

# Occupational Exposure to Dromedaries and Risk for MERS-CoV Infection, Qatar, 2013–2014

Chantal B.E.M. Reusken,<sup>1</sup>  
 Elmoubasher A.B.A. Farag,<sup>1</sup> Bart L. Haagmans,<sup>1</sup>  
 Khaled A. Mohran,<sup>1</sup> Gert-Jan Godeke,  
 V. Stalin Raj, Farhoud Alhajri, Salih A. Al-Marri,  
 Hamad E. Al-Romaihi, Mohamed Al-Thani,  
 Berend-Jan Bosch, Annemiek A. van der Eijk,  
 Ahmed M. El-Sayed, Adel K. Ibrahim,  
 N. Al-Molawi, Marcel A. Müller, Syed K. Pasha,  
 Christian Drosten, Mohd M. AlHajri,  
 Marion P.G. Koopmans

We determined the presence of neutralizing antibodies to Middle East respiratory syndrome coronavirus in persons in Qatar with and without dromedary contact. Antibodies were only detected in those with contact, suggesting dromedary exposure as a risk factor for infection. Findings also showed evidence for substantial underestimation of the infection in populations at risk in Qatar.

Since Middle East respiratory syndrome coronavirus (MERS-CoV) was first detected in 2012, approximately 1,000 human infections have been reported to the World Health Organization, all linked to residence in or travel to countries on the Arabian Peninsula (1). Dromedaries (*Camelus dromedarius*) are thought to play a central role in MERS epidemiology because widespread evidence of MERS-CoV-specific antibodies and virus shedding in camels was found (2), and highly similar viruses have been detected in humans and dromedaries at the same location (3,4). These data suggest a direct zoonotic risk for MERS-CoV infection among persons in contact with camels. We describe a comparative serologic investigation in Qatar

among persons with and without daily occupational exposure to dromedaries.

## The Study

We used 498 anonymized serum samples from persons in Qatar with and without dromedary contact (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/21/8/15-0481-Techapp1.pdf>) and control serum from Europe (National Institute for Public Health and the Environment, Bilthoven, the Netherlands; and University of Bonn, Bonn, Germany). Sampling in Qatar was cleared by the Ethics and Institutional Animal Care and Use Committees of the Medical Research Center, Hamad Medical Corporation (permit 2014-01-001). Samples from the Netherlands were used in accordance with the Dutch Federation of Medical Scientific Associations' code of conduct for proper use of human tissue. Samples from Germany were used in accordance with German national laws.

Of the 498 samples, 294 were from persons with daily occupational contact with dromedaries (cohorts A–D) and 204 were from persons without camel contact (cohorts E–G). Cohort A consisted of 109 healthy workers (5 camel slaughterers [subcohort A1] and 104 sheep slaughterers [A2]) at the central slaughterhouse in Doha, Qatar. All workers lived together and had contact with camels and sheep at the central animal market (CAM). Cohort B consisted of 8 CAM workers. Cohort C consisted of 22 healthy men living and working at the Al Shahaniya barn complex near the international dromedary racing track, and cohort D consisted of 155 healthy men living and working on a dromedary farm in Dukhan, western Qatar; molecular data showed ongoing circulation of MERS-CoV in dromedaries in these locations (online Technical Appendix). Cohort E consisted of 56 random samples from construction workers in Qatar. Cohort F consisted of 10 samples from persons working and living at a complex with 200 sheep barns in northern Qatar. Cohort G consisted of 138 samples for confirming specificity of the testing algorithm (66 samples from the Netherlands and Germany from persons with recent human CoV infection [subcohort G1] and 72 samples from the Netherlands obtained for routine testing from persons with suspected *Bordetella pertussis* infection [G2]).

