

THE LANCET Planetary Health

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: El-Kafrawy SA, Corman VM, Tolah AM, et al. Enzootic patterns of Middle East respiratory syndrome coronavirus in imported African and local Arabian dromedary camels: a prospective genomic study. *Lancet Planet Health* 2019; published online Dec 13. [http://dx.doi.org/10.1016/S2542-5196\(19\)30243-8](http://dx.doi.org/10.1016/S2542-5196(19)30243-8).

Supplementary materials

to El-Kafrawy et al., Middle East Respiratory Syndrome Coronavirus: Genetic diversity, phylogeography, infection rates and age associated patterns in imported African and local Arabian dromedary camels

Supplementary text:

Sequencing and phylogenetic methods

For initial MERS-CoV genome analyses we amplified three target regions by RT-PCR from 100 imported camel samples. These fragments were located in ORF1a (genome position 10,265 to 11,361 in EMC/2012 reference genome; Genbank Acc No. JX869059); the Spike gene (genome position 23,864 to 24,909); and ORF4b (genome position 26,025 to 27,020). Selection of samples for sequencing was based on availability of sample volume and viral RNA concentration as per initial real-time PCR testing (minimum, $C_T = 35$). All RT-PCR primers and protocols were described before¹. The three genome regions were selected to generate genomic data before and after known recombination breakpoint^{2,3}, and to include the region within ORF4b that was reported to show specific deletion patterns in African isolates⁴. The concatenated sequences of these fragments covered approximately 3140 nucleotides (~10% of a typical complete MERS-CoV genome with a size of ~30,120 nucleotides). After Sanger sequencing, we selected 22 samples to represent all clades observed in initial phylogenetic analyses using the concatenated fragments. Full genome sequences from 24 local camel samples were also generated.

Full genomes were generated using shotgun Illumina High-Throughput Sequencing (HTS) on all selected positive samples showing C_T -values of less than 26. All other samples were first amplified by RT-PCRs as in Corman et al.¹ to amplify the complete genome with overlapping PCR amplicons, followed by PCR amplicon-based HTS. For HTS from clinical sample we used 5 μ L of extracted RNA for library preparation using the KAPA RNA Hyper Prep kit (Roche Molecular Diagnostics, Basel, Switzerland) according to manufacturer's instructions. Indexed DNA libraries were measured by Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Karlsruhe, Germany), pooled together at equimolar ratios (up to 20 libraries), and normalized. Sequencing was performed using the 600-cycle MiSeq reagent v3 cartridge (Illumina, San Diego, California, U.S.) according to manufacturer's instructions. Genome regions with low coverage (<10) and sequencing gaps were amplified by bridging RT-PCR using MERS-CoV specific primers and sequenced by dye terminator chemistry (Microsynth Seqlab, Göttingen, Germany).

For the amplicon-based approach, we used a hemi-nested RT-PCR assay for amplifying and sequencing of complete genomes from samples with low RNA concentration. The RT-PCR assays were designed to amplify about 800 overlapping base pairs of all known MERS-CoV sequences and were published by our group before¹. Following amplification, PCR amplicon bands were visualized by agarose gel electrophoresis (2% agarose) with ethidium bromide and purified using KAPA Pure Beads (Roche Molecular Diagnostics). All amplicons from each single sample were pooled and each pool was quantified. Approximately 5 ng of each DNA pool was fragmented, using KAPA Frag Kit (Roche Molecular Diagnostics). Library preparation was done using the KAPA Hyper Prep kit (Roche Molecular Diagnostics) according to the manufacturer's protocol. Indexed DNA libraries were measured by Qubit dsDNA HS Assay kit (Thermo Fisher Scientific), pooled together at equimolar ratios (up to 50 libraries), and normalized. Sequencing was performed using the 600-cycle MiSeq reagent v3 cartridge (Illumina). The set-up of the instrument and the preparation of the flow cell and were performed according to manufacturer instructions.

The strategy for complete genome assembly comprised three major steps: First MERS-CoV reads in the HTS dataset were identified by using a local BLAST comparison against a database containing all MERS-CoV strains available in Genbank (as of 1st June 2019). Second, all reads matching this database were used for de-novo genome assembly by SPAdes within Geneious 9.1.8. Third, all HTS reads were used for specific reference-mapping against the de-novo assembled consensus sequences and MERS-CoV strain EMC/2012 in Geneious 9.1.8. After performing multiple iterations nearly complete consensus genomes could be generated for all samples. The de-novo and the mapping approach did not result in discordant consensus sequences. Nevertheless, genome regions with low coverage (<10 reads), sequencing gaps, and deletions as compared to reference sequences from Genbank were amplified by bridging RT-PCR using MERS-CoV specific primers and sequenced by dye terminator chemistry (Microsynth Seqlab, Göttingen, Germany).

For sequence analysis the complete or partial sequences of the MERS-CoV from this study were aligned with other MERS-CoV strains available in GenBank. The alignments included representatives of MERS-CoV lineages representing MERS-CoV clades A and B³ and all published clade C (non-A, non-B) MERS-CoV complete

genomes, as of 1st Feb 2019⁴. Alignments were made using MAFFT⁵ in Geneious R11 (www.geneious.com, Biomatters Ltd, New Zealand). Bayesian phylogeographic reconstructions were made using Beast 1.10.4⁶ under assumption of a Hasegawa, Kishino and Yano (HKY) gamma 4 nucleotide substitution model as in Dudas et al.⁷ a strict molecular clock, and a calibration of substitution rate based on recorded or database-derived sampling dates of taxa. Geographic locations were reconstructed at all tree nodes based on phylogeographic diffusion in discrete space using trait matrices that incorporate geographic centers of countries of origin (Morocco, Burkina Faso, Nigeria, Egypt, Sudan, Djibouti, Saudi Arabia, Jordan, United Arab Emirates). A Bayesian stochastic search variable selection (BSSVS) procedure was chosen, in order to limit the number of migration rates to only those that are necessary to explain the overall changes of location traits in light of the underlying phylogenetic tree structure⁸. Significance of migration rates is determined by toggled rate indicators for each migration rate in the trait change matrix, followed by Bayes factor analyses of posterior versus prior probability of the rate of interest activated. Phylogeographic rendering as well as Bayes factor analysis was done in Spread3⁹. Recombination analyses were made using SimPlot V3.5¹⁰ and RDP V4.95¹¹.

