# The 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

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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

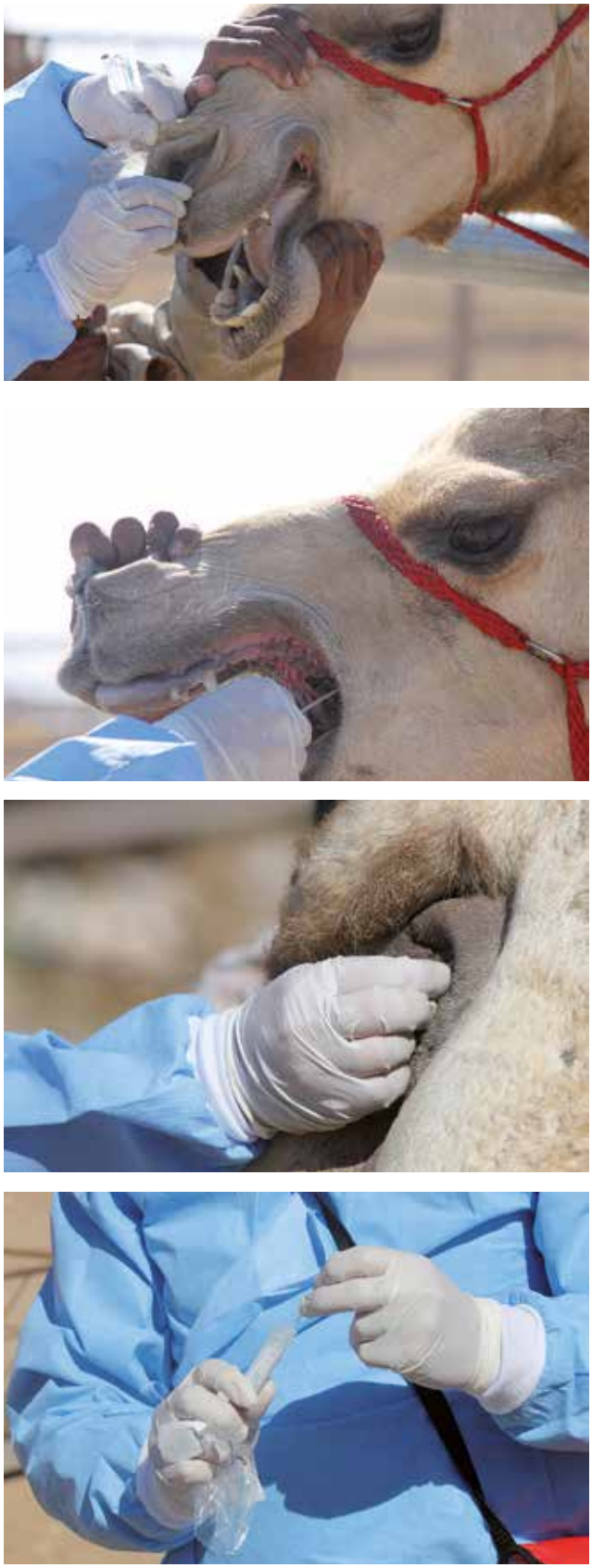


**Fig. 1**
**Sampling of dromedary camels using the universal viral transport system**

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).

