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Link of a ubiquitous human coronavirus to dromedary camels

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The four human coronaviruses (HCoVs) are globally endemic respiratory pathogens. The Middle East respiratory syndrome (MERS) coronavirus (CoV) is an emerging CoV with a known zoonotic source in dromedary camels. Little is known about the origins of endemic HCoVs. Studying these viruses' evolutionary history could provide important insight into CoV emergence. In tests of MERS-CoV–infected dromedaries, we found viruses related to an HCoV, known as HCoV-229E, in 5.6% of 1,033 animals. Human- and dromedary-derived viruses are each monophyletic, suggesting ecological isolation. One gene of dromedary viruses exists in two versions in camels, full length and deleted, whereas only the deleted version exists in humans. The deletion increased in size over a succession starting from camelid viruses via old human viruses to contemporary human viruses. Live isolates of dromedary 229E viruses were obtained and studied to assess human infection risks. The viruses used the human entry receptor aminopeptidase N and replicated in human hepatoma cells, suggesting a principal ability to cause human infections. However, inefficient replication in several mucosa-derived cell lines and airway epithelial cultures suggested lack of adaptation to the human host. Dromedary viruses were as sensitive to the human type I interferon response as HCoV-229E. Antibodies in human sera neutralized dromedary-derived viruses, suggesting population immunity against dromedary viruses. Although no current epidemic risk seems to emanate from these viruses, evolutionary inference suggests that the endemic human virus HCoV-229E may constitute a descendant of camelid-associated viruses. HCoV-229E evolution provides a scenario for MERS-CoV emergence.

coronavirus | evolution | ecology | zoonotic diseases | livestock

Coronaviruses (CoVs) (order Nidovirales, family *Coronaviridae*, subfamily *Coronavirinae*) are enveloped viruses with a large positive-strand RNA genome that infect a broad range of vertebrates, including mammals (1). Four human CoVs (HCoV-HKU1, HCoV-229E, HCoV-NL63, and HCoV-OC43) are globally endemic, causing mild to moderate respiratory tract disease. Two novel CoVs have emerged in humans during the past decade, causing outbreaks with high case fatality proportions. The severe acute respiratory syndrome (SARS)-CoV is thought to have been acquired by humans from carnivores, which, in turn, acquired the virus from rhinolophid bats (1–4). SARS-CoV is considered eradicated but SARS-related viruses carried by bats may still pose risks of human infection (5). The other emerging CoV, termed the Middle East respiratory syndrome (MERS)-CoV, is acquired as a zoonotic disease from dromedary camels, and is thought to have ancient ancestors in Old World vespertilionid bats (6–9).

Studying the origins of endemic HCoVs may provide retrospective insight into CoV emergence. Little is known about the ecological history of these ubiquitous human pathogens. However, the similarity of HCoV-OC43 to the bovine CoV suggests a primordial zoonotic acquisition from cattle (10, 11). No obvious intermediary hosts are known for the other HCoVs.

The human common cold agent HCoV-229E is an alpha-CoV that was first isolated in 1967 and has been circulating in the human population for long time with little sequence variation (12). We have recently discovered and characterized several groups of related alpha-CoVs in African bats of the genus *Hipposideros*, sharing ancient common ancestors with HCoV-229E (13, 14). Crossley et al. (15, 16) isolated a virus similar to HCoV-229E from a single captive alpaca (*Vicugna pacos*) that had died in a limited outbreak of respiratory disease among farmed alpacas in California. The biogeographic origin of this alpaca-derived coronavirus (ACoV) has remained unclear, because the virus has never been observed in feral alpacas and has only occurred from October to December 2007 in farmed alpacas linked to a single trade show in Monterey, California. Because alpacas are New World camelids, the ecological connection to ancestral viruses carried in Old World bats is difficult to explain (14).

In the context of several studies on MERS-CoV, we took samples from dromedary camels on the Arabian Peninsula and Africa (17–19). Screening of these samples by generic CoV RT-PCR

Significance

Our results raise a scenario for the natural history of a ubiquitous respiratory coronavirus (CoV) that has established itself in humans after it was likely acquired from camels. This scenario reminds us of the pandemic potential of the Middle East respiratory syndrome CoV, an agent that is thought to be acquired from camels without presently causing sustained human-to-human transmission.

