B., ... Koopmans, M. P. (2013). Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: A comparative serological study. *The Lancet. Infectious diseases*, 13(10), 859–866. [https://doi.org/10.1016/s1473-3099\(13\)70164-6](https://doi.org/10.1016/s1473-3099(13)70164-6)
- Reusken, C. B., Messadi, L., Feyisa, A., Ullaramu, H., Godeke, G. J., Danmarwa, A., ... Koopmans, M. P. (2014). Geographic distribution of MERS coronavirus among dromedary camels, Africa. *Emerging Infectious Diseases*, 20(8), 1370–1374. <https://doi.org/10.3201/eid2008.140590>
- Sabir, J. S., Lam, T. T., Ahmed, M. M., Li, L., Shen, Y., Abo-Aba, S. E., ... Guan, Y. (2016). Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. *Science*, 351(6268), 81–84. <https://doi.org/10.1126/science.aac8608>
- Song, F., Fux, R., Provacia, L. B., Volz, A., Eickmann, M., Becker, S., ... Sutter, G. (2013). Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. *Journal of Virology*, 87(21), 11950–11954. <https://doi.org/10.1128/jvi.01672-13>
- Song, H. D., Tu, C. C., Zhang, G. W., Wang, S. Y., Zheng, K., Lei, L. C., ... Zhao, G. P. (2005). Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. *Proceedings of the National Academy of Sciences of the USA*, 102(7), 2430–2435. <https://doi.org/10.1073/pnas.0409608102>
- Treangen, T. J., Ondov, B. D., Koren, S., & Phillippy, A. M. (2014). The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biology*, 15(11), 524. <https://doi.org/10.1186/PREACCEPT-2573980311437212>
- Wong, S. K., Li, W., Moore, M. J., Choe, H., & Farzan, M. (2004). A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. *Journal of Biological Chemistry*, 279(5), 3197–3201. <https://doi.org/10.1074/jbc.C300520200>
- World Health Organization. (2017), 12/4/2017. *WHO Middle East respiratory syndrome coronavirus (MERS-CoV)*. Retrieved from <http://www.who.int/emergencies/mers-cov/en/>
- Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., & Fouchier, R. A. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *New England Journal of Medicine*, 367(19), 1814–1820. <https://doi.org/10.1056/NEJMoa1211721>

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Paden CR, Yusof MFBM, Al Hammadi ZM, et al. Zoonotic origin and transmission of Middle East respiratory syndrome coronavirus in the UAE. *Zoonoses Public Health*. 2018;65:322–333. <https://doi.org/10.1111/zph.12435>