Sample of choice for detecting Middle East respiratory syndrome coronavirus in asymptomatic dromedary camels using real-time reverse-transcription polymerase chain reaction

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Summary

The newly identified Middle East respiratory syndrome coronavirus (MERS-CoV), which causes severe respiratory disease, particularly in people with comorbidities, requires further investigation. Studies in Qatar and elsewhere have provided evidence that dromedary camels are a reservoir for the virus, but the exact modes of transmission of MERS-CoV to humans remain unclear. In February 2014, an assessment was made of the suitability and sensitivity of different types of sample for the detection of MERS-CoV by real-time reverse-transcription polymerase chain reaction (RT-PCR) for three gene targets: UpE (upstream of the *E* gene), the *N* (nucleocapsid) gene and open reading frame (ORF) 1a. Fifty-three animals presented for slaughter were sampled. A high percentage of the sampled camels (79% [95% confidence interval 66.9–91.5%, standard error 0.0625]; 42 out of 53) were shown to be shedding MERS-CoV at the time of slaughter, yet all the animals were apparently healthy. Among the virus-positive animals, nasal swabs were most often positive (97.6%). Oral swabs were the second most frequently positive (35.7%), followed by rectal swabs (28.5%). In addition, the highest viral load, expressed as a cycle threshold (Ct) value of 11.27, was obtained from a nasal swab. These findings lead to the conclusion that nasal swabs are the candidate sample of choice for detecting MERS-CoV using RT-PCR technology in apparently healthy camels.

Keywords

Camel – Coronavirus – Middle East respiratory syndrome – Mucosal swab – Real-time reverse-transcription polymerase chain reaction.

Introduction

Since the identification of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 as a newly emerging disease causing severe lower respiratory tract infection in humans, cases have continued to be reported to the World Health Organization (WHO) (1). The causative agent, MERS-CoV, is a virus with a positive-

stranded RNA genome, belonging to the genus *Betacoronavirus-1* of the family *Coronaviridae*. Diagnosis in humans is based on detection of viral RNA by real-time reverse-transcription polymerase chain reaction (RT-PCR) in samples from the upper and lower respiratory tract, although positive serum samples and stool have occasionally been described (2). Although the exact sources and modes of transmission remain to be determined, dromedary camels are thought to play a role in MERS-CoV epidemiology: a high proportion of dromedary camels from regions with human cases and beyond have neutralising antibodies to MERS-CoV (3, 4, 5, 6), and virus shedding has been detected by RT-PCR and virus isolation from apparently healthy animals (7, 8, 9). Viruses isolated from camels replicate efficiently in human cells using human dipeptidyl peptidase 4 (DPP4) as an entry receptor, providing further evidence for the zoonotic potential of dromedary MERS-CoV (10). To study the epidemiology of MERS-CoV among camels, the shedding of virus by a group of 53 camels was studied, in order to determine the optimal type of sample for routine screening.

Materials and methods

Animals and sample collection

Fifty-three dromedary camels, *Camelus dromedarius*, ranging in age from four months to 11 years, that were presented for slaughter at an abattoir in Doha in February 2014 were selected randomly as part of a series of studies to identify risk factors for MERS-CoV infection in humans exposed to animals with the virus. The camels originated from different localities in Qatar, including Al-Shahanyia and Abu-Nakhla, and were kept in pens at Doha Central Market. Sample collection was done jointly by the Communicable Disease Control Team of the Supreme Health Council (CDCT-SHC) and the Animal Health Team, Qatar. Personal-protective biosafety equipment, including N95 masks, disposable gowns and gloves, was used during handling of the animals and sampling. The animals were restrained by their regular caretakers. All samples were collected using FLOQSwabs (Copan Improved Diagnostics, Brescia, Italy). Nasal,

oral and rectal swabs, as well as serum, were collected prior to slaughter. The swabs were placed into tubes containing viral transport medium-UTM (Universal Transport Medium; Copan Diagnostics, Brescia, Italy) (Fig. 1), immediately stored at 4°C, and directly transported to the Biotechnology Veterinary Laboratory, Qatar Ministry of Environment, where they were clarified by low grade centrifugation after vortexing, aliquoted and stored at –80°C until shipment on dry ice to the Department of Viroscience Laboratory, Erasmus Medical Center, the Netherlands.

