Discovery and sequence analysis of four deltacoronaviruses from birds in the Middle East suggest interspecies jumping and recombination as potential mechanism for avian-to-avian and avian-to-mammalian transmission

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Running title: Novel deltacoronaviruses in Middle East

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26

27 Word count for abstract: 233 words

28 Word count for text: 4,295 words

ABSTRACT

The emergence of Middle East respiratory syndrome showed once again that coronaviruses (CoVs) in animals are potential source for epidemics in humans. To explore the diversity of deltacoronaviruses in animals in the Middle East, we tested fecal samples from 1,356 mammals and birds in Dubai. Four novel deltacoronaviruses were detected from eight birds of four species by RT–PCR: FalCoV UAE-HKU27 from a falcon, HouCoV UAE-HKU28 from a houbara bustard, PiCoV UAE-HKU29 from a pigeon and QuaCoV UAE-HKU30 from five quails. Complete genome sequencing showed that FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 belong to the same CoV species, suggesting recent interspecies transmission between falcons and their preys, houbara bustards and pigeons possibly along the food chain. Western blot detected specific anti-FalCoV UAE-HKU27 antibodies in 33 (75%) of 44 falcon serum samples, supporting genuine infection in falcons after virus acquisition. QuaCoV UAE-HKU30 belongs to the same CoV species as PorCoV HKU15 and SpCoV HKU17 discovered previously from swine and tree sparrows respectively, supporting avian-to-swine transmission. Recombination involving the spike protein is common among deltacoronaviruses, which may facilitate cross-species transmission. FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 were originated from recombination between WECoV HKU16 and MRCoV HKU18; QuaCoV UAE-HKU30 from recombination between PorCoV HKU15/SpCoV HKU17 and MunCoV HKU13, and PorCoV HKU15 from recombination between SpCoV HKU17 and BuCoV HKU11. Birds in the Middle East are hosts for diverse deltacoronaviruses with potential for interspecies transmission.

54 **IMPORTANCE**

55 During an attempt to explore the diversity of deltacoronaviruses among mammals and
56 birds in Dubai, four novel deltacoronaviruses were detected in fecal samples from
57 eight birds of four different species: FalCoV UAE-HKU27 from a falcon, HouCoV
58 UAE-HKU28 from a houbara bustard, PiCoV UAE-HKU29 from a pigeon and
59 QuaCoV UAE-HKU30 from five quails. Genome analysis revealed evidence of recent
60 interspecies transmission between falcons and their preys, houbara bustards and
61 pigeons possibly along the food chain, as well as avian-to-swine transmission.
62 Recombination, which is known to occur frequently in some coronaviruses, was also
63 common among these deltacoronaviruses and predominantly occurred at the spike
64 region. Such recombination, involving the receptor binding protein, may contribute to
65 the emergence of new viruses capable of infecting new hosts. Birds in the Middle East
66 are hosts for diverse deltacoronaviruses with potential for interspecies transmission.
67

INTRODUCTION

Coronaviruses (CoVs) infect humans and a wide variety of animals, causing respiratory, enteric, hepatic, and neurological diseases of varying severity. Based on genotypic and serological characterization, CoVs were traditionally divided into three distinct groups (1–3). In 2008, the Coronavirus Study Group of the International Committee for Taxonomy of Viruses (ICTV) has replaced the traditional groups 1, 2 and 3 CoVs with three genera, *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus* (4). As a result of the unique mechanism of viral replication, CoVs have a high frequency of recombination (1). Their tendency for recombination and the inherently high mutation rates typical of RNA viruses may allow rapid adaptation to new hosts and ecological niches (5–9).

The severe acute respiratory syndrome (SARS) epidemic and the discovery of SARS coronavirus (SARS-CoV)-like viruses from palm civets in China have boosted interests in discovery of novel CoVs in both humans and animals (10–15). In 2004, a novel human CoV (HCoV) of the genus *Alphacoronavirus*, human coronavirus NL63 (HCoV NL63), was reported independently by two groups in 2004 (16, 17). In 2005, we also described the discovery of another novel HCoV, human coronavirus HKU1 (HCoV HKU1), under the genus *Betacoronavirus* (18–20). As for animal CoVs, we and others have described the discovery of SARS-CoV-like viruses in horseshoe bats in Hong Kong and other provinces of China (21, 22). Based on these findings, we expanded molecular surveillance studies to examine the diversity of CoVs in bats of southern China, during which at least nine other novel CoVs were discovered, including two novel lineages in *Betacoronavirus*, lineage C and D (23–25). Other novel CoVs in bats and other animals have also been discovered by various research

groups, which has broadened our knowledge on the diversity and evolution of CoVs (7, 26–29).

Birds are the reservoir of major emerging viruses, most notably, avian influenza viruses (30). Due to their flocking behavior and abilities to fly over long distances, birds have the potential to disseminate emerging viruses efficiently among themselves and to other animals and human. Yet, the number of known CoVs in birds is relatively small as compared to bats. In 2009, we described the discovery of three novel CoVs in three families of birds, named bulbul coronavirus HKU11 (BuCoV HKU11), thrush coronavirus HKU12 (ThCoV HKU12) and munia coronavirus HKU13 (MunCoV HKU13) (31). These three CoVs formed a unique group of CoV, which were subsequently classified as a novel genus of CoV, *Deltacoronavirus* (4). Recently, we have further discovered seven additional deltacoronaviruses: one from pigs, named porcine coronavirus HKU15 (PorCoV HKU15), and six from birds, named white-eye coronavirus HKU16 (WECoV HKU16), sparrow coronavirus HKU17 (SpCoV HKU17), magpie robin coronavirus HKU18 (MRCoV HKU18), night-heron coronavirus HKU19 (NHCoV HKU19), wigeon coronavirus HKU20 (WiCoV HKU20) and common-moorhen coronavirus HKU21 (CMCoV HKU21) (32). Subsequently, PorCoV HKU15 was found to be present widely in pigs and Asia and North America, and has been associated with fatal outbreaks in pig farms (33–42). The findings supported that deltacoronaviruses have the potential for avian-to-mammalian transmission and emergence in mammals. We hypothesize that there are other previously unrecognized deltacoronaviruses present in geographical locations other than Hong Kong, which may also have the potential for emergence. To test this hypothesis, we carried out a molecular epidemiology study in 1,356 mammals and birds in Dubai, The United Arab Emirates in the Middle East. Based on the results of

comparative genome and phylogenetic analyses, we propose four novel CoVs in *Deltacoronavirus*. The results of sero-epidemiological studies were also discussed.

RESULTS

Animal surveillance and identification of four novel deltacoronaviruses. A total of 1,356 fecal samples from 1,164 mammals and 192 birds were obtained (Table 1). Reverse transcription–polymerase chain reaction (RT–PCR) for a 440-bp fragment in the RNA-dependent-RNA polymerase (RdRp) genes of CoVs was positive in specimens from eight birds of four species. Sequencing results suggested the presence of four novel deltacoronaviruses: the first (falcon CoV [FalCoV] UAE-HKU27]) from one falcon (family *Falconidae*), the second (houbara CoV [HouCoV] UAE-HKU28) from one houbara bustard (family *Otididae*), the third (pigeon CoV [PiCoV] UAE-HKU29) from one pigeon (family *Columbidae*) and the fourth (quail CoV [QuaCoV] UAE-HKU30) from five quails (family *Phasianidae*) (Table 1, Fig. 1). None of the 1,164 mammals tested were positive.

Genome organization and coding potentials of the four novel deltacoronaviruses. The complete genome sequences of one strain each of FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 and two strains of QuaCoV UAE-HKU30 were obtained by assembly of the sequences of RT–PCR products from the RNA extracted from the corresponding individual specimens.

The genome sizes of the four novel CoVs ranged from 25,871 (QuaCoV UAE-HKU30 strain 1101F) to 26,162 bases (FalCoV UAE-HKU27 strain 988F) and their G + C contents ranged from 0.39 to 0.42 (Table 2). Their genome organizations are typical of CoVs, with the gene order 5′-replicase ORF1ab, spike (S), envelope (E), membrane (M), nucleocapsid (N)-3′ (Fig. 2 and Table 3). Both 5′ and 3′ ends contain short untranslated regions. The replicase ORF1ab occupies 18.363 to 18.678 kb of the genomes (Table 3). This open reading frame (ORF) encodes a number of putative proteins, including nsp3 (which contains the putative papain-like protease [PL^{pro}]),

144 nsp5 (putative chymotrypsin-like protease [3CL^{pro}]), nsp12 (putative RdRp), nsp13
145 (putative helicase) and other proteins of unknown functions. Overall, the cleavage
146 sites for the non-structural proteins in ORF1ab of FalCoV UAE-HKU27, HouCoV
147 UAE-HKU28, PiCoV UAE-HKU29 and QuaCoV UAE-HKU30 were similar to other
148 deltacoronaviruses, except for nsp3/nsp4 and nsp15/16 in FalCoV UAE-HKU27,
149 HouCoV UAE-HKU28 and PiCoV UAE-HKU29 (Table 4). In fact, the amino acids
150 downstream to the putative cleavage site at nsp3/nsp4 are quite variable across
151 different deltacoronaviruses (Table 4). As for the amino acids downstream to the
152 putative cleavage site at nsp15/nsp16, they are AL instead of SL (Table 4).