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yielded initial evidence for diverse HCoV-229E–related viruses in dromedary camels. Similar sequences have meanwhile been found by other authors (20). Here, we provide a temporally, geographically, and genetically comprehensive study of dromedary-associated 229E viruses with the aim to clarify origins of HCoV-229E. Live viruses were isolated and virologically studied to provide an estimate of human infection and epidemic risks.

Results

HCoV-229E–Related CoVs Are Endemic in Arabian and African Camels. For a targeted screening by RT-PCR, nasal swabs were obtained during 2014–2015 from 1,033 dromedary camels in the Kingdom of Saudi Arabia (KSA) and Kenya. All specimens were tested for HCoV-229E–related CoVs by a real-time RT-PCR assay capable of detecting HCoV-229E, ACoV, and genetically distant 229E-related bat CoVs (14). The RNA detection rate in dromedaries was similar in sampling sites [4.0% in KSA, 6.7% in Kenya (χ^2, $P = 0.06$)]. Mean virus concentration in respiratory specimens was 3.1×10^7 (range: 1.6×10^3–7.2×10^8) copies per milliliter of swab suspension. Fecal samples from 387 Arabian dromedaries were tested, all with negative results. Exact age information was available for 272 animals. RT-PCR–positive animals ($n = 16$, mean age = 4 mo, range: 1–24 mo) were significantly younger than RT-PCR–negative animals ($n = 246$, mean age = 23 mo, range: 0–108 mo; t test, $P < 0.001$).

To investigate the temporal and geographic range of HCoV-229E–related CoV in dromedaries, we tested 364 sera sampled during 1983–2014 in six different countries on the Arabian Peninsula and in Africa for antibodies by an indirect immunofluorescence assay (IFA) (Table 1). The oldest antibody-positive sera had been taken in 1997. Older sera from Sudan and Somalia that had previously been tested positive for MERS-CoV did not show antibodies against HCoV-229E–related CoV (18). The seroprevalence in dromedary camels from the Arabian Peninsula was significantly higher than in samples taken during the same time in Kenya (86.3% vs. 16.0%; χ^2, $P < 0.001$), and was also higher than the average of all positive-testing sample collections from Africa (86.3% vs. 25.8%; χ^2, $P < 0.001$). Younger animals had lower seroprevalence rates compared to older animals (Kenya: 6.6% vs. 33% in 15 calves and 15 adults tested; KSA: 66% vs. 73% in 39 calves and 15 adults tested).

Because HCoVs can sometimes occur as coinfections, all 58 dromedaries testing positive for HCoV-229E–related CoV were tested additionally for MERS-CoV by real-time RT-PCR (21). Two dromedaries from KSA tested positive for both CoVs (3.5%), demonstrating that coinfection with these CoVs is possible.

Isolation of HCoV-229E–Related CoV from Dromedaries in Cell Culture. Because samples from Kenya had been stored in denaturing preservation buffer, virus isolation was only attempted on fresh specimens from KSA. RT-PCR–positive samples were inoculated on human hepatoma (HuH-7), VeroE6 (monkey kidney), Caco-2 (human colon carcinoma), Caki-3 (dromedary kidney), HEF (primary human esophageal fibroblast), and LGK-1-R.B (alpaca kidney) cells, followed by daily microscopic inspection for cytopathic effects (CPEs). Four of 17 samples produced a CPE 2–3 d postinoculation on HuH-7 and Caki-3 cells. CPE formation and complete cell death were consistently observed 1 d earlier on Caki-3 cells than on HuH-7 cells. Isolation was successful down to viral RNA concentrations of *ca.* 1×10^6 copies per milliliter, which is comparable to results obtained for MERS-CoV isolation (22) (Fig. S1). Virus growth in cell culture is described in more detail in Fig. S2.

Serum Antibodies Do Not Prevent Virus Infection. Because dromedaries can be infected with MERS-CoV in the presence of high antibody titers in sera (23), all sera from dromedaries yielding virus isolates were tested by IFA using HEK-293 cells that express the HCoV-229E spike protein from a eukaryotic expression plasmid.