RNA isolation and real-time RT-PCR application

Total nucleic acids were isolated from all swabs from 200 µl swab medium using the MagnaPure 96 total nucleic acid isolation kit (Roche, Mannheim, Germany) with a final elution of 50 µl. Camel MERS-CoV RNA was quantified on the ABI prism 7700 with the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, Bleiswijk, the Netherlands) using 10 µl extracted nucleic acid, TaqMan Fast Virus 1-Step mix, 0.5 U uracil-*N*-glycosylase, primers and probes targeting the UpE (upstream of the *E* gene), the *N* (nucleocapsid) gene or open reading frame (ORF) 1a, as described (11, 12). The RNA dilutions isolated from a MERS-CoV isolate EMC stock were used as a positive control. The PCR amplification involved a reverse transcription step of 50°C for 5 min and a denaturation step of 95°C for 20 s, followed by 45 cycles of 95°C for 3 s and 60°C for 30 s.

Results

According to international consensus, samples were considered positive for MERS-CoV RNA when at least two different targets were reactive. In animals up to two years of age, nasal samples were much more frequently positive than other sample types (Fig. 2), whereas this difference was less obvious in the older animals, although the number in this group was low. All but one virus-shedding animal had a positive nasal swab.

In order to assess possible differences in viral loads for the different sample types, samples were arbitrarily grouped on the basis of the results of the *N* gene PCR (the most sensitive primer pair) into a low and high viral load category. This showed that nasal swabs more frequently had higher viral loads (cycle threshold [Ct] 30 or less) (Fig. 3) and the highest viral load sample (Ct 11.27) (Fig. 4).

Discussion

The fact that 79% of the dromedary camels examined were found to be positive for MERS-CoV RNA revealed the high prevalence of the virus in these animals at the time of the study. Further, it reflects the epidemiological role that may be played by camels in disease perpetuation, given that they are inapparent reservoir hosts of MERS-CoV. High prevalence and antibody titres have also been reported in camels from Oman and Egypt, suggesting widespread virus circulation. However, virological testing was unable to detect MERS-CoV viral sequences in these camels, probably because only faecal and serum samples were analysed (3, 5). Definitive evidence that dromedary camels can be infected with MERS-CoV was obtained when viral sequences were detected in nasal swabs from camels sampled in close proximity to outbreaks of the disease among humans in Qatar (9). Viral nucleic acids were more commonly detected in nasal swabs than in rectal specimens in a study done in Saudi Arabia (13). A near-full-genome sequence (29,908 nucleotides, > 99%) of a virus genetically very similar to human MERS-CoV was identified from a nasal swab specimen obtained from a dromedary camel in Egypt (8).

In this study, the high percentage of positive samples demonstrated by the nasal swabs, giving a sensitivity of 97.6%, in addition to the more frequent finding of a high viral load, shows that nasal swabs are the sample of choice for monitoring virus presence using RT-PCR technology in apparently healthy camels. This conclusion is supported by the fact that sequence analysis of the virus isolated from the swab with the highest viral load in this study confirmed the presence of MERS-CoV; the virus was closely related to the human

England/Qatar 1 virus isolated in 2012 in a previous study (10). An added advantage is that nasal sampling is the simplest of the three sampling methods, requiring least restraint of the animals, thereby enhancing acceptability and reducing cost. Therefore, for surveillance studies, the authors recommend limiting sample collection to nasal sampling, in young animals. A caveat is that the pattern of shedding may differ in different age groups, as suggested by the lower proportion of nasal shedders in animals over two years of age in this study. Future studies are needed to address this question.

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Fig. 1
Sampling of dromedary camels using the universal viral transport system

