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SHORT COMMUNICATION

High proportion of MERS-CoV shedding dromedaries at slaughterhouse with a potential epidemiological link to human cases, Qatar 2014

Elmoubasher A. B. A. Farag, MPH[1†], Chantal B. E. M. Reusken, PhD[2*†], Bart L. Haagmans, PhD[1†], Khaled A. Mohran, PhD[3†], V. Stalin Raj, PhD[2], Suzan D. Pas, Msc[2], Jolanda Voermans, Bsc[2], Saskia L. Smits, PhD[2], Gert-Jan Godeke, Bsc[4], Mohd. M. Al-Hajri, ABCM[1*], Farhoud H. Alhajri, Bsc[3], Hamad E. Al-Romaihi, ABCM[1], Hazem Ghobashy, PhD[3], Mamdouh M. El-Maghraby, PhD[3], Ahmed M. El-Sayed, ABCM[1], Mohamed H. J. Al Thani, ABCM[1], Salih Al-Marri, ABFM[1] and Marion P. G. Koopmans, Prof, PhD[2,4]

[1]Supreme Council of Health, Doha, Qatar; [2]Department of Viroscience, Erasmus Medical Center, Rotterdam, The Netherlands; [3]Animal Resources Department-Ministry of Environment, Doha, Qatar; [4]Center for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Two of the earliest Middle East respiratory syndrome (MERS) cases were men who had visited the Doha central animal market and adjoining slaughterhouse in Qatar. We show that a high proportion of camels presenting for slaughter in Qatar show evidence for nasal MERS-CoV shedding (62/105). Sequence analysis showed the circulation of at least five different virus strains at these premises, suggesting that this location is a driver of MERS-CoV circulation and a high-risk area for human exposure. No correlation between RNA loads and levels of neutralizing antibodies was observed, suggesting limited immune protection and potential for reinfection despite previous exposure.

Keywords: *zoonoses*; *camels*; *MERS-CoV*; *respiratory infections*

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*Correspondence to: Chantal B. E. M. Reusken, Viroscience department, ErasmusMC, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands, Email: c.reusken@erasmusmc.nl; Mohd. M. Al-Hajri, Supreme Council of Health, P.O. Box 42, Doha, Qatar, Email: malhajri1@sch.gov.qa

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Dromedary camels are likely the primary source of Middle East respiratory syndrome virus (MERS-CoV) infection in humans, but further evidence is needed to support their role in zoonotic transmission. Two of the earliest diagnosed cases in Qatar were men who had visited the Doha central animal market and the adjoining central slaughterhouse (Farag, pers. comm.). Therefore, pre- and postmortem sampling was conducted on dromedary camels ($n = 105$) at the central slaughterhouse in Doha, Qatar. Nasal, oral, and rectal swabs collected prior to slaughter were tested for the presence of MERS-CoV RNA. Most of the camels that were sampled showed evidence for MERS-CoV shedding at the time of slaughter (59%). Sequence analysis showed the circulation of at least five different virus strains at the slaughterhouse premises. An understanding of the extent and pattern of MERS-CoV shedding by dromedaries presenting for slaughter provides insight into the risks for MERS-CoV exposure of persons with occupational contact with live camels and their carcasses.

Background

Illness associated with infection with MERS-CoV is characterized primarily by mild-to-severe respiratory

[†]These authors contributed equally to the manuscript.

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complaints, most requiring hospital admission for pneumonitis or acute respiratory distress syndrome. As of June 11, 2015, ECDC has reported 1,288 laboratory-confirmed cases, including 498 deaths (1). Human-to-human transmission seems limited to family and health care settings. Overall, a large proportion of MERS cases is suspected to be a result of zoonotic transmission (1) with growing evidence for dromedary camels (*Camelus dromedarius*) as a reservoir. MERS-CoV-specific antibodies have been detected in camels across the Middle East and the African continent, suggesting a geographically widespread distribution (2). Analysis of an outbreak associated with a barn in Qatar found dromedaries and humans to be infected with nearly identical strains of MERS-CoV (3) and further support for camels as reservoir came from a study in Saudi Arabia (KSA) that found widespread circulation of different genetic variants of MERS-CoV in camels, with geographic clustering of human and camel MERS-CoV sequences (4). However, few other studies provided evidence for zoonotic transmission of MERS-CoV from camels (5). The routes of direct or indirect zoonotic transmission are yet unknown. We investigated the rate of MERS-CoV circulation in dromedaries at the slaughterhouse in Qatar, previously linked to two MERS cases in Qatar.