Table 1. HCoV-229E–related CoV seroprevalence in dromedary camels

Region	Country	Sampling year	No. tested	No. positive (%)
Middle East	KSA	2014	78	58 (74.4)
	UAE	2013	68	68 (100)
		Subtotal	146	126 (86.3)
Africa	Kenya	2013, 2014	50	8 (16)
	Somalia	1983, 1984	65	0
	Sudan	1983	60	0
	Egypt	1997	43	16 (27)
		Subtotal	218	24 (11)
Total			364	150 (41.2)

All but one serum sample showed high end-point titers against the HCoV-229E spike protein (range: 1:800–1:3,200) that did not interfere with successful virus isolation in cell culture, suggesting similarities between MERS-CoV and HCoV-229E–related viruses in dromedaries (Table S1).

Genomic and Phylogenetic Comparison of Bat-, Dromedary-, and Human-Derived 229E-Related CoVs. The full genomes of five HCoV-229E–related dromedary CoVs (four from KSA and one from Kenya), as well as a contemporary primary cell culture isolate taken from one of the authors (I.E.) during an episode of acute rhinitis in 2015 (designated HCoV-229E/BN1/GER/2015), were determined by combined next-generation sequencing and Sanger sequencing approaches. Full-genome alignments included all major genetic lineages of HCoV-229E–related bat CoVs (14) and ACoV (15, 16). Human viruses included strains isolated 46 y ago (reference strain inf-1), 23 y ago (strain USA/993-50/1993), 6 y ago (strain 0349/NED/2010), and 1 y ago (HCoV-229E/BN1/GER/2015).

The dromedary viruses differed from ACoV by 1.2% and from HCoV-229E by 7.8–8.7% of their genomic sequence, which was consistent with these viruses forming part of the same CoV species (14). Within dromedaries, HCoV-229E–related CoVs from Kenya differed by 1.4% from viruses found in KSA. Of note, while this study was finalized, Sabir et al. (20) presented a study on MERS-CoV in dromedaries from KSA, wherein HCoV-229E–related sequences were observed but not virologically characterized. All sequences presented in that study fell within the diversity of novel HCoV-229E–related viruses described here. The most mutually distant viruses from Sabir et al. (20) are included in Fig. 1*A*.

In phylogeny, all dromedary- and human-associated viruses clustered with high statistical support, sharing a common ancestor with one of the three known phylogenetic lineages of bat-associated viruses (Fig. 1*A*). Camelid- and human-associated viruses fell into two well-supported clades. The Kenyan dromedary virus clustered in a basal sister relationship to all Arabian viruses. This result suggests a phylogenetic history similar to the history of MERS-CoV, in whose phylogeny the deepest nodes can be projected to African dromedaries, whereas higher nodes are projected to dromedaries on the Arabian Peninsula (8, 14, 24, 25). The alpaca-associated virus fell within the known diversity of dromedary viruses, consistent with a viral spillover from dromedary camels to Alpacas, potentially in a husbandry context as hypothesized earlier (14). Within the HCoVs, the tree topology reflected the different times of isolation of viral strains, with older viruses branching from older nodes, suggesting a correct representation of the evolutionary process by the applied phylogenetic algorithm.

A schematic representation of dromedary-associated 229E-related CoVs is shown in Fig. 1*B*. We have shown previously that 229E-related CoVs from bats have an additional ORF8 compared with HCoV-229E (14). An intact ORF8 was found in all HCoV-229E–related dromedary viruses sequenced in this study, except the Kenyan virus, which is phylogenetically distinct from