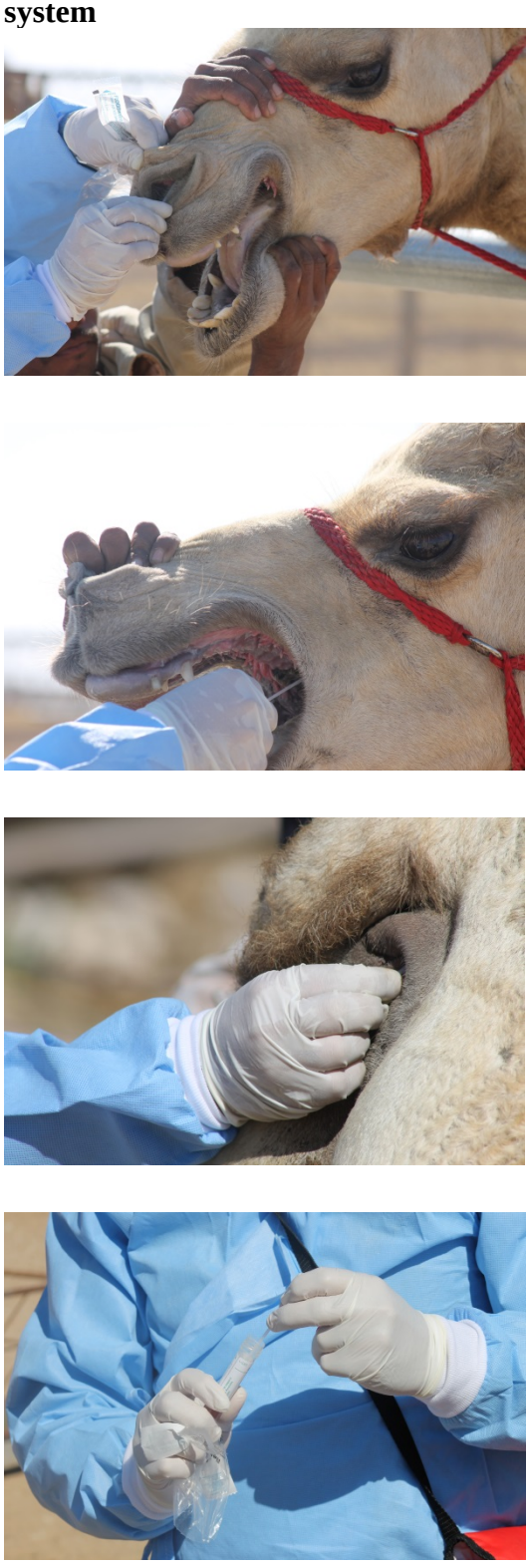
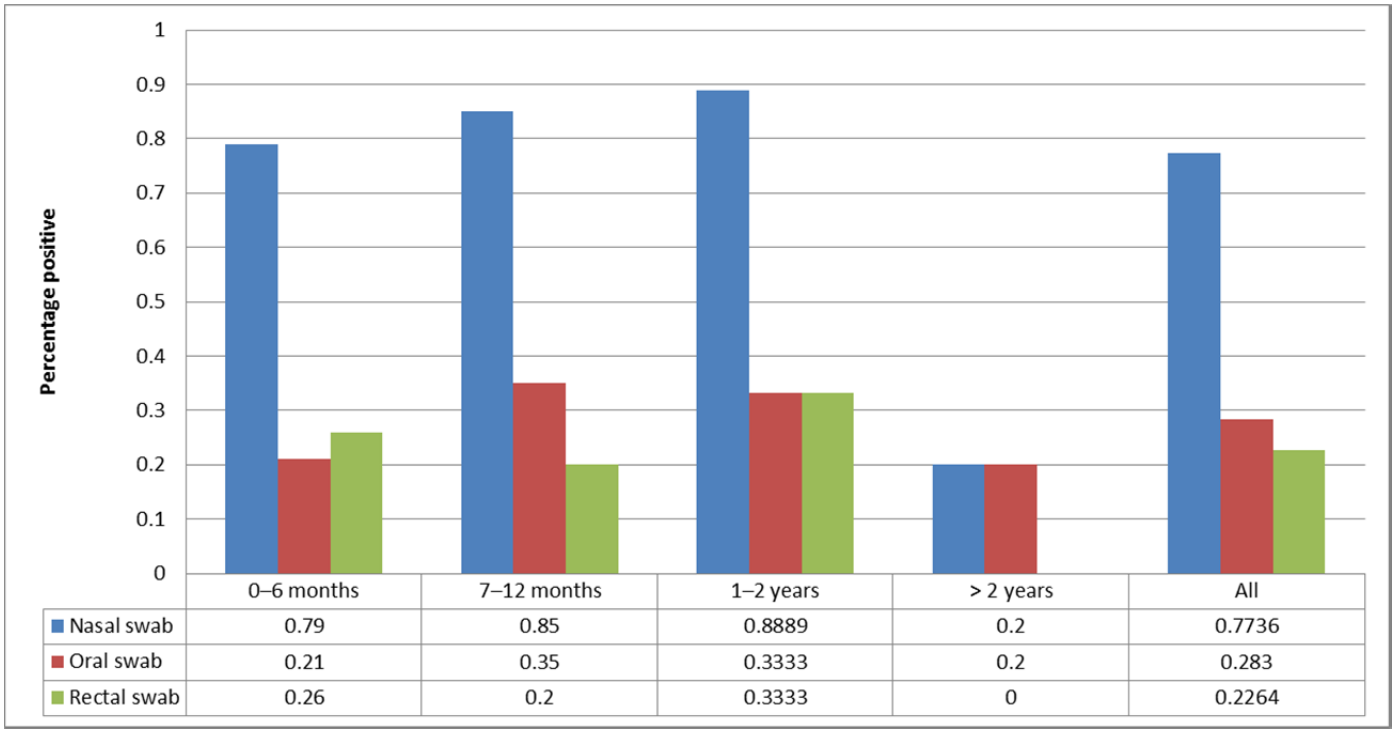