MERS virus shedding at slaughterhouse

A random group of 105 camels that presented for slaughter in February ($n = 53$) and March ($n = 52$) 2014 were sampled for MERS-CoV analysis (Table 1). Animals either had come directly from within Qatar or KSA, or had been sold through the central animal market (CM). Swabs and lymph nodes were tested for MERS-CoV RNA by internally controlled RT-PCR targeting UpE and N genes, as described (3, 6). The first camel isolate of MERS-CoV as described by Raj et al. (7) was obtained from the first group of 53 samples and among others sequences generated from this group have been used to define a general MERS-CoV typing fragment (8). In total, 59% of the camels showed evidence for virus shedding in at least one type of swab at the time of slaughter (Table 1). The percentage positive samples was the highest for nasal samples, followed by oral swabs, fecal swabs, and bronchial swabs. All but one animals with virus shedding from any sample had a positive nasal swab. For saliva (oral), the percentage of positive samples was the highest for animals between 7 and 12 months of age. Lymph nodes from 53 animals were tested, yielding five positives. Approximation of the viral loads in the samples using the Ct values obtained with the UpE target showed no significant differences between types of samples and age groups (Fig. 1) It should be noted that viral loads with $\Delta Ct > 20$ were observed only in the nasal swabs and the nasal swab sample with the highest viral load was found to contain infectious virus (7).

Diversity in MERS-CoV circulation

To obtain further insight in the diversity of the viruses that circulated in dromedary camels at the slaughterhouse, MERS-CoV strains were sequenced according to a recently developed technique that enables the identification of divergent MERS-CoV types [sequences and technique in (8)]. In total, five different sequence types were identified with three different types found at both sampling moments (Table 2). Camels either came from the large Al-Shahaniya international racing complex (ASH) or from different sources elsewhere in Qatar (indicated by the initial arrow for animals 6–8 and 10–12 in (Table 2). Subsequently, they were either brought to a showing area (Al Mazad, AM), to the barns at the CM for a holding period, or immediately sent to the slaughterhouse (SH). Therefore, the sampling for animals 1–5 and 9–13 reflects MERS-CoV sequence diversity as a result of import from other regions in Qatar, whereas virus circulation at the CM more likely explains the virus diversity for animals 6–8.

Serology

Antibodies to MERS-CoV S1 were found in 100 out of 103 animals tested by micro-array technology (9). For 53 animals, antibody levels were also determined by virus neutralization assay as described earlier (9). Almost all animals had detectable neutralizing antibodies with no obvious age pattern and no significant difference in proportion of animals with low antibody levels (<20) (Fig. 2a) . There was no correlation between antibody levels and the viral load as reflected by Ct values (Fig. 2b).

Table 1. MERS-CoV detection in pre- and postmortem samples from camels presented for slaughter in Doha, Qatar ($n = 105$)

Sample type	All ($n = 105$)	0–6 months ($n = 41$)	7–12 months ($n = 35$)	>1 years ($n = 29$)
Nasal	60 (61/101)[a]	63 (24/38)	74 (26/35)	39 (11/28)
Oral	23 (23/102)	18 (7/39)	35 (12/34)	14 (4/29)
Rectal	15 (15/103)	15 (6/39)	17 (6/35)	10 (3/29)
Bronchial	7 (7/101)	8 (3/38)	6 (2/34)	7 (2/29)
Lymph nodes	9 (5/53)	0 (0/19)	20 (4/20)	7 (1/14)

[a]Percentage positive for MERS-CoV RNA as detected by two RT-PCR targets, followed by (absolute number of samples positive/ total number tested).