For analysis of effective reproductive number of MERS-CoV circulating in Saudi-Arabia we applied Bayesian Birth Death Skyline analyses in Beast2¹² using an alignment covering a non-recombinant part of the MERS-CoV genome (corresponding to position 140 to 15985 in the MERS-CoV/EMC/2012 reference strain) and containing all MERS-CoV sequences of clades A and B as available in GenBank as of 1st April 2019. In accordance with Dudas et al.⁷, we followed the rationale that human primary cases or unique viruses observed in outbreaks represent one-time samples from the ongoing enzootic circulation in camels. We removed all human cases except primary cases and outbreak index cases. For data published up to 2015, we used the same index sequences from human outbreaks as in Dudas et al.⁷. For sequences published in GenBank after 2015, index cases could often not be identified due to a lack of associated scientific publications. We therefore identified clusters of similar human-derived sequences that fell into timeframes compatible with outbreak settings (typically, phylogenetically clustered human samples taken within up to 3 months from each other) and removed all sequences except one representative from these potential human-to-human clusters. More than one sequence per time cluster was only left in the dataset when these sequences were considerably different from each other, suggesting no transmission link between humans (=these sequences were regarded as likely to represent independent index sequences). This curation of the dataset caused reduction of the original alignment from 261 to 178 sequences (refer to supplementary material, Table 2).

The following parameter and prior settings were chosen in Beast2: Based on Dudas et al.⁷, the MRCA of the sequences under analysis falls in the end of 2011. As the origin date in BDSKY analysis has to be older than the root age, its lower bound was set to 2372 days (latest date in our sample set, May 2018). The “become uninfected” rate (the reciprocal of the duration of infection) was set to 0.05, based on Dudas et al.⁷ (we note that the true infectious period is probably shorter because Adney et al.¹³, as used for reference in Dudas et al.⁷, infected naïve camels and detected infectious nasal shedding only up to day 7 while the prior setting implicates 20 days being infectious). Priors for the sampling proportion followed the consideration that our alignment contains 178 sequences sampled over 2106 days (=5.76 years). According to a projection in Dudas et al.⁷, there are 186,750 infections per year in KSA, resulting in a sample frequency of 178 samples / 186,750 infections per year * 5.76 years = 0.0055. We chose a lognormal distribution with M= -5.2, S = 1.25 that leaves slightly more than 95% of the prior under 0.05. For the runs containing no new camel sequences from our present study, the following values were modified: Sample frequency: alignment contains 154 sequences sampled over 1690 days (=4.63 years). Accordingly: $154 / 186,750 * 4.63 = 0.0038$, parametrized as a lognormal distribution with M= -5.5, S = 1.25. The origin date was set to 2007 days minimum, adjusted to the reduced sampling timeframe caused by elimination of year 2018 sequences. Because relaxed molecular clock analyses failed to converge, all analyses were performed with a strict molecular clock assumption.

General remarks on camel importation and trade

There are approximately 14 million camels worldwide (1.6 million in KSA) of which 90% are dromedary camels. In KSA, dromedary camels are indispensable and fulfil several important domestic, commercial and nutritional demands. Camel trade is a large industry and local demands are met by importation of camels from outside KSA. Camels serve community, trading, farming, transportation, and leisure-related purposes and are also sources of milk, meat, wool and leather. The main source of dromedary camels for the Arabian Peninsula is the importation of life camels from the Horn of Africa (Somalia, Ethiopia, Sudan, Kenya and Djibouti), and North Africa (mainly Egypt, Libya) mainly through the ports of Jeddah and, to a much lesser extent, Jizan in Saudi Arabia¹⁴. In 2013,

official records in Saudi Arabia reported the import of 131,932 camels representing more than 70% of the number of animals slaughtered in the country¹⁵. Live imported camels are distributed across the Kingdom.

The increased urbanization of the human population in KSA has led to changes in patterns of camel husbandry. A report by Abdallah et al.¹⁶ described 6 types of camel farms and husbandry in Saudi Arabia; commercial farms, racing farms, use of camels for leisure for urban dwellers (weekend farms), traditional camel farms, camels for renting and camels for prestige. Camel herds are frequently moved across the Arabian Peninsula and may cross the borders of other Gulf countries for reasons such as grazing, participation in camel races and camel trade fairs. However, there is no official national identification procedure, nor is there an obligatory vaccination campaign focused on camels; it is difficult to quantify the extent of animal movements. Some general patterns are known. For instance, camels from both UAE and Qatar are moved every year during the winter season (November – February) for grazing in the Eastern region of Saudi Arabia. Detailed spatiotemporal studies of camel movement patterns are lacking, but camels are generally moved and traded over wide distances. This is also reflected in scientific data. For instance, a recent study observed little phylogeographic signal in genetic analyses of the modern dromedary population, indicative of extensive gene flow¹⁷.

The regular movement of camels across many countries may contribute to the transfer, evolution and amplification of MERS-CoV. For instance, a high degree of relatedness between MERS-CoV sequences obtained from UAE, Burydah/KSA and Al-Ahsa in camels and humans was seen¹⁸. MERS CoV has been detected in camels crossing into the UAE from Saudi Arabia^{19,20}.