Fig. 2

Kinetics of shedding, by sample type, of MERS-CoV from dromedary camels sampled at the Doha slaughterhouse

In total, 19 animals were between 0 and 6 months of age, 20 were between 6 months and 1 year, nine were aged 1–2 years, and five were older than 2 years. The table below the figure summarises the proportion of samples testing positive



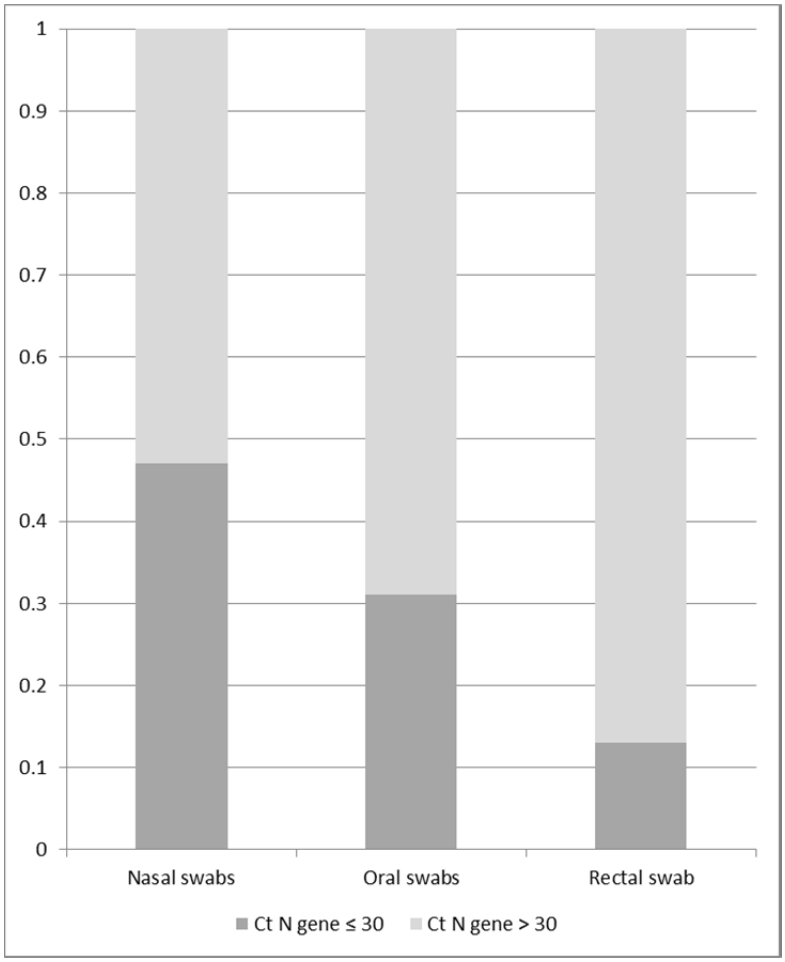
	0–6 months	7–12 months	1–2 years	> 2 years	All
Nasal swab	0.79	0.85	0.8889	0.2	0.7736
Oral swab	0.21	0.35	0.3333	0.2	0.283
Rectal swab	0.26	0.2	0.3333	0	0.2264

	0–6 months	7–12 months	1–2 years	> 2 years	All	95% CI	SE
Nasal swab	79%	85%	88.9%	20%	77.4% (41 out of 53)	66–88.6 %	0.0574
Oral swab	21%	35%	33.3%	20%	28.3% (15 out of 53)	16.2–40.4%	0.0618
Rectal swab	26%	20%	33.3%	0%	22.6% (12 out of 53)	11.4–33.9%	0.0574

Fig. 3

Distribution of viral load by sample type

Higher viral loads are indicated by a cycle threshold (Ct) value of ≤ 30.



	Ct *N* gene ≤ 30	Ct *N* gene > 30
Nasal swabs	47%	53%
Oral swabs	31%	69%
Rectal swab	13%	87%

Fig. 4
The sample with the highest viral load, as expressed by the Ct value