We used microarray technology as described (3,5,6) to analyze samples for the presence of IgG reactive with MERS-CoV S1 antigen (Table). To avoid overinterpretation of data, we set the reactivity cutoff at 30,000 relative

Author affiliations: Erasmus Medical Center, Rotterdam, the Netherlands (C.B.E.M. Reusken, B.L. Haagmans, V.S. Raj, A.A. van der Eijk, M.P.G. Koopmans); Supreme Council of Health, Doha, Qatar (E.A.B.A. Farag, S.A. Al-Marri, H.E. Al-Romaihi, M. Al-Thani, A.M. El-Sayed, M.M. AlHajri); Agricultural Research Center, Cairo, Egypt (K.A. Mohran); Ministry of Environment, Doha (K.A. Mohran, F. Alhajri); National Institute for Public Health and the Environment, Bilthoven, the Netherlands (G.-J. Godeke, M.P.G. Koopmans); Utrecht University, Utrecht, the Netherlands (B.-J. Bosch); Leawaina Camel Hospital, Dukhan, Qatar (A.K. Ibrahim, S.K. Pasha); Hamad Medical Centre, Doha (N. Al-Molawi); University of Bonn Medical Center, Bonn, Germany (M.M. Müller, C. Drosten)

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<sup>1</sup>These first authors contributed equally to this article.

**Table.** Results of MERS-CoV serologic testing of humans with and without dromedary contact, Qatar, 2013–2014\*

Exposure type, cohort	Country	Serum samples tested by		
		S1 assay, no. positive/no. tested	PRNT <sub>90</sub> , no. positive/no. tested†	S1-negative
<b>Dromedary contact</b>				
A, slaughterhouse workers		20/294	10/20	1/35
A1, camel slaughterers	Qatar	4/5	2/4 (40, 20)	NT
A2, sheep slaughterers (contact with camels/camel slaughterers)	Qatar	3/104	2/3 (20, 20)	1/16 (20)
B, central animal market workers	Qatar	1/8	0	NT
C, barn workers at international camel racing track	Qatar	4/22	3/4 (40, 40, 20)	NT
D, camel farm workers	Qatar	8/155	3/8 (40, 40, 20)	0/19
<b>No dromedary contact</b>				
E, construction workers	Qatar	0/204	NA	0/48
F, sheep farmers	Qatar	0/56	NA	0/48
G, specificity controls		0/10	NA	NT
G1, recent infection with a common hCoV	GER, NL	0/66	NA	NT
G2, suspected infection with <i>Bordetella pertussis</i>	NL	0/72	NA	NT

\*GER, Germany; hCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not applicable; NL, the Netherlands; NT, not tested; PRNT<sub>90</sub>, 90% plaque-reduction neutralization test; S1, MERS-CoV S1 antigen.

†Nos. in parentheses are reciprocal antibody titers in PRNT<sub>90</sub>.

fluorescent units for subsequent analyses (6). Samples from 20 of 294 persons with camel contact were reactive; no control or noncontact samples were reactive. Among camel handlers at the Al Shahaniya and Dukhan locations, 4 of 22 and 8 of 155, respectively, had antibodies to MERS-CoV S1. At the CAM, 1 of 8 handlers had antibodies. At the slaughterhouse location, 3 of 104 sheep slaughterers and 4 of 5 camel slaughterers were antibody-positive (Figure).

Samples from subcohort G1 (n = 66) and from all camel-contact cohorts were tested for antibodies to CoV OC43 S1, a common human CoV; all showed high seropositivity (range 89%–100%) (Figure). All 498 samples were tested for reactivity to severe acute respiratory syndrome CoV S1; none reacted (Figure).