153 The four novel CoVs display a similar genome organization and differ only in
154 the number of ORFs downstream of N (Fig. 2). Their transcription regulatory
155 sequences (TRSs) conform to the consensus motif 5′-ACACCA-3′ (Table 3), which is
156 unique to deltacoronaviruses. Interestingly, similar to other deltacoronaviruses, the
157 perfect TRS of S in the genomes of the four novel CoVs were separated from the
158 corresponding AUG by a large number (140) of bases (Table 3). This is in contrast to
159 the relatively small number of bases between the TRS for S and the corresponding
160 AUG (range: 0 base in HCoV NL63, *Rhinolophus* bat coronavirus HKU2 [Rh-
161 BatCoV HKU2], HCoV HKU1, bovine coronavirus [BCoV], human coronavirus
162 OC43 [HCoV OC43], mouse hepatitis virus [MHV], porcine hemagglutinating
163 encephalomyelitis virus, SARS-CoV and SARS-related *Rhinolophus* bat coronavirus
164 HKU3 [SARSr-Rh-batCoV HKU3] to 52 bases in infectious bronchitis virus [IBV])
165 in alphacoronaviruses, betacoronaviruses and gammacoronaviruses. Similar to other
166 deltacoronaviruses, the genomes of the four novel CoVs contain putative PL^{pro}, which
167 are homologous to PL2^{pro} of alphacoronaviruses and betacoronavirus subgroup A and
168 PL^{pro} of betacoronavirus subgroups B, C and D and gammacoronaviruses. Besides,

169 one ORF (NS6) is found between M and N of the genomes of the four novel CoVs. In
170 all the four novel CoVs, one ORF (NS7a) is present overlapping with N. For FalCoV
171 UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29, three ORFs (NS7b,
172 7c and 7d), and for QuaCoV UAE-HKU30, two ORFs (NS7b and 7c) are present
173 downstream of N. BLAST search revealed no amino acid similarities between these
174 putative non-structural proteins and other known proteins and no functional domain
175 was identified by PFAM and InterProScan, except that NS7a of FalCoV UAE-
176 HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 were homologous to NS7a
177 of WECoV HKU16, NS7b homologous to NS7b of CMCoV HKU21 and NS7c
178 homologous to NS7a of ThCoV HKU12, and that NS7a, NS7b and NS7c of QuaCoV
179 UAE-HKU30 were homologous to NS7a, NS7b and NS7c of SpCoV HKU17,
180 respectively. Transmembrane helices, predicted by TMHMM, were found only in
181 NS7c of FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 at
182 positions 4 to 23 and 30 to 52 among all the putative accessory proteins downstream
183 to the N genes of FalCoV UAE-HKU27, HouCoV UAE-HKU28, PiCoV UAE-
184 HKU29 and QuaCoV UAE-HKU30. Each of the genomes of FalCoV UAE-HKU27,
185 HouCoV UAE-HKU28, PiCoV UAE-HKU29 and QuaCoV UAE-HKU30 contains a
186 stem-loop II motif (s2m) (residues 25,924 to 25,965, 25,924 to 25,965, 25,932 to
187 25,973 and 26,649 to 26,690, respectively), a conserved RNA element downstream of
188 N and upstream of the polyA tail, similar to those in IBV, TCoV, SARSr-Rh-BatCoV,
189 SARS-CoV as well as other deltacoronaviruses.

190 Comparison of the amino acid identities of the seven conserved replicase
191 domains for species demarcation (ADRP, nsp5 [$3CL^{pro}$], nsp12 [RdRp], nsp13 [Hel],
192 nsp14 [ExoN], nsp15 [NendoU] and nsp16 [O-MT]) (4) among the four novel CoVs
193 is shown in Tables 5A and 5B. In all the seven domains, the amino acid sequences of

194 FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 showed
195 more than 90% identity among each other, but their overall amino acid sequences
196 showed only 84.4% identity to those of WECoV HKU16, indicating that these three
197 CoVs should be subspecies of a novel CoV species (Table 5A). As for QuaCoV UAE-
198 HKU30, its overall amino acid sequences showed more than 90% identity to those of
199 PorCoV HKU15 and SpCoV HKU17, indicating that QuaCoV UAE-HKU30,
200 PorCoV HKU15 and SpCoV HKU17 should be subspecies of the same CoV species
201 (Table 5B).

202 **Phylogenetic analyses.** The phylogenetic trees reconstructed using the
203 nucleotide sequences of $3CL^{pro}$, RdRp, Hel, S and N of the four novel CoVs and other
204 deltacoronaviruses are shown in Fig. 3 and the corresponding pairwise amino acid
205 identities shown in Table 2. In all five trees, FalCoV UAE-HKU27, HouCoV UAE-
206 HKU28 and PiCoV UAE-HKU29 were clustered together (Fig. 3). In the $3CL^{pro}$,
207 RdRp, Hel and N trees, FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV
208 UAE-HKU29 were clustered with WECoV HKU16, whereas QuaCoV UAE-HKU30
209 was clustered with PorCoV HKU15 and SpCoV HKU17 (Fig. 3). However, in the S
210 tree, FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 were
211 most closely related to MRCoV HKU18, whereas QuaCoV UAE-HKU30 was most
212 closely related to MunCoV HKU13 (Fig. 3).

213 **Recombination analysis.** For the FalCoV UAE-HKU27, HouCoV UAE-
214 HKU28 and PiCoV UAE-HKU29 cluster and QuaCoV UAE-HKU30, bootscan
215 analysis showed possible recombination sites in the S gene (positions 20,300–24,300
216 for the FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29
217 cluster [Fig. 4A] and positions 19,900–23,300 for QuaCoV UAE-HKU30 [Fig. 4B]).

Estimation of divergence dates. Using the Bayesian Skyline under a relaxed clock model with an uncorrelated lognormal distribution, the mean evolutionary rate of CoVs was estimated as 1.027×10^{-4} nucleotide substitutions per site per year for the RdRp gene. Molecular clock analysis using the RdRp gene showed that the mean time to the most recent common ancestor (tMRCA) of *Deltacoronavirus* was estimated to be April 82 (95% highest posterior density [HPD], 822 BC to 1824), that of HouCoV UAE-HKU28/PiCoV UAE-HKU29 to be March 2011 (95% HPD, October 2009 to February 2013), that of FalCoV UAE-HKU27/HouCoV UAE-HKU28/PiCoV UAE-HKU29 to be June 1981 (95% HPD, January 1964 to June 2010) and that of QuaCoV UAE-HKU30 to be February 1954 (95% HPD, October 1921 to September 2007) (Fig. 5).

Western blot analysis. Prominent immunoreactive bands were visible for 33 (75%) of 44 falcon serum samples. The band size observed (42 kDa) was consistent with the expected size of 41.1 kDa for the full-length His_6-tagged recombinant N protein (Fig. 6).

DISCUSSION

Similar to birds in Hong Kong, birds in the Middle East are also hosts for a diversity of deltacoronaviruses. In 2009, we reported the discovery of three novel avian CoVs that were phylogenetically distinct from infectious bronchitis virus (31). These three CoVs were found in fecal samples of bulbuls, thrushes and munias in Hong Kong (31). In 2011, the ICTV approved the classification of these three avian CoVs as a novel genus *Deltacoronavirus* in the *Coronaviridae* family (4). In 2012, in a large epidemiological study, we discovered seven additional deltacoronaviruses (32). Six of them were from fecal samples of Japanese white eyes, tree sparrows, original magpie robins, black-crowned night herons, Eurasian wigeons, and common moorhens respectively and one from fecal samples of pigs in Hong Kong (32). In the last few years, PorCoV HKU15 (now officially named *Coronavirus HKU15*) has been widely detected in fecal samples of pigs in Canada, China, Laos, Mexico, South Korea, Thailand, Vietnam and the USA (33–41). Recently, we have also found PorCoV HKU15 in respiratory samples of pigs which may have implications on the possible routes and sites of infections (42). In this study, four additional novel deltacoronaviruses were detected in the fecal samples of falcons, houbara bustards, pigeons and quails in Dubai. Similar to the other deltacoronaviruses, FalCoV UAE-HKU27, HouCoV UAE-HKU28, PiCoV UAE-HKU29 and QuaCoV UAE-HKU30 also have large genome sizes of 25,871 to 26,162 bases, owing to the presence of multiple ORFs downstream to the N gene. Continuous surveillance studies on birds in the Middle East and other regions will help better understand the viral and host diversity of deltacoronaviruses, and their potential for emergence in mammals.