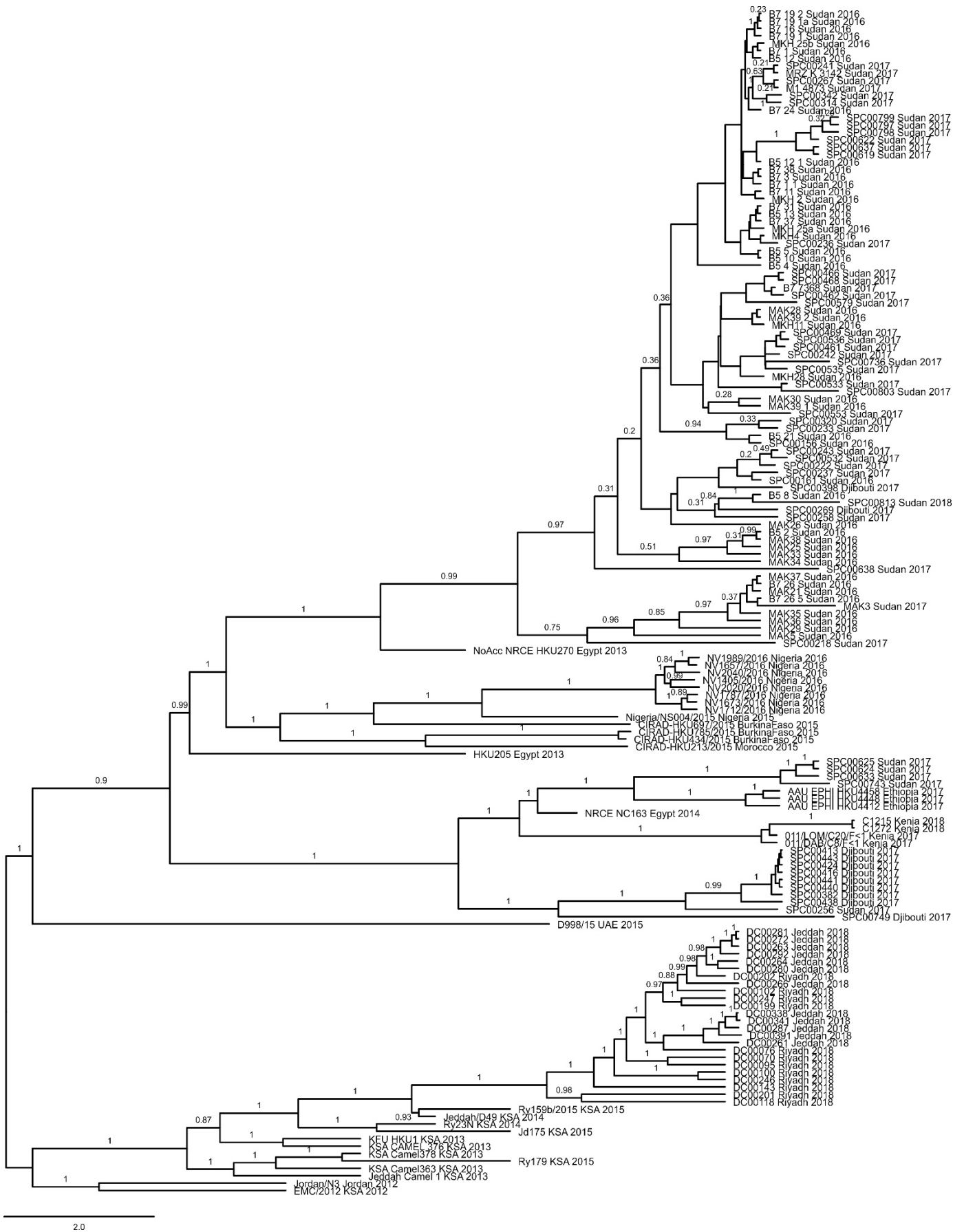


Figure S1: Tree as in Figure 1 without collapsing of clades. Numbers show the number of trees that have the node of interest represented in the 95% highest posterior density. Values below 0.2 are omitted.

Table S1. Sequences used for phylogenetic and phylogeographic analyses as shown in Figure 1, including accession numbers for viruses isolated in the present study.