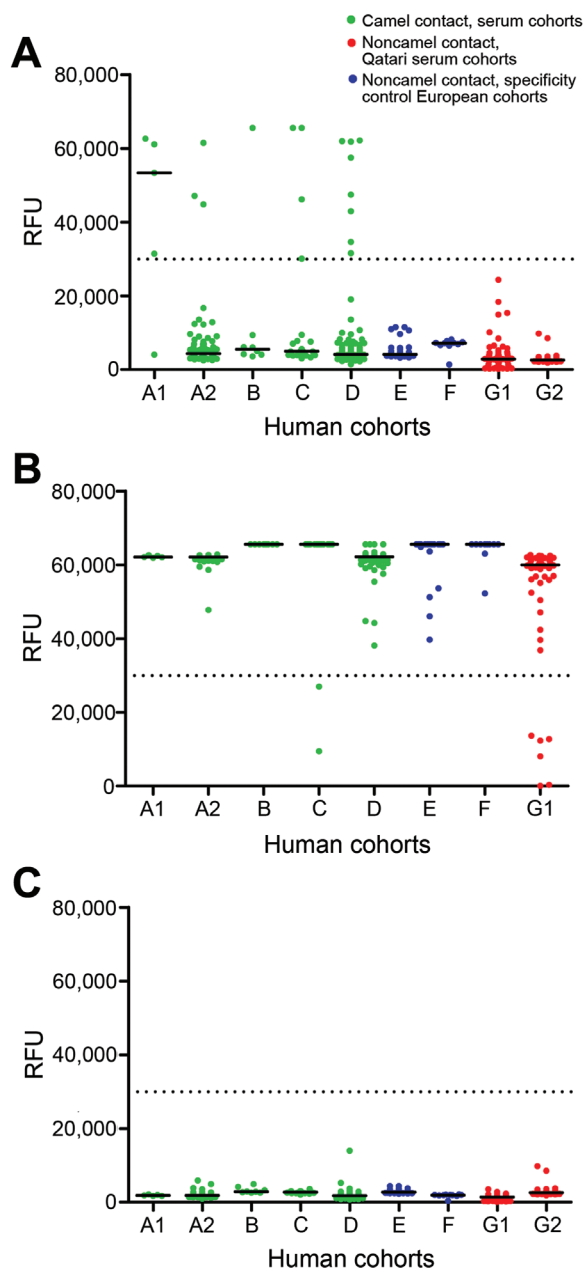
We used a 90% plaque-reduction neutralization test (PRNT<sub>90</sub>) to confirm the presence of MERS-CoV-specific antibodies in serum samples from camel handlers. For testing, we used the 20 samples that were reactive to MERS-CoV S1 and a random selection of nonreactive samples from camel-contact (n = 35) and noncontact (n = 48) cohorts. Results were positive for 10 of the 20 MERS-CoV S1 antibody-positive samples (reciprocal titers of 20 or 40) (Table).

All but 1 of the 35 samples from persons with camel contact who had negative S1 ELISA screening results were negative by PRNT<sub>90</sub>; the positive sample had a reciprocal titer of 20 (Table). All 48 samples from the noncontact cohorts were negative by PRNT<sub>90</sub>. This finding may indicate an underestimation of MERS-CoV seroprevalence by S1 testing. Furthermore, 6 samples from S1-positive and 2 from S1-negative persons with camel contact showed a reciprocal titer of 10, but titers of 10 were not observed in the noncontact cohorts. Five of these 8 reactive samples were also positive in a whole-virus MERS-CoV immunofluorescence assay at dilution 1:100; however, we regarded these as negative to avoid overinterpretation of data (data not shown).

## Conclusions

We detected MERS-CoV neutralizing antibodies in healthy persons who had daily occupational contact with dromedaries but not in persons without such contact. Only limited evidence is available regarding the presence of MERS-CoV antibodies in the general human population or in specific population cohorts. However, an overall seroprevalence of 0.15% was found in a cross-sectional study in Saudi Arabia, and among slaughterhouse workers, neutralizing antibodies were detected in 5 of 140 participants (7). This finding is similar to our finding among slaughterhouse workers: 7 of 109 were MERS-CoV antibody-positive. Four other studies lacked serologic evidence of MERS-CoV infection in humans with occupational exposure to dromedaries (8–11). However, only 1 of those studies documented actual MERS-CoV circulation in dromedaries during human contact, and it was concluded that MERS-CoV was not highly transmissible from camels to humans, although only 7 persons had regular contact with only 1 herd (8). On several occasions, the percentage of camels shedding MERS-CoV was high (60%) at the CAM and slaughterhouse (C.B.E.M. Reusken, unpub. data). Thus, locations with a continuous flow of dromedaries with different places of origin and different immune statuses may enable prolonged circulation of MERS-CoV and sustained exposure of dromedary handlers to the virus; in Qatar, such locations would include the CAM, slaughterhouse, and barns near the international racing tracks.