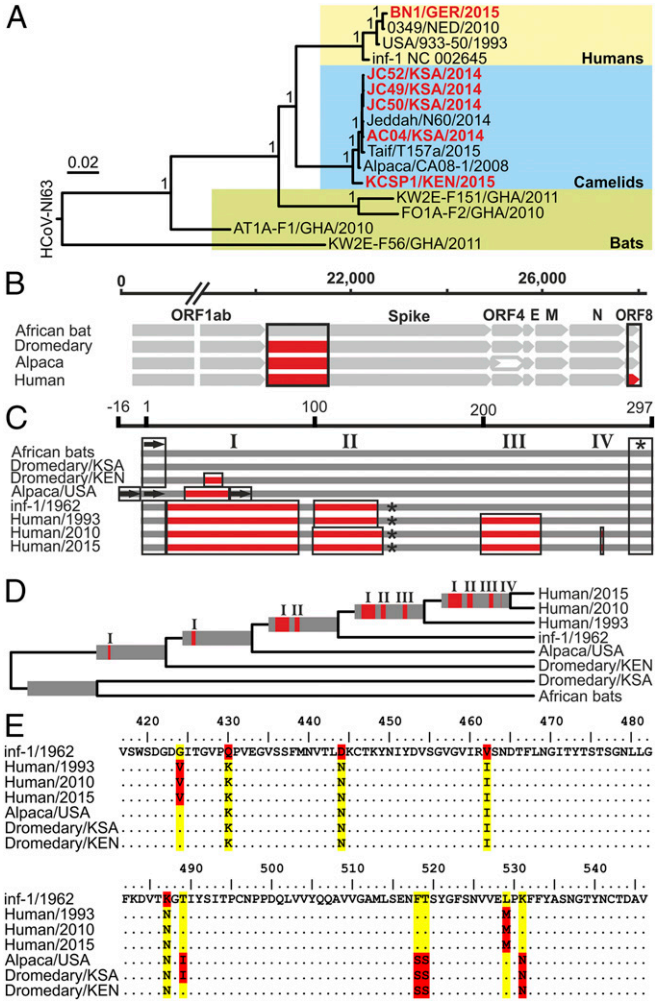

Fig. 1. (*A*) Phylogeny of HCoV-229E–related CoVs. Nodes illustrate posterior probabilities. Sequences from this study are shown in red. GER, Germany; NED, Netherlands; USA, United States of America; KEN, Kenya; GHA, Ghana. HCoV-NL63 (branch-truncated) is an outgroup. Taif/T157a/2015 and Jeddah/N60/2014 were used as described by Sabir et al. (20). (*B*) Genomic organization of HCoV-229E–related CoVs. ORF1ab was truncated due to graphical reasons. Boxes illustrate the regions with major genetic differences between HCoV-229E–related viruses. Red bars indicate deletions. (*C* and *D*) Deletion patterns in ORF8 homologs of HCoV-229E–related CoVs. Red lines indicate regions with deletions (numbered I to IV). Asterisks indicate triplets that would act as in-frame stop codons. Arrows represent start codons. (*E*) RBD of HCoV-229E and HCoV-229E–related viruses. Black dots illustrate conserved amino acid residues compared with HCoV-229E. Variables sites are shown in red (minority) or yellow (majority).

Arabian viruses and contains a small in-frame deletion (I in Fig. 1 *C* and *D*, details are provided in Fig. S3). At this position (I), the alpaca-associated virus shows a slightly larger deletion that is further extended in size in the human viruses (Fig. 1*C*). A parsimonious model for the acquisition of the deletion suggests that the initial deletion event occurred in a virus lineage that was ancestral to HCoV-229E, the alpaca virus, and the Kenyan virus (Fig. 1*D*). The Arabian viruses might stem from another part of the pool of African viruses wherein ORF8 is intact. Expression of an ORF8 was demonstrated by RT-PCR and sequencing of a typical subgenomic RNA (sgRNA) with fused message leader and body elements (Fig. S4).

Three other ORF8 deletions are only present in human viruses and may have occurred de novo in humans, because they were enlarged in plausible chronological sequence via older to contemporary human strains (Fig. 1*D*). A putatively recent timing of the spillover of ACoV from dromedaries into captive alpacas is consistent with the presence of the largest of all ORF8 deletions among camelid viruses in ACoV.

The spike protein S1 domains of all camelid-associated viruses were similar to those domains in human viruses, and contained deletions as opposed to bat viruses (14). According to phylogeny, the deletion would have occurred in a common ancestor to all dromedary and human viruses (Fig. 1*B*).

The nucleocapsid genes of HCoV-229E–related CoVs differ per host and geographic region in a pattern that suggests African and Arabian virus lineages to have been in isolation from each other, as well as from human viruses, for a considerable time (Fig. S5).