GenBank Acc. No.	Country	Sampling date	Strain	ORF4b deletion types	Clade
JX869059	KSA	30/06/2012	EMC/2012_KSA_2012	0	A
KC776174	Jordan	30/06/2012	Jordan/N3_Jordan_2012	0	A
MN541187	KSA	14/05/2018	DC00070_Riyadh_2018	0	B
MN541188	KSA	14/05/2018	DC00076_Riyadh_2018	0	B
MN541186	KSA	14/05/2018	DC00095_Riyadh_2018	0	B
MN541185	KSA	14/05/2018	DC00100_Riyadh_2018	0	B
MN541189	KSA	14/05/2018	DC00102_Riyadh_2018	0	B
MN541181	KSA	14/05/2018	DC00118_Riyadh_2018	0	B
MN541183	KSA	14/05/2018	DC00143_Riyadh_2018	0	B
MN541190	KSA	14/05/2018	DC00199_Riyadh_2018	0	B
MN541182	KSA	14/05/2018	DC00201_Riyadh_2018	0	B
MN541198	KSA	14/05/2018	DC00202_Riyadh_2018	0	B
MN541184	KSA	14/05/2018	DC00246_Riyadh_2018	0	B
MN541191	KSA	14/05/2018	DC00247_Riyadh_2018	0	B
MN541193	KSA	18/07/2018	DC00261_Jeddah_2018	0	B
MN541202	KSA	18/07/2018	DC00263_Jeddah_2018	0	B
MN541199	KSA	14/07/2018	DC00264_Jeddah_2018	0	B
MN541192	KSA	18/07/2018	DC00266_Jeddah_2018	0	B
MN541203	KSA	18/07/2018	DC00272_Jeddah_2018	0	B
MN541200	KSA	18/07/2018	DC00280_Jeddah_2018	0	B
MN541204	KSA	18/07/2018	DC00281_Jeddah_2018	0	B
MN541195	KSA	19/07/2018	DC00287_Jeddah_2018	0	B
MN541201	KSA	19/07/2018	DC00292_Jeddah_2018	0	B
MN541196	KSA	15/07/2018	DC00338_Jeddah_2018	0	B
MN541197	KSA	25/07/2018	DC00341_Jeddah_2018	0	B
MN541194	KSA	01/08/2018	DC00391_Jeddah_2018	0	B
KT368866	KSA	30/06/2015	Jd175_KSA_2015	0	B
KT368841	KSA	30/06/2014	Jeddah/D49_KSA_2014	0	B
KF917527	KSA	30/06/2013	Jeddah-Camel-1_KSA_2013	0	B
KJ650297	KSA	30/06/2013	KFU-HKU1_KSA_2013	0	B
KJ713298	KSA	30/06/2013	KSA_Camel363_KSA_2013	0	B
KJ713296	KSA	30/06/2013	KSA_Camel378_KSA_2013	0	B
KJ713299	KSA	30/06/2013	KSA-CAMEL-376_KSA_2013	0	B
KT368870	KSA	30/06/2015	Ry159b/2015_KSA_2015	0	B
KT368875	KSA	30/06/2015	Ry179_KSA_2015	0	B
KT368825	KSA	30/06/2014	Ry23N_KSA_2014	0	B
MG923469	Morocco	03/03/2015	CIRAD-HKU213/2015_Morocco_2015	II	C1.1
MG923470	BurkinaFaso	23/02/2015	CIRAD-HKU434/2015_BurkinaFaso_2015	III	C1.1
MG923471	BurkinaFaso	18/03/2015	CIRAD-HKU785/2015_BurkinaFaso_2015	III	C1.1
MG923472	Nigeria	13/01/2015	Nigeria/NS004/2015_Nigeria_2015	V	C1.1
MG923473	BurkinaFaso	16/03/2015	CIRAD-HKU697/2015_BurkinaFaso_2015	IV	C1.1
MG923474	Nigeria	24/01/2016	NV1405/2016_Nigeria_2016	II	C1.1
MG923475	Nigeria	03/02/2016	NV1657/2016_Nigeria_2016	V	C1.1
MG923476	Nigeria	15/02/2016	NV1989/2016_Nigeria_2016	V	C1.1
MG923477	Nigeria	17/02/2016	NV2040/2016_Nigeria_2016	V	C1.1
MG923478	Nigeria	03/02/2016	NV1673/2016_Nigeria_2016	V	C1.1
MG923479	Nigeria	05/02/2016	NV1712/2016_Nigeria_2016	V	C1.1
MG923480	Nigeria	07/02/2016	NV1787/2016_Nigeria_2016	V	C1.1
MG923481	Nigeria	16/02/2016	NV2020/2016_Nigeria_2016	V	C1.1
MN541246	Sudan	12/12/2016	B5-10_Sudan_2016	VI	C1.2
MN541286	Sudan	12/12/2016	B5-12_Sudan_2016	VI	C1.2
MN541247	Sudan	12/12/2016	B5-12-1_Sudan_2016	VI	C1.2
MN541248	Sudan	12/12/2016	B5-13_Sudan_2016	VI	C1.2
MN541233	Sudan	12/12/2016	B5-2_Sudan_2016	VI	C1.2
MN541249	Sudan	12/12/2016	B5-21_Sudan_2016	VI	C1.2
MN541287	Sudan	12/12/2016	B5-4_Sudan_2016	VI	C1.2
MN541245	Sudan	12/12/2016	B5-5_Sudan_2016	VI	C1.2
MN541243	Sudan	12/12/2016	B5-8_Sudan_2016	0	C1.2
MN541250	Sudan	12/12/2016	B7-1_Sudan_2016	VI	C1.2
MN541285	Sudan	13/12/2016	B7-11_Sudan_2016	VI	C1.2
MN541252	Sudan	13/12/2016	B7-1-I_Sudan_2016	VI	C1.2
MN541290	Sudan	13/12/2016	B7-16_Sudan_2016	VI	C1.2
MN541291	Sudan	13/12/2016	B7-19-1_Sudan_2016	VI	C1.2
MN541292	Sudan	13/12/2016	B7-19-1a_Sudan_2016	VI	C1.2
MN541289	Sudan	13/12/2016	B7-19-2_Sudan_2016	VI	C1.2
MN541253	Sudan	13/12/2016	B7-24_Sudan_2016	VI	C1.2
MN541228	Sudan	13/12/2016	B7-26_Sudan_2016	0	C1.2
MN541222	Sudan	13/12/2016	B7-26-5_Sudan_2016	0	C1.2
MN541251	Sudan	13/12/2016	B7-3_Sudan_2016	VI	C1.2
MN541254	Sudan	13/12/2016	B7-31_Sudan_2016	VI	C1.2
MN541255	Sudan	13/12/2016	B7-37_Sudan_2016	VI	C1.2
MN541256	Sudan	13/12/2016	B7-38_Sudan_2016	VI	C1.2
MN541257	Sudan	21/02/2017	B7-7368_Sudan_2017	0	C1.2
KJ477103	Egypt	01/01/2013	HKU270_Egypt_2013	0	C1.2
MN541293	Sudan	07/03/2017	M1-4873_Sudan_2017	VI	C1.2
MN541224	Sudan	11/12/2016	MAK21_Sudan_2016	0	C1.2
MN541231	Sudan	11/12/2016	MAK25_Sudan_2016	0	C1.2
MN541235	Sudan	11/12/2016	MAK26_Sudan_2016	0	C1.2
MN541258	Sudan	11/12/2016	MAK28_Sudan_2016	VI	C1.2
MN541221	Sudan	11/12/2016	MAK29_Sudan_2016	0	C1.2
MN541223	Sudan	11/12/2017	MAK3_Sudan_2017	0	C1.2
MN541288	Sudan	11/12/2016	MAK30_Sudan_2016	0	C1.2
MN541232	Sudan	11/12/2016	MAK33_Sudan_2016	0	C1.2
MN541230	Sudan	11/12/2016	MAK34_Sudan_2016	0	C1.2
MN541225	Sudan	11/12/2016	MAK35_Sudan_2016	0	C1.2
MN541226	Sudan	11/12/2016	MAK36_Sudan_2016	0	C1.2
MN541227	Sudan	11/12/2016	MAK37_Sudan_2016	0	C1.2
MN541234	Sudan	11/12/2016	MAK38_Sudan_2016	0	C1.2
MN541260	Sudan	11/12/2016	MAK39-1_Sudan_2016	0	C1.2
MN541259	Sudan	11/12/2016	MAK39-2_Sudan_2016	0	C1.2
MN541220	Sudan	11/12/2016	MAK5_Sudan_2016	0	C1.2
MN541264	Sudan	28/12/2016	MKH11_Sudan_2016	VI	C1.2
MN541265	Sudan	28/12/2016	MKH-2_Sudan_2016	VI	C1.2
MN541284	Sudan	28/12/2016	MKH-25a_Sudan_2016	VI	C1.2
MN541262	Sudan	28/12/2016	MKH-25b_Sudan_2016	VI	C1.2
MN541263	Sudan	28/12/2016	MKH28_Sudan_2016	0	C1.2
MN541261	Sudan	28/12/2016	MKH4_Sudan_2016	VI	C1.2