Recent interspecies jumping events were observed in the deltacoronaviruses from falcons, houbara bustards and pigeons, which were likely a result of predator-

and-prey relationship along the food chain. According to the ICTV definition for demarcation of CoV species, where CoVs that share an overall amino acid identity of more than 90% in their seven conserved replicase domains (ADRP, nsp5 [$3CL^{pro}$], nsp12 [RdRp], nsp13 [Hel], nsp14 [ExoN], nsp15 [NendoU] and nsp16 [O-MT]) should be regarded as the same species, FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 should be subspecies of a novel CoV species. Notably, the S proteins of FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29, which are responsible for CoV receptor binding, also shared 94.5–99.8% amino acid identities, suggesting that the viruses have not evolved much yet to adapt to the corresponding avian host after jumping from one species to another. In fact, molecular clock analysis estimated that the tMRCA of HouCoV UAE-HKU28 and PiCoV UAE-HKU29 was just around seven years ago and that of FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 was just around 38 years ago (Fig. 5). Nevertheless, it should be noted that results of molecular clock estimation are only speculative for RNA viruses which are known for episodic evolution and adaptation to different environments (43). It is interesting to note that falcons, houbara bustards and pigeons are three radically different types of birds with unique behaviors and habitats. Falcons (order *Falconiformes*, family *Falconidae*) are medium-sized birds of prey traditionally used for hunting wild quarry in the Arabian region. Houbara bustards (order *Otidiformes*, family *Otididae*) are large birds that are geographically restricted to arid habitats. Pigeons (order *Columbiformes*, family *Columbidae*) are relatively smaller and are globally distributed. Yet, these birds are ecologically closely related because falcons are known predators of pigeons and houbara bustards, and are also fed with the meat of these birds (44, 45). Moreover, falcons are trained by Arabian falconers to hunt houbara bustards because Arabs and

other Asians like Pakistanis believe their meat possesses aphrodisiac qualities and the meat is sold at high prices, despite banning of such controversial practices in some countries. Therefore, interspecies transmission of this CoV species is likely a result of the predator-and-prey relationship. Serological testing confirmed the presence of specific antibodies against FalCoV UAE-HKU27 in 75% of field falcon sera collected in Dubai. Such a relatively high seroprevalence is similar to those observed in other coronaviruses found in other animals, such as MERS-CoV and dromedary CoV UAE-HKU23 in dromedaries and rabbit CoV HKU14 in rabbits (7, 46), supporting widespread genuine infection among the falcon population and excluding the possibility of remnant viruses in falcon fecal samples resulting from ingestion of pigeons and houbara bustards. The detection of viruses from only one pigeon was not surprising, as viral shedding is often transient during the acute infection phase. Further molecular studies will reveal whether these three closely related deltacoronaviruses share a common receptor in these three phylogenetically distant birds, where viral adaptation to an evolutionarily conserved host-cell receptor might help offer facile interspecies transmissibility (47).

Recombination involving the S protein is likely a common phenomenon among deltacoronaviruses, which may facilitate interspecies transmission and adaptation to new animal hosts. Phylogenetic analysis showed that the CoV species comprising FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 was most closely related to WECoV HKU16 in the $3CL^{pro}$, RdRp, Hel and N genes, but was only distantly related to WECoV HKU16 and most closely related to MRCoV HKU18 in the S gene, suggesting recombination events around the S gene region as demonstrated by bootscan analysis (Fig. 4). As for QuaCoV UAE-HKU30, PorCoV HKU15 and SpCoV HKU17, according to the ICTV definition for demarcation of

CoV species, these three CoVs should be classified as subspecies of the same CoV species. However, in contrast to FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 which are clustered together in all phylogenetic trees, QuaCoV UAE-HKU30, PorCoV HKU15 and SpCoV HKU17 showed phylogenetic positions shifting in different phylogenetic trees. QuaCoV UAE-HKU30 was clustered with PorCoV HKU15 and SpCoV HKU17 in the $3CL^{pro}$, RdRp, Hel and N genes, whereas it was most closely related to MunCoV-HKU13 in the S gene (Fig. 3). Recombination around the S gene region was also demonstrated by bootscan analysis (Fig. 4). Similarly, PorCoV HKU15 and/or Asian leopard cat CoV (ALCCoV) were most closely related to SpCoV HKU17 in the $3CL^{pro}$, RdRp, Hel and N genes, but were only distantly related to SpCoV HKU17 and most closely related to BuCoV HKU11 in the S gene. This suggests that these mammalian deltacoronaviruses may have arisen from recombination events between SpCoV HKU17 and BuCoV HKU11 or related viruses. Recombination is not uncommon in other CoVs, and was found to be responsible for the emergence of SARS-CoV (48–51), generation of new genotypes or strains of other CoVs including human CoV HKU1, human CoV OC43 and feline CoV type II strains (5, 6, 52). The present results suggest that recombination is also common among deltacoronaviruses, with the S gene being a frequent recombination site. Further studies may reveal if this may be an important mechanism for overcoming the mammalian species barrier through more efficient receptor binding to swine or other mammalian cells in PorCoV HKU15 and related viruses.

MATERIALS AND METHODS

Ethical statement. Collection of animal fecal samples and field falcon sera in this study were approved by the Animal Ethic Committee of Central Veterinary Research Laboratory and Ministry of Climate Change and Environment, UAE according to the Ministerial Decree No. 384 of year 2008 on the executive by-law of the Federal Law No. 16 of year 2007 concerning animal welfare. All the experimental procedures were performed in accordance with the International Guiding Principles for Biomedical Research Involving Animals regarding the care and use of animals.

Animal surveillance and sample collection. All animal fecal samples were left-over specimens submitted to the Central Veterinary Research Laboratory in Dubai, The United Arab Emirates for pathogen screening over a 24-month period (January 2013 to December 2014). A total of 1,356 fecal samples from 1,164 mammals and 192 birds were tested (Table 1). Serum samples from falcons in the field were collected in Dubai over a 4-month period (November 2015–February 2016). All samples were taken from the caudal tibial vein (*vena metatarsalis plantaris superficialis*) under isoflurane-anesthesia in serum tubes, centrifuged and stored at –20°C until use.

RNA extraction. Viral RNA was extracted from the fecal samples using RNeasy Mini Kit (Qiagen, Germany). The RNA was eluted in 50 µl of RNase-free water and was used as the template for RT–PCR.

RT–PCR of RdRp gene of CoVs using deltacoronavirus-conserved primers and DNA sequencing. Initial CoV screening was performed by amplifying a 440-bp fragment of the RdRp gene of CoVs using deltacoronavirus-conserved primers (LPW16472 5′-GTGGVTGTMTTAATGCACAGTC-3′ and LPW 16473 5′-TACTGYCTGTTRGTCATRGTG-3′) we published previously (32). Reverse

transcription was performed using the SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA). The PCR mixture (25 μl) contained cDNA, PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$ and 0.01% gelatin), 200 μM of each dNTPs and 1.0 U *Taq* polymerase (Applied Biosystem, Foster City, CA). The mixtures were amplified in 60 cycles of 94°C for 1 min, 48°C for 1 min and 72°C for 1 min and a final extension at 72°C for 10 min in an automated thermal cycler (Applied Biosystem). Standard precautions were taken to avoid PCR contamination and no false-positive was observed in negative controls.

The PCR products were gel-purified using the QIAquick Gel Extraction Kit (Qiagen). Both strands of the PCR products were sequenced twice with the ABI Prism 3130*xl* Genetic Analyzer (Applied Biosystems), using the two PCR primers. The sequences of the PCR products were compared with known sequences of the RdRp genes of CoVs in the DDBJ/ENA/GenBank sequence databases. A phylogenetic tree was reconstructed using 371-bp fragments of the RdRp gene with the maximum likelihood method using the substitution model general time reversible with gamma distributed rate variation as well as estimated proportion of invariable sites (GTR+G+I) by PhyML.3.0.

Complete genome sequencing. One complete genome each of FalCoV UAE-HKU27, HouCoV UAE-HKU28 and PiCoV UAE-HKU29 and two complete genomes of QuaCoV UAE-HKU30 were amplified and sequenced using the RNA extracted from the original fecal specimens as templates. The RNA was converted to cDNA by a combined random-priming and oligo(dT) priming strategy. The cDNA was amplified by degenerate primers designed by multiple alignments of the genomes of other CoVs with complete genomes available, using strategies described in our previous publications (7, 18, 29). Additional primers were designed from the results

of the first and subsequent rounds of sequencing. The 5′ ends of the viral genomes were confirmed by rapid amplification of cDNA ends using the SMARTer 5'/3' RACE kit (Clontech, Mountain View, CA). Sequences were assembled and manually edited to produce final sequences of the viral genomes.