Dromedary-Derived Viruses Use the HCoV-229E Entry Receptor. Viral receptor use is a major barrier against cross-host transmission. The exact receptor-binding domain (RBD) of HCoV-229E is unknown (26–28), but its location can be mapped to the C terminus of the spike S1 subunit. As shown in Fig. 1*D*, camelid viruses are highly similar to human HCoV-229E strains in this genomic region (five to seven different sites, 94.7–96.2% identity).

The receptor for HCoV-229E is human aminopeptidase N (hAPN). A comparison of human, dromedary, feline, and porcine aminopeptidase N (APN) sequences yielded no immediate insights into receptor compatibility, except that the degree of sequence identity was highest between hAPN and dromedary APN (Fig. S6). The putative spike-interacting domain was not conserved between human and dromedary APN genes. To assess the ability of the dromedary-related viruses to use the HCoV-229E receptor functionally, infection experiments with HEK-293 cells expressing hAPN were conducted. In contrast to unmodified HEK-293 cells, which were susceptible to neither HCoV-229E nor the dromedary-derived viruses, HEK-293-hAPN cells enabled replication of HCoV-229E and dromedary viruses, demonstrating that both viruses use hAPN (Fig. 2*A*). To confirm these findings, we infected HuH-7 cells in presence of a polyclonal hAPN antibody to block the RBD on the cell surface. Infection with both tested HCoV-229E–related dromedary viruses was inhibited by hAPN antibodies, whereas untreated cells showed virus replication (Fig. 2*B*).

Dromedary-Derived Viruses Are Sensitive to the Interferon Response in Human Cells. In addition to receptor-mediated cell entry, the type I interferon (IFN) response may constitute an important barrier against human infection with HCoV-229E–related camel CoV. In HuH-7 cells pretreated with pan-species IFN at low (100 IU/mL) and high (1,000 IU/mL) concentrations, all tested viruses showed reduced production of viral RNA in comparison to untreated cells (Fig. 2*C*). The cell culture-adapted strain HCoV-229E inf-1 showed the strongest suppression of replication compared with the contemporary HCoV-229E/BN1/GER/2015 and both dromedary-derived viruses. Greatest inhibition by IFN pretreatment was observed for all viruses 24 h postinfection (hpi). After high IFN dose preincubation, the dromedary viruses were inhibited *ca.* 50-fold and the wild-type human virus HCoV-229E/BN1/GER/2015 was inhibited *ca*. 80-fold, which we do not consider a significant difference (Fig. 2*C*). By 48 hpi, the human virus had recovered its replication level in cells pretreated with even high IFN concentration, whereas replication of both dromedary viruses was still reduced by up to 15-fold after pretreatment with high IFN doses.

Dromedary-Derived Viruses Do Not Replicate Efficiently in Cell Culture Surrogates of Mucosal Infection. Because permanent cell cultures show differences and defects in organ-specific gene expression, we conducted replication experiments in cell cultures derived from mucosal tissues: Caco-2 cells from human colon carcinoma, as well as A549 cells derived from human lung adenocarcinoma. In addition, we used polarized human airway epithelial (HAE) cell cultures derived from primary lung cells whose phenotype is maintained in such a way that