In this study, PRNT<sub>90</sub>-derived antibody titers were relatively low compared with those from earlier studies of MERS patients and dromedaries (2); B.L. Haagmans, unpub. data). The lower titers might reflect the apparent asymptomatic manifestation of MERS-CoV infection, individual differences in susceptibility, or both (2). Also, primary infections may result in a short-lived antibody peak followed by a rapid waning of antibody, depending on virus



**Figure.** Reactivity of human serum samples, from persons with and without dromedary contact, with S1 antigens of various coronaviruses (CoVs), Qatar, 2013–2014. A) Middle East respiratory syndrome CoV S1; B) human CoV OC43 S1; C) severe acute respiratory syndrome CoV S1. Relative fluorescent units (RFU) are shown at a serum dilution of 1:20. Black lines indicate median; dotted black lines at 30,000 RFU depict cutoff for analysis. Human cohorts: A1, camel slaughterers; A2, sheep slaughterers who had contact with dromedaries and camel slaughterers; B, workers at the central animal market; C, barn workers at the international camel racing track; D, workers on camel farms; E, construction workers; F, sheep farmers; G1, persons recently infected with a common human CoV (serum samples from the Netherlands and Germany); G2, persons with suspected *Bordetella pertussis* infection (serum samples from the Netherlands).

and host properties (12), as seen in influenza A(H5N1) virus infection: antibody levels are higher in symptomatic than asymptomatic H5N1-infected persons, and antibodies wane more quickly during asymptomatic infection (13). MERS-CoV antibody kinetics and the persistence of antibodies detected by different serologic methods are not known. Such parameters are needed to estimate the force of infection on the basis of serologic data (14).

MERS-CoV–seropositive participants in this study did not report severe health problems, giving evidence for frequent unrecognized human infections. Assuming the health histories are accurate, this finding implies that the current overall MERS-CoV–associated death rate of 37.1% (1) is most likely an overestimation of the actual rate and that most infections may be asymptomatic or mild. A major issue to be resolved is whether, and to what extent, asymptomatic cases contribute to the spread of MERS-CoV; it is well recognized that variability in disease transmission exists among humans (15).

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Dr. Reusken is a public health virologist at the Viroscience department of Erasmus Medical Center. Her research interests include viruses operating at the animal–human interface.

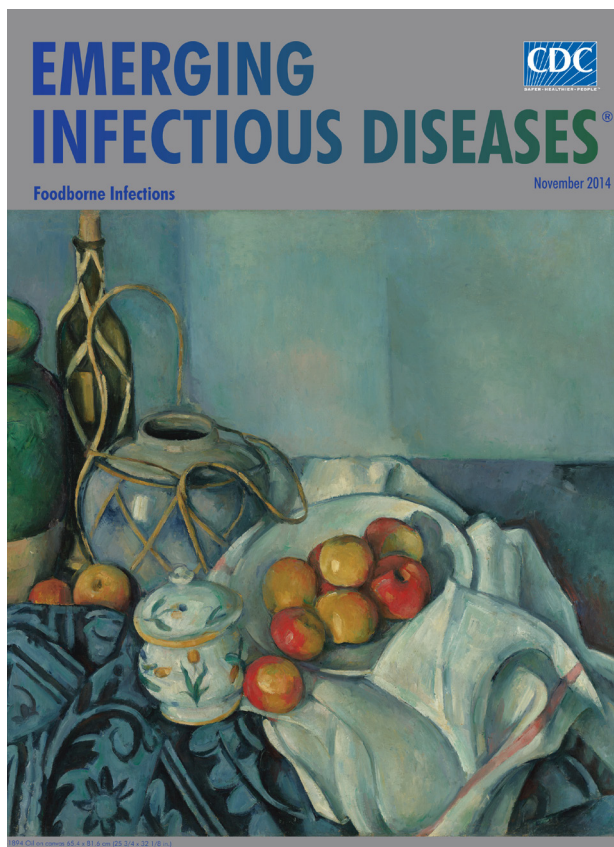
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Address for correspondence: Chantal B.E.M. Reusken, Erasmus Medical Center, Department of Viroscience, Wytemaweg 8, 3015CN, Rotterdam, the Netherlands; email: c.reusken@erasmusmc.nl; and Mohd M AlHajri, Supreme Council of Health, PO Box 42, Doha, Qatar; email: malhajri1@sch.gov.qa

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## Technical Appendix

### Description of Human Serum Cohorts

The human serum cohorts consisted of the following:

A) Anonymized serum samples from 109 healthy males (immigrants) collected in 2014 and working at the central slaughterhouse in Doha, Qatar. Only five workers exclusively work in camel slaughter in Qatar, with 3–20 years of experience (A1). All five were sampled. The other workers exclusively slaughter sheep (A2). However, all workers live together and have contact with the animals (camels, sheep) at the central animal market. The percentage of camels presented for slaughter that shed MERS-CoV was high at several occasions (1) (C.B.E.M. Reusken, unpub. data).