Genome analysis. The nucleotide sequences of the genomes and the deduced amino acid sequences of the ORFs were compared to those of other CoVs using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/). Phylogenetic tree reconstruction was performed using the maximum likelihood (ML) method with PhyML3.0. The best-fit substitution models were selected using PhyML with Smart Model Selection. Protein family analysis was performed using PFAM and InterProScan (53, 54). Prediction of transmembrane domains was performed using TMHMM (55).

Recombination analysis. To detect possible recombination events, bootscan analysis was performed by using the nucleotide alignment of the genome sequences of the novel deltacoronaviruses with Simplot version 3.5.1, as previously described (56). The analysis was conducted using a sliding window of 1,000 nucleotides moving in 200 nucleotide steps with 1,000 bootstrap values. Possible recombination sites suggested by the bootscan analysis were confirmed through multiple sequence alignments.

Estimation of divergence dates. Divergence times for the genus *Deltacoronavirus* was calculated using a Bayesian Markov Chain Monte Carlo (MCMC) approach as implemented in BEAST (Version 1.7.4) as described previously (50, 57). RdRp gene sequence data were selected for analyses under the substitution model GTR+G+I using an unrelaxed log-normal distributed (Ucld) relaxed molecular clock and a Bayesian Skyline coalescent model. MCMC run was 5

405 × 10^7 steps long, sampling every 1,000 steps. Convergence was assessed on the basis
406 of the effective sampling size after a 10% burn-in using Tracer version 1.6. tMRCA
407 and the HPD regions at 95% were calculated. The trees were summarized in a target
408 tree by the Tree Annotator program included in the BEAST package by choosing the
409 tree with the maximum sum of posterior probabilities (maximum clade credibility)
410 after a 10% burn-in.

411 **Cloning and purification of His_6-tagged recombinant N protein of FalCoV**
412 **UAE-HKU27.** Cloning and purification of the His_6-tagged recombinant N protein of
413 FalCoV UAE-HKU27 was performed using a protocol we described previously (46,
414 58). To produce a plasmid for protein purification, primers LPW36358 (5′-
415 GGAATTCCATATGATGAGCACTCCCACAGTCCCT-3′) and LPW36359 (5′-
416 CCGCTCGAGATGCAGTTGAATCTCCATCCTG-3′) were used to amplify the gene
417 encoding the N protein of FalCoV UAE-HKU27 by RT–PCR. The sequence coding
418 for amino acid residues 1 to 344 of the N protein was amplified and cloned into the
419 *Nde*I and *Xho*I sites of the expression vector pET-28b(+) (Merck, Germany) in-frame
420 and upstream of the histidine residue series. The recombinant N protein was
421 expressed and purified using Ni-NTA agarose (Qiagen) according to the
422 manufacturer's instructions.

423 **Western blot analysis.** Western blot analysis was performed according to our
424 published protocol (46, 58). Briefly, 600 ng of purified His_6-tagged recombinant N
425 protein of FalCoV UAE-HKU27 was loaded into the well of a sodium dodecyl sulfate
426 (SDS)–10% polyacrylamide gel and subsequently electrophoresed and then
427 electroblotted onto a nitrocellulose membrane (Bio-Rad, Hercules, CA). The blot was
428 cut into strips and each separated strip was incubated with an individual serum sample,
429 in a dilution of 1:1,000, obtained from falcons in Dubai. Antigen-antibody interaction

was detected with an in-house developed polyclonal guinea pig anti-falcon IgY antibody (59) at a dilution of 1:2,000, a horseradish peroxidase (HRP)-conjugated rabbit anti-guinea pig IgG antibody (Invitrogen) at a dilution of 1:4,000, and the WesternBright Quantum HRP substrate (Advansta, USA). Monoclonal anti-His_6 antibody (clone HIS.H8; Invitrogen) at a concentration of 0.5 µ g/mL, with HRP-conjugated goat anti-mouse IgG antibody (Invitrogen) at a dilution of 1:4,000 as the secondary antibody, was used as the positive control.

Nucleotide sequence accession numbers. The nucleotide sequences of the five complete genomes of FalCoV UAE-HKU27, HouCoV UAE-HKU28, PiCoV UAE-HKU29 and QuaCoV UAE-HKU30 have been lodged into the DDBJ/ENA/GenBank sequence databases with the accession numbers LC364342–LC364346.

444 **ACKNOWLEDGEMENTS**

445 This work is partly supported by the Theme-based Research Scheme (Project No.
446 T11/707/15), University Grant Committee, Hong Kong; University Development
447 Fund, The University of Hong Kong, Hong Kong and the Collaborative Innovation
448 Center for Diagnosis and Treatment of Infectious Diseases, Ministry of Education,
449 China.

450

451 **CONFLICT OF INTEREST**

452 Patrick C. Y. Woo has provided scientific advisory/laboratory services for Gilead
453 Sciences, Incorporated; International Health Management Associates, Incorporated;
454 Merck & Corporation, Incorporated and Pfizer, Incorporated. The other authors report
455 no conflict of interest. These commercial companies had no role in study design, data
456 collection, analysis, interpretation or writing of the report. The authors alone are
457 responsible for the content and the writing of the manuscript.

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696 **LEGENDS TO FIGURES**

697 **FIG. 1.** Phylogenetic analysis of amino acid sequences of the 371-bp fragment
698 (excluding primer sequences) of RNA-dependent RNA polymerase (RdRp) of
699 coronaviruses (CoVs) identified from birds from Dubai in the present study. The tree
700 was reconstructed by the maximum-likelihood method using PhyML 3.0 with the
701 substitution model general time reversible with gamma distributed rate variation and
702 estimated proportion of invariable sites (GTR+G+I). Bootstrap values were calculated
703 from 1,000 trees. The scale bar indicates the number of nucleotide substitutions per
704 site. The eight newly identified coronaviruses are shown in bold. List of viruses and
705 their respective DDBJ/ENA/GenBank accession numbers are as follow: ALCCoV,
706 Asian leopard cat coronavirus (EF584908); Badger SARS-CoV, SARS-related
707 Chinese ferret badger CoV (AY545919); BCoV, bovine CoV (NC_003045); BdCoV
708 HKU22, bottlenose dolphin CoV HKU22 (KF793826); BuCoV HKU11, bulbul CoV
709 HKU11 (FJ376619); BWCoV SW1, Beluga whale CoV SW1 (NC_010646); Camel
710 MER-CoV, Camel Middle East respiratory syndrome CoV (KT751244); ChRCoV
711 HKU24, China Rattus CoV HKU24 (KM349742); Civet SARS-CoV, SARS-related
712 palm civet CoV (AY304488); CMCoV HKU21, common-moorhen CoV HKU21
713 (NC_016996); DcCoV HKU23, dromedary camel CoV HKU23 (KF906251); FalCoV
714 UAE-HKU27, falcon CoV UAE-HKU27; FIPV, feline infectious peritonitis virus
715 (AY994055); GiCoV, giraffe CoV (EF424622); HouCoV UAE-HKU28, houbara
716 CoV UAE-HKU28; HCoV 229E, human CoV 229E (NC_002645); HCoV HKU1,
717 human CoV HKU1 (NC_006577); HCoV NL63, human CoV NL63 (NC_005831);
718 HCoV OC43, human CoV OC43 (NC_005147); Human MERS-CoV, human Middle
719 East respiratory syndrome CoV (JX869059); Human SARS-CoV, severe acute
720 respiratory syndrome–related human CoV (NC_004718); IBV, infectious bronchitis

721 virus (NC_001451); IBV-partridge, partridge coronavirus (AY646283); IBV-peafowl,
722 peafowl coronavirus (AY641576); MHV, murine hepatitis virus (NC_001846);
723 MRCoV HKU18, magpie robin CoV HKU18 (NC_016993); MunCoV HKU13,
724 munia CoV HKU13 (FJ376622); NHCoV HKU19, night-heron CoV HKU19
725 (NC_016994); PEDV, porcine epidemic diarrhea virus (NC_003436); PHEV, porcine
726 hemagglutinating encephalomyelitis virus (NC_007732); PiCoV UAE-HKU29,
727 pigeon CoV UAE-HKU29; Pi-BatCoV HKU5, *Pipistrellus* bat CoV HKU5
728 (NC_009020); PorCoV HKU15, porcine CoV HKU15 (NC_016990); PRCV, porcine
729 respiratory CoV (DQ811787); QuaCoV UAE-HKU30, quail CoV UAE-HKU30;
730 RbCoV HKU14, rabbit CoV HKU14 (JN874559); Rh-BatCoV HKU2, *Rhinolophus*
731 bat CoV HKU2 (EF203064); Ro-BatCoV HKU9, *Rousettus* bat CoV HKU9
732 (NC_009021); SACoV, sable antelope CoV (EF424621); SARSr-Rs-BatCoV HKU3,
733 SARS-related *Rhinolophus* bat CoV HKU3 (DQ022305); Sc-BatCoV 512,
734 *Scotophilus* bat CoV 512 (NC_009657); SpCoV HKU17, sparrow CoV HKU17
735 (NC_016992); TGEV, transmissible gastroenteritis virus (NC_002306); ThCoV
736 HKU12, thrush CoV HKU12 (FJ376621); Ty-BatCoV HKU4, *Tylonycteris* bat CoV
737 HKU4 (NC_009019); WECoV HKU16, white-eye CoV HKU16 (NC_016991);
738 WiCoV HKU20, wigeon CoV HKU20 (NC_016995).