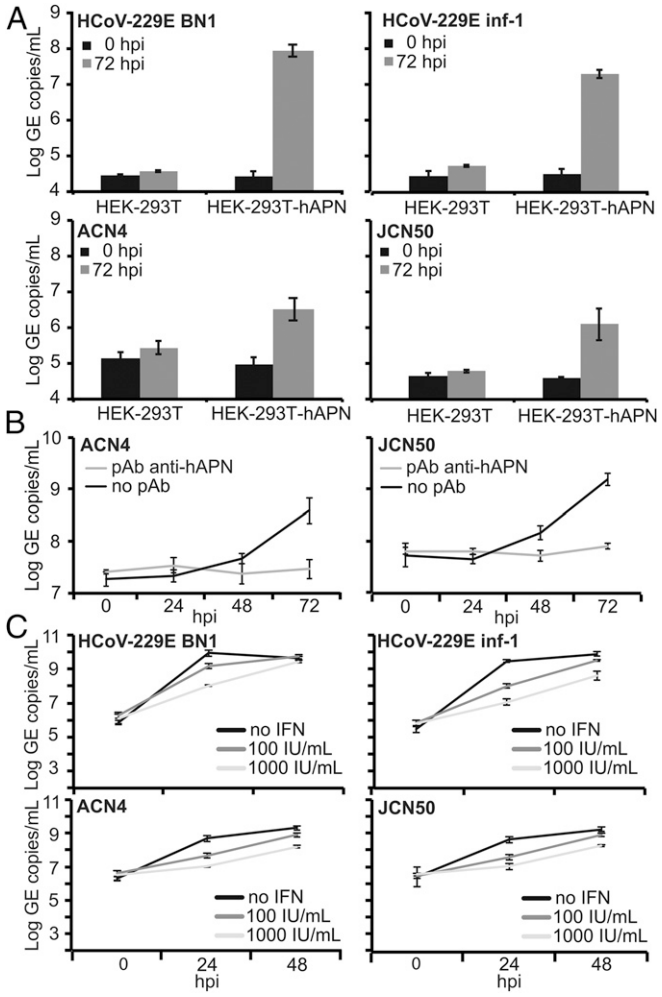

Fig. 2. (*A*) Receptor use of HCoV-229E–related viruses. HEK-293T-hAPN cells stably express hAPN. (*B*) Receptor blocking with polyclonal anti-hAPN antibodies. (*C*) IFN susceptibility of HCoV-229E and HCoV-229E–related viruses. GE, genome equivalent.

cilial and mucus-producing functions are recovered in an in vitro mucosal model. HAE, Caco-2, and A549 cells are known to be susceptible to infection with HCoV-229E inf-1. Infection experiments were conducted with the dromedary-derived viruses and HCoV-229E, using high virus concentrations (multiplicity of infection = 0.1). For HAE cells, we used increasing virus infection doses up to 50,000 plaque-forming units and assessed different growth temperatures, resembling the temperatures in the upper and lower airways. Although HCoV-229E replicated efficiently, none of the dromedary-associated viruses replicated in any of the used culture models (Fig. 3 *A* and *B*). To begin to understand the nature of the replication block, the intracellular occurrence of sgRNA transcripts was tested by RT-PCR (Fig. 3 *C* and *D*). In A549 cells and HAE cultures, sgRNA was transcribed by all viruses, suggesting that replication was not prevented in the stage of viral entry and initial stages of replication.

Dromedary-Derived Viruses Are Neutralized in Vitro by Human Anti–HCoV-229E Serum Antibodies. To assess the capability of human antibodies to neutralize the HCoV-229E–related dromedary viruses, a microneutralization assay was established. Human neutralizing antibody titers against HCoV-229E are known to be very low in general (29). We screened our serum archive for human sera that had measurable neutralization titers against HCoV-229E. Among 96 antibody-positive sera, eight were identified that were able to neutralize HCoV-229E at titers of at least 1:20, which, according to our experience and according to Miyazaki et al. (29), is considered a high neutralizing titer against HCoV-299E. Three of these sera neutralized infection by the dromedary-derived 229E CoVs ACN4 and JCN50 at measurable titers of at least 1:10, consistent with a close antigenic relatedness of HCoV-229E and dromedary-derived 229E CoVs (Table S2).

Discussion

Here, we characterize a diverse group of alpha-CoVs related to HCoV-229E with regard to evolution, history of host associations, and potential to cause infections in the human system.

The presence of 229E antibodies in dromedaries over a large geographic area suggests widespread and long-established viral circulation. This observation matches the greater genetic diversity of dromedary-associated viruses compared with human viruses. Seroprevalence rates were generally lower in Africa as opposed to the Arabian Peninsula, pointing at population density effects associated with intense husbandry in Arabia. The absence of antibodies in older samples taken in 1983 and 1984 should not be regarded as evidence against the presence of the virus in dromedaries at that time, because the size and geographic coverage of these samples were limited. The density and connectivity of