MN541296	Sudan	06/03/2017	MRZ-K-3142_Sudan_2017	VI	C1.2
MN541266	Sudan	13/12/2016	SPC00156_Sudan_2016	0	C1.2
MN541236	Sudan	13/12/2016	SPC00161_Sudan_2016	VI	C1.2
MN541219	Sudan	21/02/2017	SPC00218_Sudan_2017	0	C1.2
MN541237	Sudan	21/02/2017	SPC00222_Sudan_2017	0	C1.2
MN541267	Sudan	05/03/2017	SPC00233_Sudan_2017	0	C1.2
MN541268	Sudan	05/03/2017	SPC00236_Sudan_2017	VI	C1.2
MN541269	Sudan	05/03/2017	SPC00237_Sudan_2017	0	C1.2
MN541294	Sudan	06/03/2017	SPC00241_Sudan_2017	VI	C1.2
MN541270	Sudan	06/03/2017	SPC00242_Sudan_2017	VI	C1.2
MN541238	Sudan	06/03/2017	SPC00243_Sudan_2017	VI	C1.2
MN541241	Sudan	06/03/2017	SPC00258_Sudan_2017	VI	C1.2
MN541295	Sudan	07/03/2017	SPC00267_Sudan_2017	VI	C1.2
MN541242	Djibouti	05/03/2017	SPC00269_Djibouti_2017	0	C1.2
MN541297	Sudan	21/03/2017	SPC00314_Sudan_2017	VI	C1.2
MN541271	Sudan	21/03/2017	SPC00320_Sudan_2017	VI	C1.2
MN541298	Sudan	23/03/2017	SPC00342_Sudan_2017	VI	C1.2
MN541239	Djibouti	27/03/2017	SPC00398_Djibouti_2017	VI	C1.2
MN541272	Sudan	02/04/2017	SPC00461_Sudan_2017	0	C1.2
MN541273	Sudan	02/04/2017	SPC00462_Sudan_2017	0	C1.2
MN541274	Sudan	02/04/2017	SPC00466_Sudan_2017	0	C1.2
MN541275	Sudan	02/04/2017	SPC00468_Sudan_2017	0	C1.2
MN541276	Sudan	02/04/2017	SPC00469_Sudan_2017	0	C1.2
MN541240	Sudan	20/04/2017	SPC00532_Sudan_2017	0	C1.2
MN541277	Sudan	20/04/2017	SPC00533_Sudan_2017	0	C1.2
MN541278	Sudan	20/04/2017	SPC00535_Sudan_2017	VI	C1.2
MN541279	Sudan	20/04/2017	SPC00536_Sudan_2017	0	C1.2
MN541280	Sudan	07/05/2017	SPC00553_Sudan_2017	0	C1.2
MN541281	Sudan	06/06/2017	SPC00579_Sudan_2017	0	C1.2
MN541299	Sudan	20/09/2017	SPC00619_Sudan_2017	VI	C1.2
MN541300	Sudan	20/09/2017	SPC00622_Sudan_2017	VI	C1.2
MN541301	Sudan	20/09/2017	SPC00637_Sudan_2017	VI	C1.2
MN541229	Sudan	20/09/2017	SPC00638_Sudan_2017	VI	C1.2
MN541282	Sudan	12/11/2017	SPC00736_Sudan_2017	0	C1.2
MN541304	Sudan	26/12/2017	SPC00797_Sudan_2017	VI	C1.2
MN541302	Sudan	26/12/2017	SPC00798_Sudan_2017	VI	C1.2
MN541303	Sudan	26/12/2017	SPC00799_Sudan_2017	VI	C1.2
MN541283	Sudan	26/12/2017	SPC00803_Sudan_2017	0	C1.2
MN541244	Sudan	01/01/2018	SPC00813_Sudan_2018	0	C1.2
MK357908	Kenya	01/03/2017	011/DAB/C8/F<1_Kenya_2017	0	C2
MK357909	Kenya	01/03/2017	011/LOM/C20/F<1_Kenya_2017	0	C2
MG923466	Ethiopia	15/03/2017	AAU-EPHI-HKU4412_Ethiopia_2017	0	C2
MG923467	Ethiopia	15/03/2017	AAU-EPHI-HKU4448_Ethiopia_2017	0	C2
MG923468	Ethiopia	15/03/2017	AAU-EPHI-HKU4458_Ethiopia_2017	0	C2
MH734114	Kenya	13/03/2018	C1215_Kenya_2018	VII	C2
MH734115	Kenya	13/03/2018	C1272_Kenya_2018	VII	C2
KU740200	Egypt	30/06/2014	NRCE_NC163_Egypt_2014	0	C2
MN541206	Sudan	06/03/2017	SPC00256_Sudan_2017	VII	C2
MN541214	Djibouti	27/03/2017	SPC00382_Djibouti_2017	VII	C2
MN541213	Djibouti	27/03/2017	SPC00413_Djibouti_2017	VII	C2
MN541208	Djibouti	27/03/2017	SPC00416_Djibouti_2017	VII	C2
MN541212	Djibouti	27/03/2017	SPC00424_Djibouti_2017	VII	C2
MN541207	Djibouti	27/03/2017	SPC00438_Djibouti_2017	VII	C2
MN541209	Djibouti	27/03/2017	SPC00440_Djibouti_2017	VII	C2
MN541210	Djibouti	27/03/2017	SPC00441_Djibouti_2017	VII	C2
MN541211	Djibouti	27/03/2017	SPC00443_Djibouti_2017	VII	C2
MN541218	Sudan	20/09/2017	SPC00624_Sudan_2017	0	C2
MN541217	Sudan	20/09/2017	SPC00625_Sudan_2017	0	C2
MN541216	Sudan	20/09/2017	SPC00633_Sudan_2017	0	C2
MN541215	Sudan	12/11/2017	SPC00743_Sudan_2017	0	C2
MN541205	Djibouti	05/12/2017	SPC00749_Djibouti_2017	0	C2
KJ477102	Egypt	01/01/2013	HKU205_Egypt_2013	0	C3
KX108943	UAE	23/04/2015	D998/15_UAE_2015	0	Unclassified (NonA/B)