B) Anonymized sera from eight healthy males (originally from India, Nepal, Pakistan and Sudan) working at the Central animal market and collected in 2014. The market serves mainly camels and sheep but goats, cows, horses and donkeys as well. The market comprises  $\approx$ 100 pens for camels, with  $\approx$ 20 animals per pen. The camels originate from Australia, Iran, Oman, Pakistan, Qatar, Saudi-Arabia, Somalia, Sudan and Syria, and are sold for slaughter or use (pet, breeding, milk production) in Qatar. Animals introduced from Australia are presumed to be naive for MERS-CoV (2). This cohort has daily close contact with animals and their secretions.

C) Serum samples from 22 healthy males living and working at the Al Shahaniya barn complex near the Qatar international dromedary racing track. During the racing season from October through March, camels from around the Gulf area visit and stay together with Qatari camels at the Al Shahaniya barn complex. The barn complex consists of 749 barns with an estimated total of 14,000 camels. These persons originate from India, Nepal, Pakistan or Sudan and have daily close contact (nurturing, cleaning, grooming, training for racing) with camels residing at the barns. The sera were collected in 2013 and 2014. Published and

unpublished molecular data of camel samples taken in the period 2012–2014 and the connection of the first two human cases in Qatar in 2015 with this barn complex show ongoing circulation of MERS-CoV in the complex (3–6).

D) Serum samples from 155 healthy males living and working at a camel farm in the Dukhan area, West Qatar, collected in 2013 and 2014. The farm consists of milking, breeding and racing herds with an estimated 6.000 camels (4). The camel handlers originated from India, Nepal, Pakistan or Sudan, were 20–35 years of age and had daily, intensive contact with camels (nurturing, cleaning, grooming, veterinary care, training for races). The herds at the Dukhan farm showed molecular evidence for circulation of MERS-CoV at several occasions during 2013 and 2014 (A.K. Ibrahim, unpub. data).

E) Random, anonymized serum samples collected in 2014 from 56 males working for construction companies (laborers, metal workers, guards, plumbers, crane operators, drivers) and living in company camps in Doha, Qatar. All workers were healthy when samples were collected. The workers were <35 years of age and of Asian origin. There is no occupational contact with dromedaries, no ownership of dromedaries.

F) Anonymized serum samples from ten healthy males (immigrants) working and living at a complex with 200 sheep barns in North Qatar. At the barns >95% of the animals are sheep, other animals are goats, chickens, ducks. There is no contact with camels. Samples were collected in 2014.

G) Control group for specificity of the testing algorithm. G1) Anonymized serum samples from patients with a recent common human coronavirus (hCoV) infection (n = 66). Serum samples of 10 children, ages ranging from 9–14 months (2x hCoV-HKU1, 2x hCoV-OC43, 3x hCoV-229E and 3x hCoV-NL63 IgG positive sera) and obtained in 2001 in the Netherlands. Four anonymized hCoV-OC43 PCR positive sera from adults obtained in Germany in 2013. Fifty-four anonymized serum samples from adults obtained in Erasmus MC, the Netherlands in the period 2010–2014 and taken >2wks-<1 year after a respiratory tract sample tested positive for hCoVs using real-time RT-PCR technology (23x hCoV-OC43, 16x hCoV-229E, 15x hCoV-NL63. Serum had been collected at a later stage during hospitalization and subsequent routine visits to the out-patient clinic, the majority of these patients had recurrent health problems due to immune-deficiency, and was stored at Erasmus MC at –20°C. The study was approved by the local medical ethical committee (MEC approval: 2014–414). G2) Anonymized serum samples from 72 persons ranging in age from

0.1 year to 95.3 years sampled during 2008 for routine *Bordetella pertussis* serology in the Netherlands. This serum set represents a cohort biased toward patients with non-influenza-like respiratory symptoms (7).

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