739 **FIG. 2.** Genome organization of members of *Deltacoronavirus*. Open reading frames
740 downstream of spike (S) gene are magnified to show the differences among the
741 genomes of the 10 CoVs. Papain-like protease (PL^{pro}), chymotrypsin-like protease
742 ($3CL^{pro}$), and RNA-dependent RNA polymerase (RdRp) genes are represented by
743 green boxes. S, envelope (E), membrane (M) and nucleocapsid (N) genes are
744 represented by orange boxes. Putative accessory proteins are represented by blue

745 boxes. The novel coronaviruses discovered in this study are shown in bold.
746 Abbreviations for the viruses are the same as those in Fig. 1.

747 **FIG. 3.** Phylogenetic analyses of chymotrypsin-like protease ($3CL^{pro}$), RNA-
748 dependent RNA polymerase (RdRp), helicase (Hel), spike (S) protein and
749 nucleocapsid (N) protein of Falcon CoV-HKU27, Houbara CoV-HKU28, Pigeon
750 CoV-HKU29 and Quail CoV-HKU30. The trees were reconstructed by the maximum-
751 likelihood method using PhyML 3.0 with the substitution models Le and Gascuel (LG)
752 with gamma distributed rate variation (G) ($3CL^{pro}$); LG with G, estimated proportion
753 of invariable sites (I) and empirical frequencies (F) (RdRp); LG+G+F (Hel and N);
754 and Whelan and Goldman (WAG)+G+I+F (S) with bootstrap values calculated from
755 1,000 trees. 314, 944, 599, 1561 and 392 amino acid positions in $3CL^{pro}$, RdRp, Hel,
756 S and N, respectively, were included in the analyses. The scale bars indicate the
757 number of amino acid substitutions per site. Viruses characterized in this study are in
758 bold. Abbreviations for the viruses are the same as those in Fig. 1.

759 **FIG. 4.** Detection of possible recombination by bootscan analysis. Bootscanning was
760 conducted with Simplot version 3.5.1 (F84 model; window size, 1,000 bp; step,
761 200 bp). (A) Falcon CoV UAE-HKU27 (FalCoV UAE-HKU27) was used as the
762 query sequence and compared with the genome sequences of white-eye coronavirus
763 HKU16 (WECoV HKU16), magpie robin coronavirus HKU18 (MRCoV HKU18) and
764 ThCoV HKU12 thrush coronavirus HKU12 (ThCoV HKU12). (B) Quail CoV UAE-
765 HKU30 (QuaCoV UAE-HKU30) was used as the query sequence and compared with
766 the genome sequences of sparrow coronavirus HKU17 (SpCoV HKU17), munia
767 coronavirus HKU13 (MunCoV HKU13) and ThCoV HKU12.

768 **FIG. 5.** Estimation of the mean time to the most recent common ancestor (tMRCA)
769 for *Deltacoronavirus*. The time-scaled phylogeny was summarized from all Markov

Chain Monte Carlo (MCMC) phylogenies of the RNA-dependent RNA polymerase (RdRp) gene data set analyzed under the relaxed-clock model with an uncorrelated log-normal distribution in BEAST version 1.7.4. Viruses characterized in this study are in bold. Abbreviations for the viruses are the same as those in Fig. 1.

FIG. 6. Western blot analysis of falcon CoV UAE-HKU27 (FalCoV UAE-HKU27) using nucleocapsid (N) protein expressed in *Escherichia coli*. Lane 1, purified recombinant FalCoV UAE-HKU27 N protein; Lane 2, falcon serum sample (FS7) strongly positive for antibody against FalCoV UAE-HKU27 N protein; Lane 3: falcon serum sample (FS5) negative for antibody against FalCoV UAE-HKU27 N protein.

781 **Table 1.** Animals screened and their associated coronaviruses (CoVs) in the present
782 study
783

Animals	No. of specimens tested	No. (%) of specimens positive for CoV	CoV
Birds	192	8 (4.16%)	
Black swan	1	0	-
Crowned crane	1	0	-
Eclectus parrot	1	0	-
Falcon	34	1 (2.94%)	Falcon CoV UAE-HKU27 (n=1)
Flamingo	3	0	-
Grey parrot	1	0	-
Heuglin's bustard	10	0	-
Houbara bustard	36	1 (2.77%)	Houbara CoV UAE-HKU28 (n=1)
Kori bustard	4	0	-
Myna	1	0	-
Ostrich	4	0	-
Peacock	1	0	-
Pigeon	18	1 (7.14%)	Pigeon CoV UAE-HKU29 (n=1)
Rhea	1	0	-
Thick-knee	1	0	-
Stone curlew	22	0	-
Partridge	8	0	-
Chicken	19	0	-
Duck	2	0	-
Guineafowl	6	0	-
Pheasant	7	0	-
Quail	10	5 (50%)	Quail CoV UAE-HKU30 (n=5)
Sand grouse	1	0	-
Mammals	1,164	0	
Antelope	90	0	-
Cat	59	0	-
Cattle	3	0	-
Camel	754	0	-
Dog	145	0	-
Goat	34	0	-
Horse	44	0	-
Lion	7	0	-
Monkey	6	0	-
Rabbit	9	0	-
Rodent	13	0	-

784 **Table 2.** Comparison of genomic features and amino acid identities between the four novel deltacoronaviruses, representative members
785 of alpha-, beta- and gammacoronaviruses and other deltacoronaviruses
786
787