Samples in bold type were sequenced for this study.

Table S2: List of 178 sequences as used in the Bayesian birth death skyline analysis

Accession No	Source*	Host	Location	Date
MG011353	refstrain	human	KSA	01/06/2016
MG011355	refstrain	human	KSA	01/07/2016
MG011341	refstrain	human	KSA	06/03/2016
MH310912	refstrain	human	KSA	15/05/2017
MH432120	refstrain	human	KSA	28/02/2017
MN541189	DC00102	camel	Riyadh	14/05/2018
MN541186	DC00095	camel	Riyadh	14/05/2018
MN541191	DC00247	camel	Riyadh	14/05/2018
MN541188	DC00076	camel	Riyadh	14/05/2018
MN541198	DC00202	camel	Riyadh	14/05/2018
MN541192	DC00266	camel	Jeddah	18/07/2018
MN541183	DC00143	camel	Riyadh	14/05/2018
KT806044	refstrain	human	KSA	09/02/2015
KX154686	refstrain	human	KSA	01/02/2016
MN541199	DC00264	camel	Jeddah	18/07/2018
MN541202	DC00263	camel	Jeddah	18/07/2018
MN541204	DC00281	camel	Jeddah	18/07/2018
MN541201	DC00292	camel	Jeddah	19/07/2018
MN541194	DC00391	camel	Jeddah	01/08/2018
MN541195	DC00287	camel	Jeddah	19/07/2018
MN541190	DC00199	camel	Riyadh	14/05/2018
MN541203	DC00272	camel	Jeddah	18/07/2018
MN541196	DC00338	camel	Jeddah	25/07/2018
MN541197	DC00341	camel	Jeddah	25/07/2018
KT368870	refstrain	camel	KSA	01/03/2015
KT806048	refstrain	human	KSA	07/02/2015
KT368835	refstrain	camel	KSA	01/12/2014
KT368836	refstrain	camel	KSA	01/12/2014
KT368837	refstrain	camel	KSA	01/12/2014
KT368838	refstrain	camel	KSA	01/12/2014
KT368839	refstrain	camel	KSA	01/12/2014
KT368840	refstrain	camel	KSA	01/12/2014
KT368841	refstrain	camel	KSA	01/12/2014
KT368842	refstrain	camel	KSA	01/12/2014
KT368846	refstrain	camel	KSA	01/11/2014
KT368847	refstrain	camel	KSA	01/11/2014
KT368848	refstrain	camel	KSA	01/11/2014
KX154684	refstrain	human	KSA	01/02/2016
MN541200	DC00280	camel	Jeddah	18/07/2018
KT368833	refstrain	camel	KSA	01/12/2014
KY688118	refstrain	human	KSA	07/02/2015
MN541185	DC00100	camel	Riyadh	14/05/2018
MN541184	DC00246	camel	Riyadh	14/05/2018
KT368834	refstrain	camel	KSA	01/12/2014
KT368873	refstrain	camel	KSA	01/03/2015
KT368874	refstrain	camel	KSA	01/03/2015
KT026453	refstrain	human	KSA	10/02/2015
KT806047	refstrain	human	KSA	27/03/2015
KT806050	refstrain	human	KSA	12/02/2015
KT806052	refstrain	human	KSA	02/02/2015
MG011354	refstrain	human	KSA	16/06/2016
MG011359	refstrain	human	KSA	29/03/2016
MG011362	refstrain	human	KSA	25/12/2016
MN541187	DC00070	camel	Riyadh	14/05/2018
MN541193	DC00261	camel	Jeddah	18/07/2018
KT368889	refstrain	camel	KSA	01/03/2015
KT368826	refstrain	camel	KSA	01/07/2014
KT368878	refstrain	camel	KSA	01/03/2015
KT368868	refstrain	camel	KSA	01/03/2015
KT368876	refstrain	camel	KSA	01/03/2015
KT368877	refstrain	camel	KSA	01/03/2015
KT806046	refstrain	human	KSA	10/05/2015
MG011361	refstrain	human	KSA	10/01/2017
KF600628	refstrain	human	KSA	04/06/2013
KT368872	refstrain	camel	KSA	01/03/2015
MG757597	refstrain	human	KSA	24/08/2015
KT026454	refstrain	human	KSA	01/03/2015
KT806053	refstrain	human	KSA	02/02/2015
KY688120	refstrain	human	KSA	10/05/2015
KX154689	refstrain	human	KSA	01/01/2016
MH395139	refstrain	human	KSA	04/12/2016
KT368884	refstrain	camel	KSA	01/04/2015
KT368885	refstrain	camel	KSA	01/04/2015
KT368886	refstrain	camel	KSA	01/04/2015
KT368858	refstrain	camel	KSA	01/01/2015
KT368859	refstrain	camel	KSA	01/01/2015
KT368860	refstrain	camel	KSA	01/01/2015
KT368861	refstrain	camel	KSA	01/01/2015
KT368862	refstrain	camel	KSA	01/01/2015
KT368863	refstrain	camel	KSA	01/01/2015