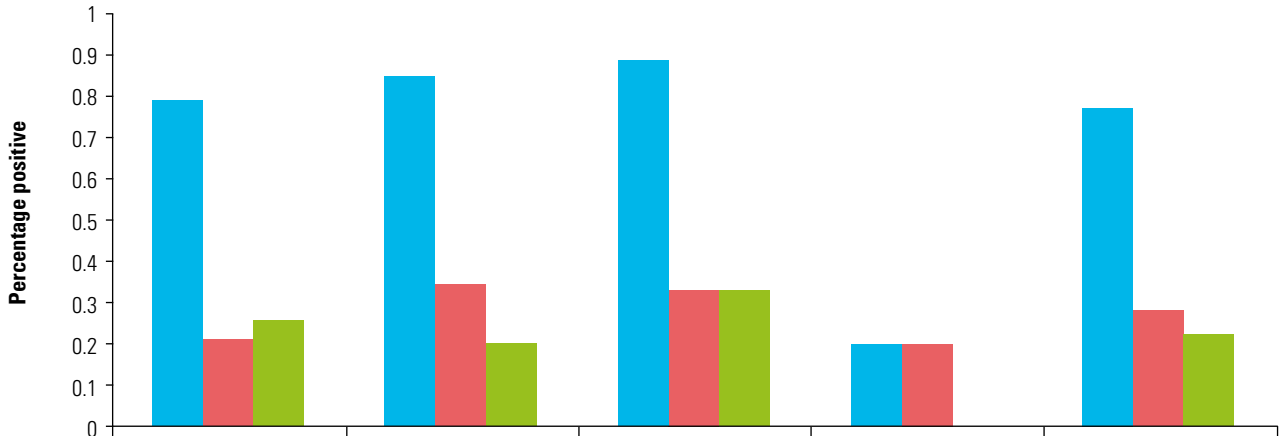
# Discussion

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

**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

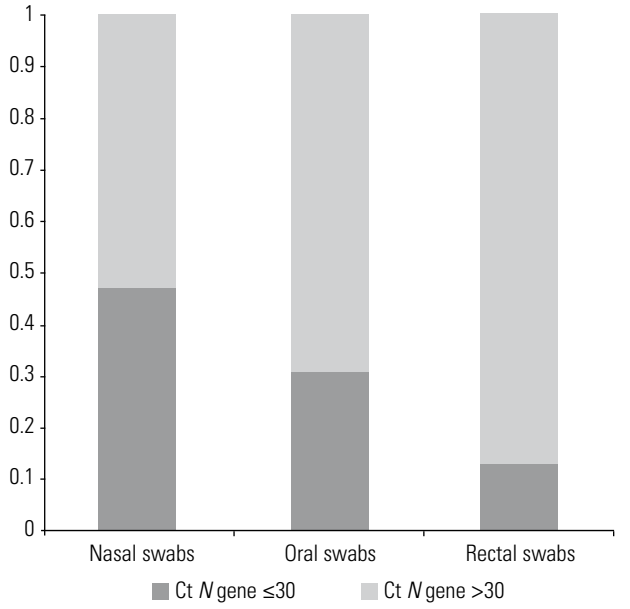



| | 0–6 months | 7–12 months | 1–2 years | >2 years | All |
|---|---|---|---|---|---|
| Nasal swab | 0.79 | 0.85 | 0.8889 | 0.2 | 0.7736 |
| Oral swab | 0.21 | 0.35 | 0.3333 | 0.2 | 0.283 |
| Rectal swab | 0.26 | 0.2 | 0.3333 | 0 | 0.2264 |

| | 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 |

CI: confidence interval
SE: standard error

**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 swabs** | 13% | 87% |

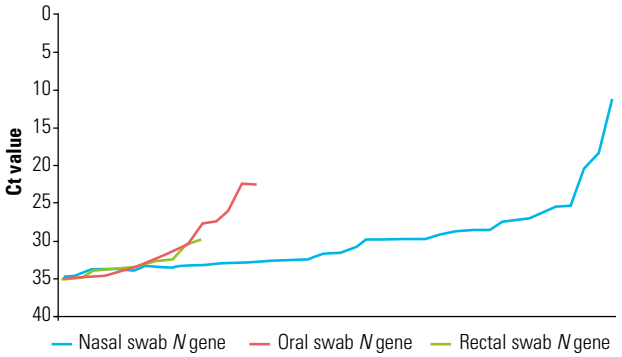



**Fig. 4**
**The sample with the highest viral load, as expressed by the cycle threshold (Ct) value**

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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# Choix du type d'échantillon à prélever pour détecter le coronavirus responsable du syndrome respiratoire du Moyen-Orient chez des dromadaires asymptomatiques au moyen d'une amplification en chaîne par polymérase couplée à une transcription inverse en temps réel

K.A. Mohran, E.A.B.A. Farag, C.B.E.M. Reusken, V.S. Raj, M.M. Lamers, S.D. Pas, J. Voermans, S.L. Smits, M.M. AlHajri, F. Alhajri, H.E. Al-Romaihi, H. Ghobashy, M.M. El-Maghraby, S.H.S. Al Dhahiry, N. Al-Mawlawi, A.M. El-Sayed, M. Al-Thani, S.A. Al-Marri, B.L. Haagmans & M.P.G. Koopmans