CoV	Genome size (base)	G+C content	Amino acid identity (%)																			
			FalCoV UAE-HKU27					HouCoV UAE-HKU28					PiCoV UAE-HKU29					QuaCoV UAE-HKU30				
			$3CL^{pro}$	RdRp	Hel	S	N	$3CL^{pro}$	RdRp	Hel	S	N	$3CL^{pro}$	RdRp	Hel	S	N	$3CL^{pro}$	RdRp	Hel	S	N
Alphacoronavirus																						
HCoV 229E	27,317	0.38	35.8	49.1	47.7	44.8	21.4	35.5	49.0	47.7	44.8	21.4	35.8	49.1	47.7	44.8	21.4	34.8	48.9	50.6	41.1	22.3
HCoV NL63	27,553	0.34	36.7	48.9	47.5	39.3	19.8	36.7	48.8	47.5	39.5	20.5	37.0	48.9	47.5	39.4	20.5	36.0	48.8	49.6	37.7	21.5
Betacoronavirus																						
Lineage A																						
βCoV	31,028	0.37	36.5	51.9	47.2	24.9	22.7	36.5	52.0	47.2	24.9	22.5	38.1	52.1	47.2	25.0	22.5	37.2	51.4	48.6	25.4	23.4
HCoV HKU1	29,926	0.32	38.1	51.6	47.2	25.5	20.1	37.8	51.6	47.2	25.8	20.1	36.9	51.7	47.2	25.8	20.1	38.1	51.2	48.3	25.0	21.3
Lineage B																						
SARS-CoV	29,751	0.41	35.8	50.2	50.2	25.9	24.2	35.5	50.2	50.0	24.5	24.0	35.8	50.3	50.0	24.5	24.0	34.8	51.1	51.9	25.0	25.3
Lineage C																						
MERS-CoV	30,119	0.41	36.7	50.7	48.9	25.8	24.2	36.4	50.7	48.9	25.0	23.5	36.7	50.8	48.9	25.0	23.5	35.8	51.4	50.2	25.3	23.1
Ty-BatCoV HKU4	30,286	0.38	36.0	51.2	48.9	25.1	24.2	36.3	51.2	48.9	25.3	24.1	36.6	51.3	48.9	25.3	24.1	35.7	50.6	49.7	24.0	23.8
Lineage D																						
Ro-Bat-CoV HKU9	29,114	0.41	38.0	52.5	49.2	26.7	24.3	38.0	52.4	49.0	26.6	24.5	38.3	52.5	49.0	26.6	24.5	37.4	51.7	51.1	26.9	24.2
Gammacoronavirus																						
IBV	27,608	0.38	42.9	54.8	53.4	27.9	28.2	42.9	54.9	53.4	28.5	28.4	43.3	55.0	53.4	28.6	28.4	44.6	54.6	56.1	29.5	29.0
Deltacoronavirus																						
BuCoV HKU11	26,476	0.39	85.0	88.8	94.5	45.2	71.5	84.4	89.1	94.7	45.0	72.1	85.0	88.9	94.7	45.2	72.1	81.1	87.5	89.4	68.1	73.4
ThCoV HKU12	26,396	0.38	84.7	86.9	94.5	46.3	78.6	84.7	87.0	94.4	47.0	79.1	85.3	86.8	94.4	46.8	79.1	82.4	87.2	89.9	47.9	79.9
MunCoV HKU13	26,552	0.43	77.9	87.6	88.3	46.2	74.1	77.5	87.6	88.1	46.5	74.4	77.9	87.4	88.1	46.4	74.4	84.0	89.9	96.1	73.0	77.4
PorCoV HKU15	25,421	0.43	80.8	87.4	88.1	45.7	75.4	80.1	87.5	88.0	46.0	75.7	80.8	87.3	88.0	45.9	75.7	91.2	95.2	98.5	71.8	90.9
WECoV HKU16	26,027	0.40	86.6	91.9	96.8	47.1	82.7	86.3	91.9	97.0	47.7	83.6	87.0	91.7	97.0	47.8	83.6	78.5	86.7	88.4	62.2	75.5
SpCoV HKU17	26,067	0.45	80.8	87.5	88.3	67.3	75.9	80.1	87.6	88.1	67.9	76.8	80.8	87.4	88.1	67.7	76.8	93.8	95.8	98.7	44.7	91.8
MRCoV HKU18	26,674	0.47	78.8	87.1	88.3	68.6	73.4	78.5	87.1	88.1	68.2	73.6	78.8	86.9	88.1	68.2	73.6	85.7	90.3	96.3	45.0	78.2
NHCoV HKU19	26,064	0.38	58.3	72.3	75.9	45.6	50.4	57.6	72.3	76.2	46.0	50.7	57.9	72.3	76.2	46.2	50.7	53.7	72.3	78.4	41.8	51.6
WiCoV HKU20	26,211	0.39	58.3	72.1	73.8	45.6	49.3	57.7	72.0	73.8	45.7	50.8	58.3	71.9	73.8	45.7	50.8	59.0	71.3	75.4	43.0	51.4
CMCoV HKU21	26,212	0.35	76.2	84.8	89.1	47.1	64.7	75.9	84.7	89.2	46.5	65.3	76.5	84.5	89.2	46.5	65.3	71.7	83.5	84.6	51.5	61.6
FalCoV UAE-HKU27	26,155	0.39	-	-	-	-	-	99.3	98.9	99.5	94.5	100	99.3	98.7	99.5	94.5	99.1	81.8	86.3	88.3	45.8	74.2
HouCoV UAE-HKU28	26,155	0.39	98.7	98.9	99.5	94.5	99.1	-	-	-	-	-	99.3	99.8	100	99.8	100	81.1	86.4	88.1	46.0	74.8
PiCoV UAE-HKU29	26,162	0.39	99.3	98.7	99.5	94.5	99.1	98.7	99.8	100	99.8	99.1	-	-	-	-	-	81.8	86.2	88.1	46.1	74.8
QuaCoV UAE-HKU30	25,871	0.42	80.8	86.3	88.3	45.8	74.8	80.1	86.4	88.1	46.0	74.8	80.8	86.2	88.1	46.1	74.8	-	-	-	-	-

788 **Table 3.** Coding potential and putative transcription regulatory sequences of novel
789 deltacoronavirus genomes
790

CoVs	ORF	Location	Frame	Length (aa)	Length (nt)	TRS location	TRS sequence (distance in bases to AUG)
FalCoV UAE-HKU27	1ab	595–19,271	+1, +3	6226	18,678	71	ACACCA (523) AUG
	S	19,253–22,855	+2	1201	3,603	19112	ACACCA (140) AUG
	E	22,849–23,097	+1	83	249	22828	ACACCU (20) AUG
	M	23,090–23,746	+2	219	657	23072	ACACCA (17) AUG
	NS6	23,746–24,027	+1	94	282	23726	ACGCCA (19) AUG
	N	24,051–25,085	+3	345	1,035	24042	ACACCA (8) AUG
	NS7a	24,079–24,735	+1	219	657	24042	ACACCA (36) AUG
	NS7b	25,066–25,272	+1	69	207	25042	ACAACG (18) AUG
	NS7c	25,259–25,636	+2	126	378	25244	AGACCU (14) AUG
	NS7d	25,629–25,826	+3	66	198	25618	AUACCA (10) AUG
HouCoV UAE-HKU28	1ab	588–19,264	+3,+2	6226	18,678	70	ACACCA (517) AUG
	S	19,246–22,848	+1	1201	3,603	19105	ACACCA (140) AUG
	E	22,842–23,090	+3	83	249	22821	ACACCU (20) AUG
	M	23,083–23,739	+1	219	657	23065	ACACCA (17) AUG
	NS6	23,739–24,020	+3	94	282	23719	ACGCCA (19) AUG
	N	24,044–25,078	+2	345	1,035	24035	ACACCA (8) AUG
	NS7a	24,072–24,728	+3	219	657	24035	ACACCA (36) AUG
	Ns7b	25,059–25,265	+3	69	207	25040	ACAACG (18) AUG
	NS7c	25,252–25,629	+1	126	378	25237	AGACCU (14) AUG
	Ns7d	25,622–25,819	+2	66	198	25611	AUACCA (10) AUG
PiCoV UAE-HKU29	1ab	588–19,264	+3,+2	6226	18,678	70	ACACCA (517) AUG
	S	19,246–22,848	+1	1201	3,603	19105	ACACCA (140) AUG
	E	22,842–23,090	+3	83	249	22821	ACACCU (20) AUG
	M	23,083–23,739	+1	219	657	23065	ACACCA (17) AUG
	NS6	23,739–24,020	+3	94	282	23719	ACGCCA (19) AUG
	N	24,044–25,078	+2	345	1,035	24035	ACACCA (8) AUG
	NS7a	24,072–24,728	+3	219	657	24035	ACACCA (36) AUG
	Ns7b	25,059–25,265	+3	69	207	25040	ACAACG (18) AUG
	NS7c	25,252–25,629	+1	126	378	25237	AGACCU (14) AUG
	Ns7d	25,622–25,819	+2	66	198	25611	AUACCA (10) AUG
QuaCoV UAE-HKU30	1ab	522–19,312	+3, +2	6121	18,363	62	ACACCA (459) AUG
	S	19,294–22,770	+1	1159	3,477	19153	ACACCA (140) AUG
	E	22,764–23,015	+3	84	252	22740	CAACCA (23) AUG
	M	23,008–23,661	+1	218	654	22987	ACACCA (20) AUG
	NS6	23,661–23,942	+3	94	282	23614	ACACCA (46) AUG
	N	23,967–24,992	+3	342	1,026	23959	ACACCA (7) AUG
	NS7a	24,061–24,663	+1	201	603	23978	GCTCCA (82) AUG
	NS7b	25,003–25,419	+1	139	417	24998	ACACCA (4) AUG
	NS7c	25,358–25,579	+2	74	222	25347	ACACCA (10) AUG