KT368864	refstrain	camel	KSA	01/01/2015
KT368865	refstrain	camel	KSA	01/01/2015
KT368888	refstrain	camel	KSA	01/04/2015
MG757604	refstrain	human	KSA	31/01/2016
MG011349	refstrain	human	KSA	13/04/2016
KY688123	refstrain	human	KSA	13/05/2015
KT806051	refstrain	human	KSA	05/02/2015
KT368880	refstrain	camel	KSA	01/04/2015
KT368881	refstrain	camel	KSA	01/04/2015
KT368882	refstrain	camel	KSA	01/04/2015
KT368883	refstrain	camel	KSA	01/04/2015
KU851864	refstrain	human	KSA	24/08/2015
MG520075	refstrain	human	KSA	13/10/2015
MH306207	refstrain	human	KSA	01/08/2016
MG011358	refstrain	human	KSA	21/09/2016
MG757600	refstrain	human	KSA	01/09/2015
MH310909	refstrain	human	KSA	05/05/2017
KU851859	refstrain	human	KSA	12/07/2015
MG366880	refstrain	human	KSA	02/06/2015
KT368871	refstrain	camel	KSA	01/03/2015
MG011347	refstrain	human	KSA	13/03/2016
KX154691	refstrain	human	KSA	25/02/2016
KT368887	refstrain	camel	KSA	01/04/2015
KU851863	refstrain	human	KSA	27/08/2015
MG757595	refstrain	human	KSA	24/08/2015
MG912607	refstrain	human	KSA	11/06/2017
MG757601	refstrain	human	KSA	01/09/2015
KF600652	refstrain	human	KSA	30/10/2012
KT368869	refstrain	camel	KSA	01/03/2015
KX154690	refstrain	human	KSA	01/01/2016
KX154685	refstrain	human	KSA	01/02/2016
MG011356	refstrain	human	KSA	12/07/2016
MG011351	refstrain	human	KSA	10/04/2016
MG011346	refstrain	human	KSA	14/03/2016
KJ156910	refstrain	human	KSA	05/08/2013
KT368879	refstrain	camel	KSA	01/03/2015
KT368852	refstrain	camel	KSA	01/10/2014
KT368890	refstrain	camel	KSA	01/03/2015
MG011345	refstrain	human	KSA	18/03/2016
MG011344	refstrain	human	KSA	13/03/2016
MG011340	refstrain	human	KSA	05/03/2016
KF600630	refstrain	human	KSA	13/05/2013
MG011357	refstrain	human	KSA	21/09/2016
KF186566	refstrain	human	KSA	21/04/2013
KT368849	refstrain	camel	KSA	01/10/2014
KT368850	refstrain	camel	KSA	01/10/2014
KT368854	refstrain	camel	KSA	01/09/2014
KT368866	refstrain	camel	KSA	01/02/2015
KT368867	refstrain	camel	KSA	01/02/2015
KT806055	refstrain	human	KSA	10/02/2015
KT368825	refstrain	camel	KSA	01/07/2014
KU710264	refstrain	human	KSA	04/11/2014
KX154687	refstrain	human	KSA	01/02/2016
KT368827	refstrain	camel	KSA	01/09/2014
KT368855	refstrain	camel	KSA	01/09/2014
KT368856	refstrain	camel	KSA	01/09/2014
KT368857	refstrain	camel	KSA	01/09/2014
KT368851	refstrain	camel	KSA	01/10/2014
MG011342	refstrain	human	KSA	07/03/2016
KT368828	refstrain	camel	KSA	01/12/2014
KT368843	refstrain	camel	KSA	01/12/2014
KT368844	refstrain	camel	KSA	01/12/2014
KT368845	refstrain	camel	KSA	01/12/2014
MN541182	DC00201	camel	Riyadh	14/05/2018
KJ650295	refstrain	camel	KSA	30/12/2013
KJ650296	refstrain	camel	KSA	30/12/2013
KJ650297	refstrain	camel	KSA	30/11/2013
KJ713299	refstrain	camel	KSA	01/11/2013
KJ156869	refstrain	human	KSA	17/07/2013
KM027262	refstrain	human	KSA	22/04/2014
KT368853	refstrain	camel	KSA	01/09/2014
MG912595	refstrain	human	KSA	31/05/2017
KF600612	refstrain	human	KSA	23/10/2012
MG366881	refstrain	human	KSA	07/06/2015
MN541181	DC00118	camel	Riyadh	14/05/2018
KM027256	refstrain	human	KSA	03/04/2014
KJ156952	refstrain	human	KSA	01/03/2013
KJ713298	refstrain	camel	KSA	01/11/2013
KJ713295	refstrain	camel	KSA	01/11/2013
KJ713297	refstrain	camel	KSA	01/11/2013
KT877351	refstrain	camel	KSA	01/09/2014
KT877350	refstrain	camel	KSA	01/09/2014
MH013216	refstrain	human	KSA	15/10/2015
KJ713296	refstrain	camel	KSA	01/11/2013

KJ156944	refstrain	human	KSA	02/07/2013
KJ156881	refstrain	human	KSA	12/06/2013
KF600613	refstrain	human	KSA	05/02/2013
KF917527	refstrain	camel	KSA	08/11/2013
KF958702	refstrain	human	KSA	05/11/2013
KJ156949	refstrain	human	KSA	12/06/2013
KT368829	refstrain	camel	KSA	01/12/2014
KT368830	refstrain	camel	KSA	01/12/2014
KT368832	refstrain	camel	KSA	01/12/2014
KT368831	refstrain	camel	KSA	01/12/2014
KT368875	refstrain	camel	KSA	01/03/2015
KT368824	refstrain	camel	KSA	01/05/2014
KR011266	refstrain	human	KSA	06/01/2015
KY688119	refstrain	human	KSA	01/05/2015