**Résumé**

Des travaux de recherche approfondis sont encore nécessaires concernant le coronavirus responsable du syndrome respiratoire du Moyen-Orient (MERS-CoV), un virus identifié récemment et qui provoque des troubles respiratoires sévères en particulier chez les individus atteints de pathologies multiples. Les études effectuées au Qatar et ailleurs ont démontré que les dromadaires font office de réservoirs du virus ; toutefois, les modalités précises de la transmission du MERS-CoV à l'être humain demeurent obscures. En février 2014, une équipe de chercheurs a évalué l'adéquation et la sensibilité de plusieurs types d'échantillons pour détecter le MERS-CoV en utilisant l'amplification en chaîne par polymérase couplée à une transcription inverse en temps réel (RT-PCR) spécifique pour trois cibles génétiques, à savoir la séquence UpE (en amont du gène *E*), le gène *N* (nucléocapside) et le cadre de lecture ORF1a. Pour ce faire, divers prélèvements ont été effectués sur 53 dromadaires destinés à l'abattage. Un fort pourcentage de ces dromadaires (79 % [intervalle de confiance à 95 % compris entre 66,9 et 91,5 %, erreur standard : 0,0625], soit 42 sur 53) excrétaient le MERS-CoV au moment de l'abattage, mais aucun ne présentait le moindre signe clinique. Les échantillons dans lesquels le plus de cas positifs ont été détectés étaient les écouvillons nasaux (97,6 %). Venaient ensuite les écouvillons oraux, qui ont détecté 35,7 % de cas positifs, puis les écouvillons rectaux (28,5 % de cas positifs détectés). Par ailleurs, ce sont les écouvillons nasaux qui ont permis d'obtenir l'intensité la plus élevée de la réponse de la RT-PCR, exprimée en une valeur du seuil de cycles de 11,27. Ces résultats permettent de conclure que les écouvillons nasaux sont les échantillons à privilégier pour la détection du MERS-CoV par RT-PCR chez les dromadaires asymptomatiques.

**Mots-clés**

Amplification en chaîne par polymérase couplée à une transcription inverse en temps réel – Coronavirus – Dromadaire – Écouvillon pour prélèvement microbiologique – Syndrome respiratoire du Moyen-Orient.

# Muestras idóneas para detectar el coronavirus del síndrome respiratorio de Oriente Medio en dromedarios asintomáticos por reacción en cadena de la polimerasa acoplada a transcripción inversa en tiempo real

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**Resumen**

Es preciso investigar más a fondo el coronavirus del síndrome respiratorio de Oriente Medio (MERS-CoV), recién identificado, que provoca una grave enfermedad respiratoria, sobre todo en personas con afecciones concomitantes. Estudios realizados en Qatar y otros lugares han deparado pruebas de que los dromedarios son un reservorio del virus, pero aún no están del todo claros los modelos exactos de transmisión del MERS-CoV al ser humano. Los autores describen un análisis realizado en febrero de 2014 de la idoneidad y sensibilidad de distintos tipos de muestra para detectar el MERS-CoV mediante una reacción en cadena de la polimerasa acoplada a transcripción inversa en tiempo real (RT-PCR) dirigida contra tres genes: el gen UpE (*upstream of the E gene*: en dirección 5' desde el gen *E*); el gen *N* (nucleocápside) y el marco de lectura abierto (ORF) 1a. Para ello se tomaron muestras de 53 animales enviados al sacrificio. Se comprobó que un elevado porcentaje de los dromedarios analizados (un 79% [intervalo de confianza al 95%: 66,9–91,5%; error estándar: 0,0625], esto es, 42 de 53) excretaban virus en el momento del sacrificio, pese a que todos los animales parecían estar sanos. Entre los ejemplares positivos para el MERS-CoV, las muestras que con más frecuencia arrojaban resultado positivo eran los frotis nasales (97,6%). Las segundas, por orden de frecuencia, eran los frotis bucales (35,7%), seguidos de los frotis rectales (28,5%). Además, la carga viral más alta, expresada por un valor de ciclo umbral (Ct) (o punto de cruce) de 11,27, se obtuvo a partir de un frotis nasal. Estos resultados llevan a la conclusión de que los frotis nasales son el tipo de muestra más adaptado para detectar el MERS-CoV en dromedarios aparentemente sanos mediante la técnica de RT-PCR.

**Palabras clave**

Coronavirus – Dromedario – Frotis de mucosas – Reacción en cadena de la polimerasa con transcripción inversa en tiempo real – Síndrome respiratorio de Oriente Medio.

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