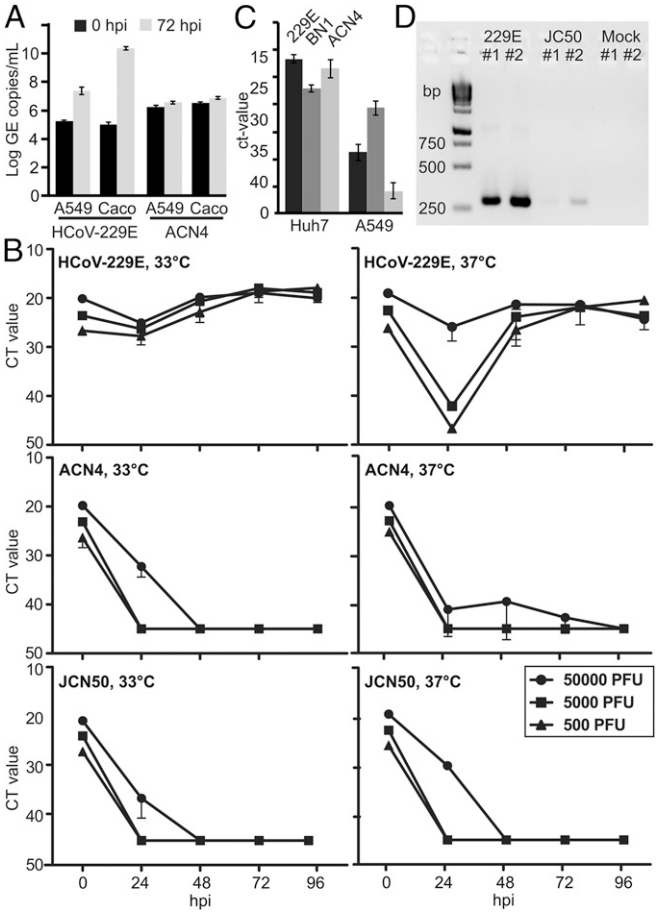

Fig. 3. (*A*) Production of virus in cell culture supernatant. A549, human lung adenocarcinoma cells; Caco, human colon carcinoma cells. Multiplicity of infection = 0.1. (*B*) Infection of polarized primary HAE cells at two different temperatures. (*C*) Nucleocapsid gene sgRNA transcription in permissive HuH-7 cells and nonpermissive A549 cells (quadruplicate data, real-time RT-PCR). (*D*) sgRNA in HAE cultures from two different donors (#1 and #2). ACN4, JCN50: dromedary-derived 229E CoVs; 229E: HCoV-229E strain inf-1; BN1: HCoV-229E BN1/GER/2015. CT, cycle threshold; PFU, plaque-forming units.

African flocks sampled in 19983/1984 may have been too low to ensure widespread infection.

Comprehensive assessment of genetic diversity provides new implications regarding the putative evolutionary history of HCoV-229E. Camelid-associated viruses are likely to have diverged from a larger viral diversity in bats as shown previously (14). The habitat ranges of the involved bat species suggest a geographic origin on the African continent (8, 9, 13, 14). Because camels were not introduced to Africa earlier than 5,000 y ago (30), the acquisition and zoonotic spillover to camels would have taken place after this time, providing an important biogeographic reference for studies of the evolution of human CoVs. Emergence of these viruses in camelids would have coincided with a deletion in the spike S1 subunit. Because human- and camelid-associated viruses are monophyletic, phylogeny does not allow differentiation between an ancient acquisition of human viruses from camelids and an acquisition by both hosts from a common source (e.g., bats). However, the ORF8 gene of HCoV-229E–related CoVs from African dromedaries contains a signature deletion around nucleotide positions 27–38 that reoccurs in an enlarged version in all human viruses. It also occurs in an enlarged version in ACoV. Whereas human viruses carry only the deleted version, dromedary viruses contain at least two different ORF8 versions (full and deleted). Among the possible explanations, it seems most likely that the ancestral human virus would have been sampled from a larger viral diversity existing in camelids. The transmission may or may not have involved additional hosts. Two alternative hypotheses for this gene's derivation in human viruses can largely be excluded. First, a direct acquisition from a bat reservoir is unlikely because ORF8 genes with the signature deletion have not been found in bats. Second, acquisition of a deleted ORF8 gene from camelid viruses at a later point is largely excluded by the monophyly and chronologically ordered internal branching of human viruses, suggesting ecological isolation and absence of opportunities for recombination. The gradual expansion of the ORF8 signature deletion in human viruses and the acquisition of additional ORF8 deletions are ongoing from old through recent isolates, suggesting a host transition that is not ancient and still involves genetic change. Of note, a gradual deletion of an accessory gene of unknown function was also observed for the SARS-CoV after transition to humans during the middle and late phases of the SARS epidemic (31).