*refstrain = strain downloaded from GenBank; DCXXXXX = strain from present study

Table S3: Bayes factor support values for migration parameter matrix

Migration		Support
From	To	Bayes factor*
KSA	Burkina	1.560062757
KSA	Nigeria	3.241761123
KSA	Sudan	0.338070613
KSA	Djibouti	0.234949677
KSA	Kenya	0.295927703
KSA	Ethiopia	0.360259191
KSA	Jordan	18.94969109
KSA	UAE	17.40214172
KSA	Morocco	0.868004162
KSA	Egypt	0.390922955
Burkina	Nigeria	5.404771122
Burkina	Sudan	1.952542644
Burkina	Djibouti	0.355912476
Burkina	Kenya	1.187489927
Burkina	Ethiopia	1.748378817
Burkina	Jordan	0.418098321
Burkina	UAE	0.499879625
Burkina	Morocco	37291.32954**
Burkina	Egypt	0.710172379
Nigeria	Sudan	2.847235533
Nigeria	Djibouti	0.59787071
Nigeria	Kenya	2.7528712
Nigeria	Ethiopia	5.047123431
Nigeria	Jordan	0.67318858
Nigeria	UAE	0.899855386
Nigeria	Morocco	4.650547005
Nigeria	Egypt	1.481778823
Sudan	Djibouti	0.237521292
Sudan	Kenya	0.260282433
Sudan	Ethiopia	0.467729749
Sudan	Jordan	0.239065711
Sudan	UAE	0.226740761
Sudan	Morocco	2.102620602
Sudan	Egypt	0.367886186
Djibouti	Kenya	38.33427865
Djibouti	Ethiopia	0.261322651
Djibouti	Jordan	0.209398611
Djibouti	UAE	0.205338089
Djibouti	Morocco	0.185651038
Djibouti	Egypt	1.081438108
Kenya	Ethiopia	0.273320577
Kenya	Jordan	0.178126474
Kenya	UAE	0.153232422
Kenya	Morocco	0.439961612
Kenya	Egypt	4.300139729
Ethiopia	Jordan	0.263925348
Ethiopia	UAE	0.228277587
Ethiopia	Morocco	0.666355543
Ethiopia	Egypt	0.4097448
Jordan	UAE	0.858594184
Jordan	Morocco	0.196734583
Jordan	Egypt	0.285384042
UAE	Morocco	0.263925348
UAE	Egypt	0.269662121
Morocco	Egypt	0.283281316

*Numbers in bold type represent significant migrations

**Note that this rate is based on a single sequence and therefore uncertain

Supplementary bibliography

1. Corman VM, Ithete NL, Richards LR, et al. Rooting the phylogenetic tree of middle East respiratory syndrome coronavirus by characterization of a conspecific virus from an African bat. *J Virol* 2014; **88**(19): 11297-303.
2. Wang Y, Liu D, Shi W, et al. Origin and Possible Genetic Recombination of the Middle East Respiratory Syndrome Coronavirus from the First Imported Case in China: Phylogenetics and Coalescence Analysis. *MBio* 2015; **6**(5): e01280-15.
3. Sabir JS, Lam TT, Ahmed MM, et al. Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. *Science* 2016; **351**(6268): 81-4.
4. Chu DKW, Hui KPY, Perera R, et al. MERS coronaviruses from camels in Africa exhibit region-dependent genetic diversity. *Proc Natl Acad Sci U S A* 2018; **115**(12): 3144-9.
5. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013; **30**(4): 772-80.
6. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol* 2018; **4**(1): vey016.
7. Dudas G, Carvalho LM, Rambaut A, Bedford T. MERS-CoV spillover at the camel-human interface. *Elife* 2018; **7**.
8. Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. *PLoS Comput Biol* 2009; **5**(9): e1000520.
9. Bielejec F, Baele G, Vrancken B, Suchard MA, Rambaut A, Lemey P. SpreaD3: Interactive Visualization of Spatiotemporal History and Trait Evolutionary Processes. *Mol Biol Evol* 2016; **33**(8): 2167-9.
10. Lole KS, Bollinger RC, Paranjape RS, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 1999; **73**(1): 152-60.
11. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evol* 2015; **1**(1): vev003.
12. Stadler T, Kuhnert D, Bonhoeffer S, Drummond AJ. Birth-death skyline plot reveals temporal changes of epidemic spread in HIV and hepatitis C virus (HCV). *Proc Natl Acad Sci U S A* 2013; **110**(1): 228-33.
13. Adney DR, van Doremalen N, Brown VR, et al. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. *Emerg Infect Dis* 2014; **20**(12): 1999-2005.
14. Faye B. Camel Farming Sustainability: The Challenges of the Camel Farming System in the XXIth Century. *Journal of Sustainable Development* 2013; **6**(12).
15. @influenza_bio, Mackay IM. Are MERS cases in Saudi Arabia and the UAE linked to camel imports? 2014. <http://virologydownunder.blogspot.com/2014/06/are-mers-cases-in-saudi-arabia-and-uae.html>.
16. Abdallah HR, Faye B. Typology of camel farming system in Saudi Arabia. *Emir J Food Agr* 2013; **25**(4): 250-60.
17. Almathen F, Charruau P, Mohandesan E, et al. Ancient and modern DNA reveal dynamics of domestication and cross-continental dispersal of the dromedary. *Proc Natl Acad Sci U S A* 2016; **113**(24): 6707-12.
18. Hemida MG, Alnaeem A, Chu DK, et al. Longitudinal study of Middle East Respiratory Syndrome coronavirus infection in dromedary camel herds in Saudi Arabia, 2014-2015. *Emerg Microbes Infect* 2017; **6**(6): e56.
19. Paden CR, Yusof M, Al Hammadi ZM, et al. Zoonotic origin and transmission of Middle East respiratory syndrome coronavirus in the UAE. *Zoonoses Public Health* 2018; **65**(3): 322-33.
20. Yusof MF, Eltahir YM, Serhan WS, et al. Prevalence of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels in Abu Dhabi Emirate, United Arab Emirates. *Virus Genes* 2015; **50**(3): 509-13.