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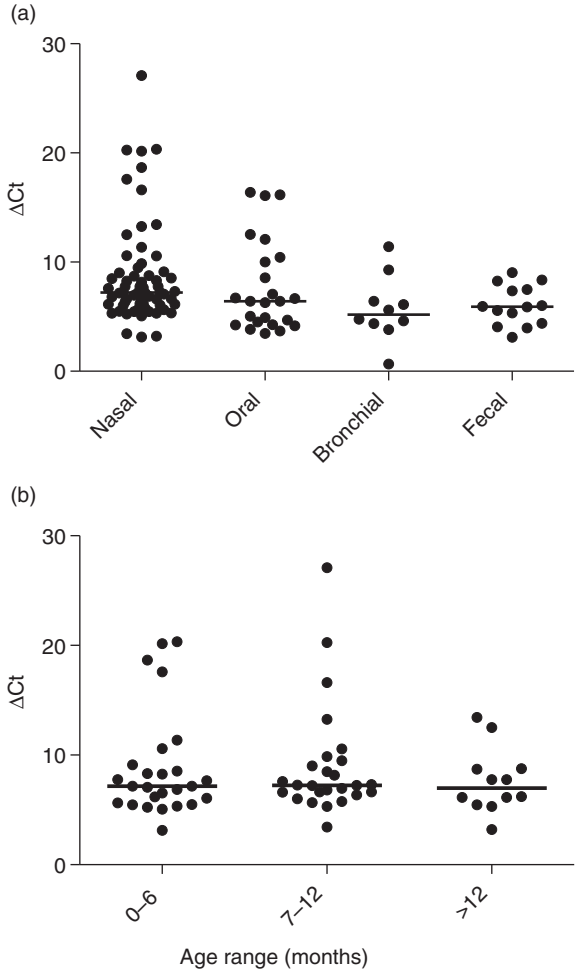

Fig. 1. MERS-CoV RNA shedding by dromedary camels at the central slaughterhouse, Qatar, depicted by sample type (a) and age group for nasal swabs (b). Viral loads in samples are approximated using Ct values obtained with the Up-E target and are expressed as ΔCt (40-Ct_{sample}). Black lines indicate medians.

Discussion

A high proportion of dromedary camels shed MERS-CoV RNA when presented for slaughter on two occasions at the central abattoir in Qatar. Co-circulation of multiple MERS-CoV variants demonstrates multiple virus introductions through flow of new animals traded into this group of animals, reflecting the virus diversity in wider Qatar, including animals imported from Australia, the Middle East region and East Africa. This suggests that CM is a driver of MERS-CoV circulation and a high-risk site for human exposure. Indeed two cases in Qatar were linked to visits to this area, and serology data on the only five workers that exclusively work in camel slaughter in Qatar illustrated this potential burden as four of the five slaughterers had IgG antibodies specific for MERS-CoV (10)

A study at four slaughterhouses in Egypt showed an overall RNA prevalence in nasal swabs of 3.6% among 110 camels (11), which is significantly lower than in our study. A comparison of the organization of the meat markets between Egypt and Qatar could provide insight in the observed differences. The camels that are put together for a holding period of weeks prior to slaughter in Doha have a wide variety of origins with varying initial immune status, which might provide a platform for extensive virus circulation. These include naïve camels from Australia (12) and camels from areas in the Horn of Africa and the Gulf region with known differences in immune status (2, 13, 14). We observed a positivity rate in rectal swabs of 15 out of 103 animals that were analyzed (of which 61 were positive in nasal swabs). Other studies observed none to very low numbers of camels shedding MERS-CoV RNA in feces (3, 15). However, the total numbers of animals in these studies were too low to make a significant comparison with the data presented here. In the current views on MERS-CoV epidemiology, young camels ($\leq$1year) with primary infections are thought to play a bigger role in MERS-CoV transmission than older animals for which less frequent shedding is observed (4, 15) and who demonstrate higher rates of seroconversion reviewed in (Ref. 2). However, we observed no significant differences in MERS-CoV RNA shedding between different age groups. Moreover, the lack of correlation between viral RNA loads and levels of neutralizing antibodies in the animals suggests limited protection and potential for reinfection despite previous exposure, similar to the situation in humans with the four common human CoVs and as observed in a camel herd in KSA (15). A problem is that the time since onset of infection could not be determined as the animals did not show overt symptoms. Therefore, it remains to be determined how the kinetics of infection are. In theory, the observed shedding of virus in the