791 **Table 4.** Putative cleavage sites at the junctions between non-structural proteins in FalCoV UAE-HKU27, HouCoV UAE-HKU28, PigCoV
792 UAE-HKU29 and QuaCoV UAE-HKU30 compared with other deltacoronaviruses
793

nsp	PorCoV HKU15	WECoV HKU16	SpCoV HKU17	MRCoV HKU18	NHCoV HKU19	WiCoV HKU20	CMCoV HKU21	FalCoV UAE-HKU27	HouCoV UAE-HKU28	PigCoV UAE-HKU29	QuaCoV UAE-HKU30
nsp2/nsp3	AG/SD	AG/SD	AG/SD	AG/AD	VG/GL	DG/VY	AG/VS	AG/SD	AG/SD	AG/SD	AG/SD
nsp3/nsp4	AG/AP	AG/AR	AG/AP	AG/AM	TG/GN	GG/SK	AG/KF	AG/RK	AG/RK	AG/RK	AG/AP
nsp4/nsp5	LQ/AG	LQ/AG	LQ/AG	LQ/AG	VQ/AG	VQ/SG	VQ/AG	LQ/AG	LQ/AG	LQ/AG	LQ/AG
nsp5/nsp6	LQ/SG	LQ/SN	LQ/SG	LQ/SG	LQ/GT	LQ/AN	LQ/AS	LQ/SG	LQ/SG	LQ/SG	LQ/SG
nsp6/nsp7	VQ/NK	VQ/NK	VQ/NK	VQ/NK	VQ/NK	VQ/NR	VQ/NR	VQ/NR	VQ/NR	VQ/NR	VQ/NK
nsp7/nsp8	VQ/AV	VQ/AV	VQ/AV	VQ/AV	LQ/VV	LQ/VV	LQ/VV	LQ/AV	LQ/AV	LQ/AV	VQ/AV
nsp8/nsp9	LQ/NN	LQ/NN	LQ/NN	LQ/NN	LQ/NN	CQ/NN	LQ/NN	LQ/NN	LQ/NN	LQ/NN	LQ/NN
nsp9/nsp10	LQ/AS	LQ/AN	LQ/AN	LQ/AN	LQ/SS	LQ/AN	LQ/AT	LQ/AN	LQ/AN	LQ/AN	LQ/AN
nsp10/nsp11	LQ/NS	LQ/GS	LQ/NS	LQ/NS	LQ/LG	LQ/SN	LQ/NT	LQ/GS	LQ/GS	LQ/GS	LQ/NS
nsp12/nsp13	LQ/AS	LQ/AS	LQ/AS	LQ/AS	LQ/AT	LQ/AT	LQ/AS	LQ/AS	LQ/AS	LQ/AS	LQ/AS
nsp13/nsp14	LQ/SS	LQ/SS	LQ/SS	LQ/AG	VQ/SL	VQ/AE	VQ/CS	LQ/SS	LQ/SS	LQ/SS	LQ/SG
nsp14/nsp15	LQ/NL	LQ/NL	LQ/NL	LQ/NL	LQ/TL	LQ/TL	LQ/TI	LQ/NL	LQ/NL	LQ/NL	LQ/NL
nsp15/nsp16	LQ/SL	VQ/SL	LQ/SL	LQ/SL	VQ/AL	LQ/SL	VQ/SL	VQ/AL	VQ/AL	VQ/AL	LQ/SL

794

795 **Table 5A.** Comparison of amino acid identities (%) of the seven conserved
796 replicase domains for species demarcation among FalCoV UAE-HKU27, HouCoV
797 UAE-HKU28, PiCoV UAE-HKU29, and WECoV-HKU16
798

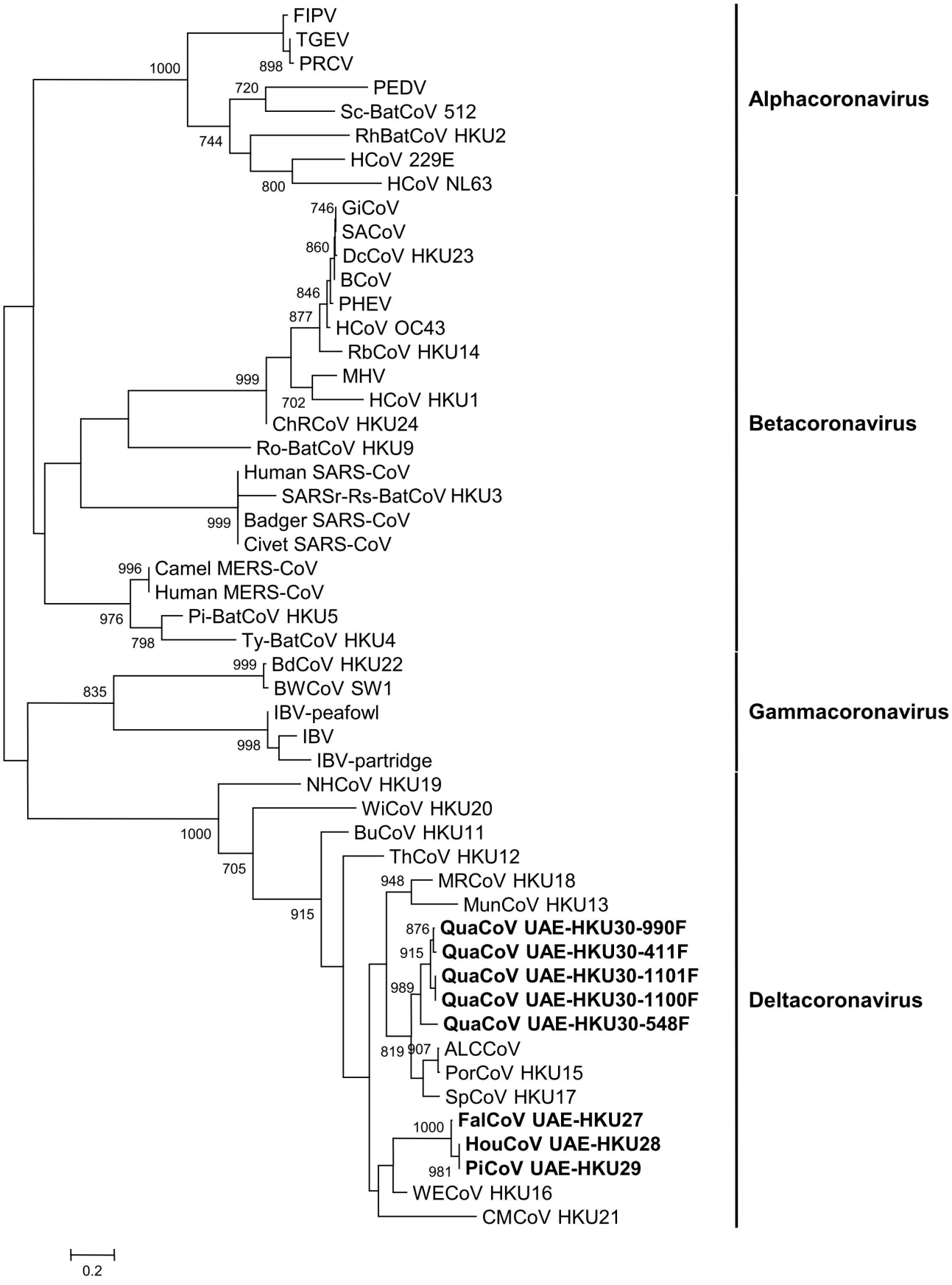
CoV	Domains	WECoV HKU16	FalCoV UAE-HKU27	HouCoV UAE-HKU28	PiCoV UAE-HKU29
WECoV HKU16	ADRP	-	68.4	68.7	68.8
	$3CL^{pro}$	-	86.6	86.3	87.0
	RdRp	-	91.9	91.9	91.7
	Hel	-	96.8	97.0	97.0
	ExoN	-	93.6	93.8	93.8
	NendoU	-	86.2	85.6	85.6
	O-MT	-	89.2	89.2	88.9
	Concatenated	-	84.4	84.4	84.4
FalCoV UAE-HKU27	ADRP	-	-	98.6	98.6
	$3CL^{pro}$	-	-	98.7	98.7
	RdRp	-	-	98.9	98.7
	Hel	-	-	99.5	99.5
	ExoN	-	-	99.8	99.8
	NendoU	-	-	99.1	99.1
	O-MT	-	-	100	99.6
	Concatenated	-	-	99.1	99.1
HouCoV UAE-HKU28	ADRP	-	-	-	99.9
	$3CL^{pro}$	-	-	-	99.3
	RdRp	-	-	-	99.8
	Hel	-	-	-	100
	ExoN	-	-	-	100
	NendoU	-	-	-	100
	O-MT	-	-	-	99.6
	Concatenated	-	-	-	99.9