It remains to be determined whether the loss of gene function in SARS-CoV and HCoV-229E has involved an attenuation of pathogenicity during circulation in humans. As a general concept, it has been hypothesized that viruses that are long associated with their hosts develop an attenuated pathogenicity during adaptation (32). In dromedary camels, the MERS-CoV seems to cause no or only mild disease (33–36). In the present study, all samples were collected from healthy-looking animals, suggesting low pathogenicity in camels for HCoV-229E–related viruses as well. Also, other aspects, such as the higher rate of infection in calves versus adult animals, point to an infection pattern similar to MERS-CoV. The observation of severe respiratory disease and abortion in alpacas from which ACoV was originally isolated could reflect a lack of adaptation to alpacas (15, 16). Because ACoV was observed only in one spatiotemporally restricted outbreak and not in feral alpacas, it seems possible that alpacas acquired the virus in captivity (e.g., in husbandry together with dromedary camels). Experimental infection studies will be necessary to understand the symptoms caused by 229E infection in camelids and alpacas better.

Considering the high infection prevalence, as well as the high virus concentrations in the dromedary respiratory tract, it appears likely that humans in Africa and the Middle East are frequently exposed to HCoV-229E–related dromedary viruses. The observed virus concentrations are in the same range as concentrations observed for MERS-CoV (33, 37), which should suffice to infect humans. Because 229E viruses endemic in humans and dromedaries are distinct on the genome level, discriminative molecular and serological markers might enable epidemiological laboratory studies to confirm or exclude the sporadic acquisition of HCoV-229E–related dromedary viruses by humans. ORF8 and the nucleocapsid gene may constitute appropriate markers for this purpose.

Because ORF8 might affect virulence, dromedary-associated 229E viruses could pose a threat to humans. The availability of live-virus isolates in the present study has enabled a provisional assessment of indicators of virulence and transmissibility. Our data show that HCoV-229E–related viruses are able to use the same cell entry receptor as HCoV-229E (i.e., hAPN), suggesting a principal capability of infecting human cells. However, failure to replicate in mucosa-derived cell cultures and differentiated HAE cultures predicts a low overall capability of the novel viruses to replicate in the human respiratory or enteric tract. Furthermore, the novel viruses were at least as sensitive as wild-type HCoV-229E against type I IFN in cell culture, predicting the existence of a relevant level of innate immunity. Finally, the demonstration of serological cross-neutralization points to the existence of population immunity, which predicts limited epidemic risks associated with these viruses in contemporary human populations. The primordial virus acquisition in humans would have met an immunologically naive human population in which limited onward transmission may have been established easier, leading to adaptive changes and an optimization of viral traits facilitating transmission.

In summary, these findings provide important implications on the origin of a human respiratory virus. Phenotypic assessment of live viruses suggests an existing but limited epidemic risk for humans. Our findings raise a scenario for the emergence of MERS-CoV, which also represents a dromedary-associated virus that has limited potential to infect humans but has not as yet established sustained human-to-human transmission. Livestock species, including species that are of regional importance such as camels, should be systematically tested for viruses capable of infecting humans.

Methods

Respiratory swabs from dromedaries were sampled in Kenya and KSA in 2014 and 2015. Stored sera from KSA as well as the United Arab Emirates, Kenya, Somalia, Sudan, and Egypt were available from previous studies (17–19). Sera were sampled between 1983 and 2015. Viral RNA was purified using the MagNA Pure 96 system (Roche). Testing for 229E-CoV RNA was done as described previously (14). Sequences were deposited under GenBank accession nos. KT253324-27 and KU291448-49. Serology by recombinant HCoV-229E spike protein IFA has been described previously (38, 39). Sampling of dromedaries, usage of stored biological specimens, and international shipment thereof were done under governmental research clearances or approved by the Institutional Animal Care and Use Committee (reference no. 2014.05.15) at the International Livestock Research Institute (ILRI), Nairobi. Details on animal handling, sampling, cell culture methodology, and virus and antibody detection are provided in *SI Methods*.

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