Table 2. Summary of background information from slaughter camels for which sequences could be obtained from nasal swabs

Animal ID #	Origin	Age	Sampling moment	Sequence type
1	ASH→AM→SH	6 months	1	1
2	ASH→AM→SH	6 months	1	1
3	ASH→AM→SH	6 months	2	1
4	ASH→AM→SH	8 months	2	1
5	ASH→AM→SH	7 months	2	1
6	→CM→SH	6 months	1	2
7	→CM→SH	6 months	1	3
8	→CM→SH	8 months	1	3
9	ASH→SH	2 years	1	3
10	→AM→CM	6 months	2	3
11	→AM→CM	10 month	1	4
12	→AM→CM	6 months	2	4
13	ASH→SH	8 months	2	5

ASH = Al-Shahaniya, AM = Al Mazad, SH = slaughterhouse, CM = central market.

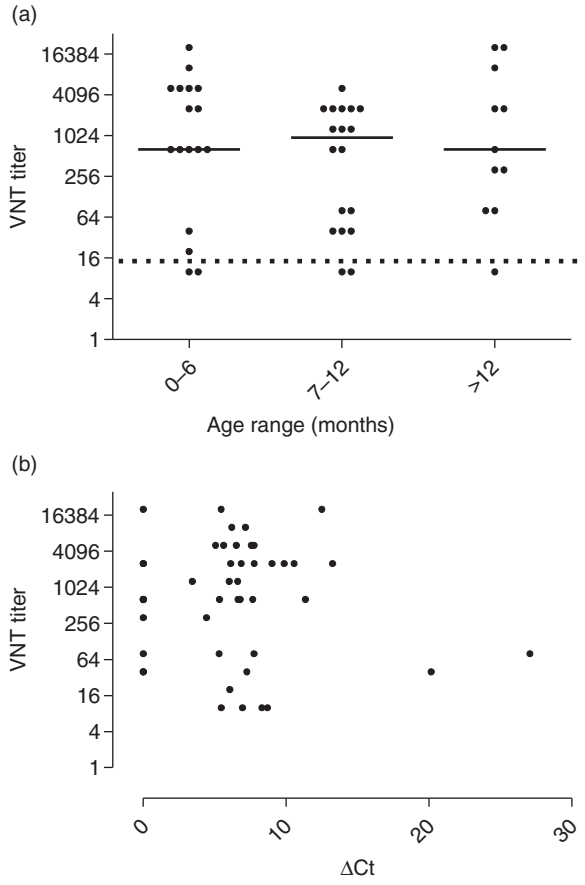

Fig. 2. Reciprocal MERS-CoV-neutralizing antibodies titers by age group (a) and correlated with ΔCt (40-Ct_{sample}) (b) for 53 camels at central slaughterhouse, Qatar.

presence of neutralizing antibodies could represent sampling toward the end of an infection cycle. Alternatively, the data may reflect limited mucosal immunity as has been shown for other animal coronaviruses (16). The possibility of camel vaccination has been suggested as a possible approach to controlling MERS-CoV transmission to humans. However, this may prove to be a challenging task in light of the above observations.

Given the high numbers of animals shedding these viruses in dynamic environments like the Doha market and abattoir, potential human health risks need to be considered and the implementation of management alternatives (e.g. separation of naïve animals from previously exposed animals and personal protective equipment for employees) might reduce the burden of MERS-CoV exposure to humans.

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