799

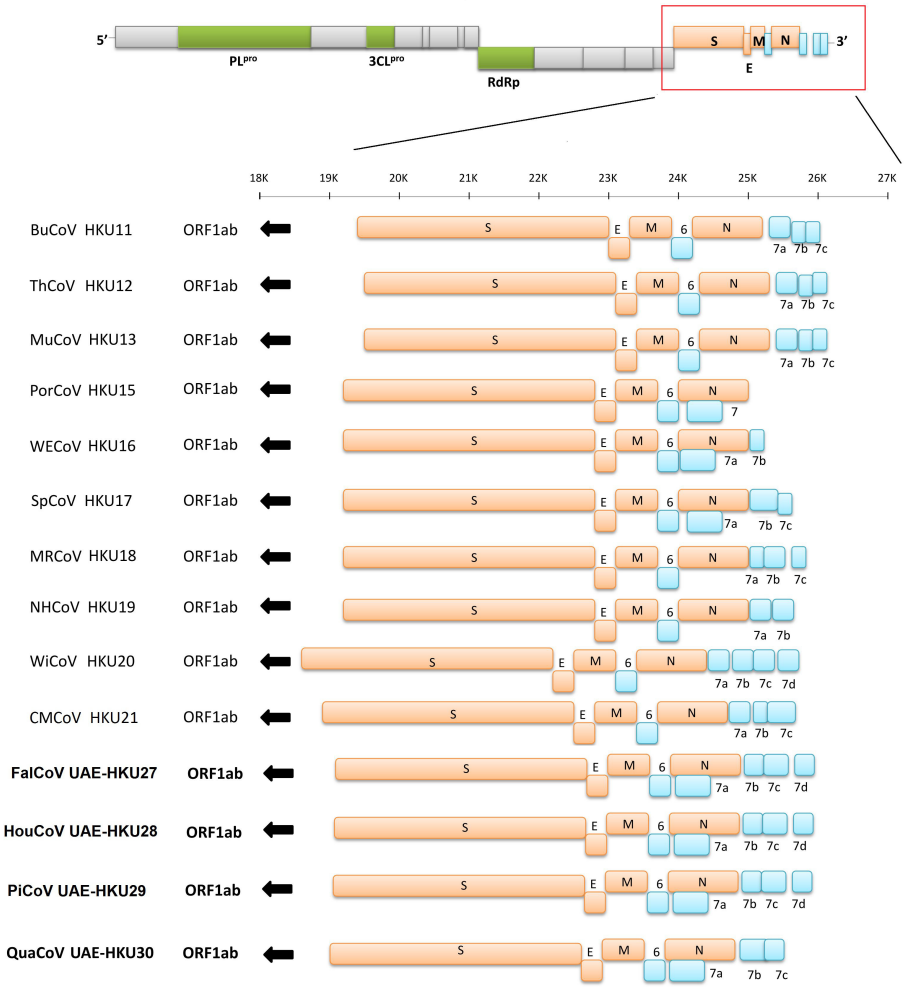
800 **Table 5B.** Comparison of amino acid identities (%) of the seven conserved
801 replicase domains for species demarcation among QuaCoV UAE-HKU30, PorCoV-
802 HKU15, and SpCoV-HKU17
803

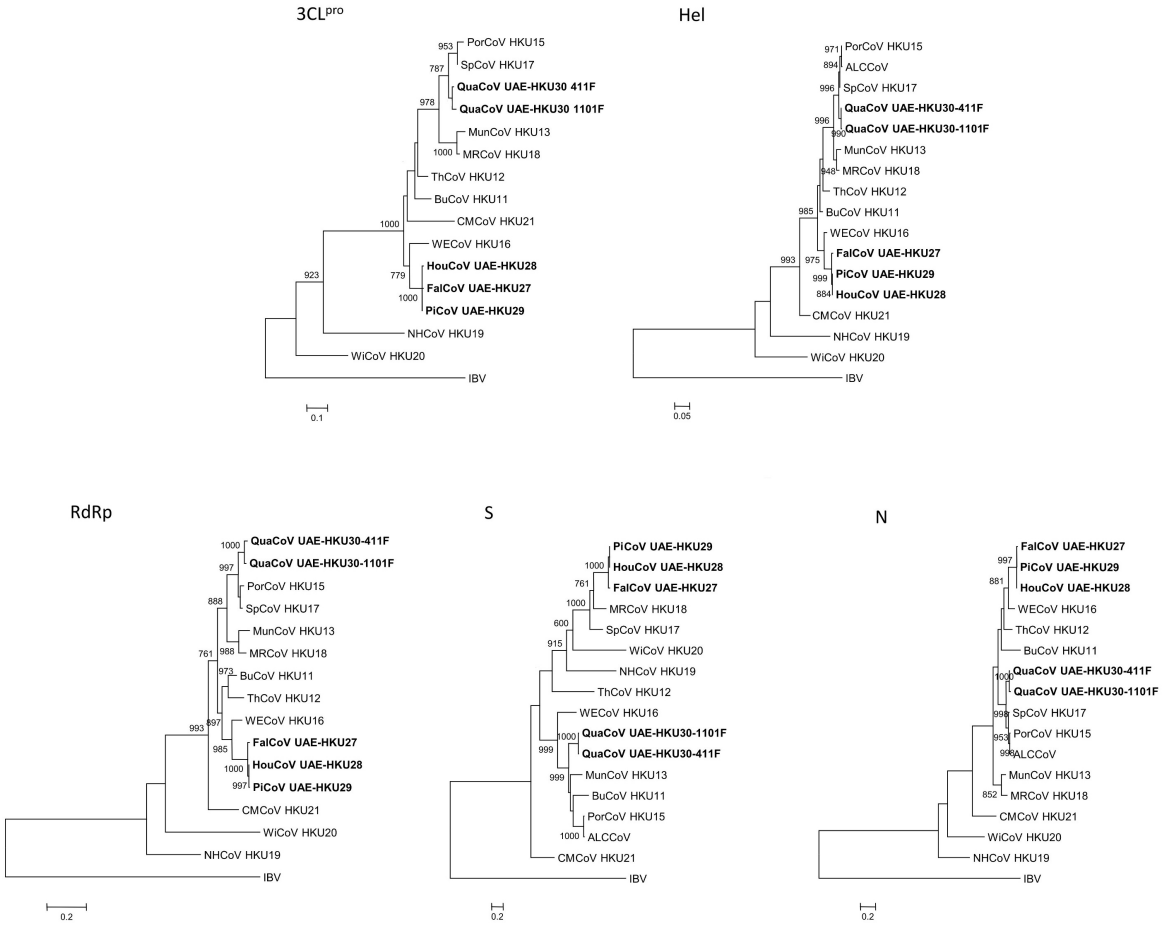
CoV	Domains	PorCoV HKU15	SpCoV HKU17	QuaCoV UAE-HKU30
PorCoV HKU15	ADRP	-	89.3	83.0
	$3CL^{pro}$	-	97.1	91.2
	RdRp	-	97.8	95.2
	Hel	-	99.2	98.5
	ExoN	-	97.3	96.1
	NendoU	-	96.3	91.1
	O-MT	-	97.5	96.4
	Concatenated	-	95.0	91.3
SpCoV HKU17	ADRP	-	-	83.0
	$3CL^{pro}$	-	-	93.8
	RdRp	-	-	95.8
	Hel	-	-	98.7
	ExoN	-	-	97.1
	NendoU	-	-	92.0
	O-MT	-	-	95.7
	Concatenated	-	-	91.8

804



Journal of Virology





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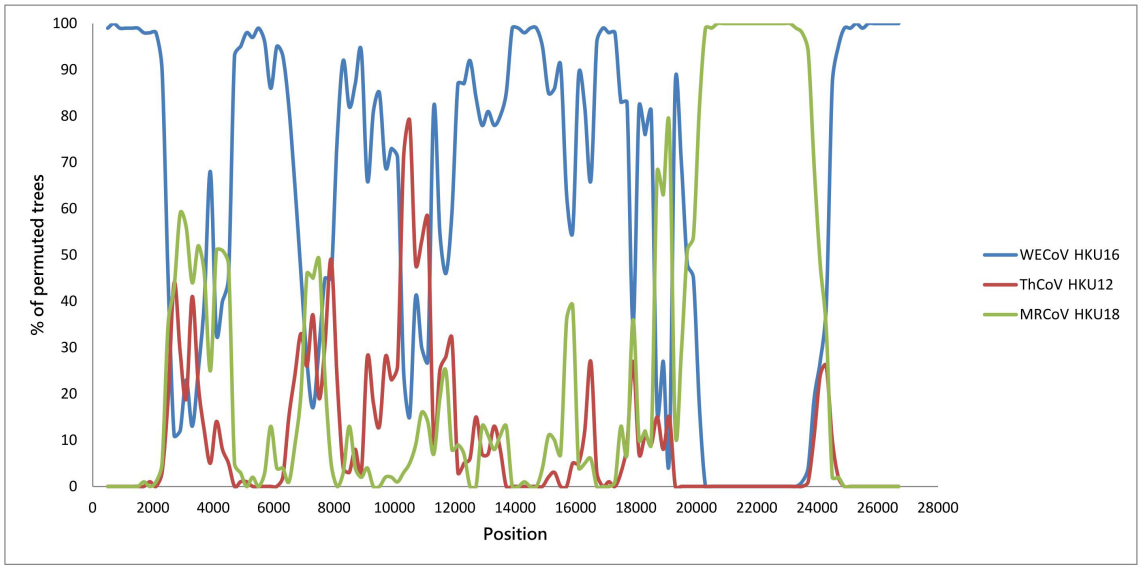

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