# MERS-CoV in Camels but Not Camel Handlers, Sudan, 2015 and 2017

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We tested samples collected from camels, camel workers, and other animals in Sudan and Qatar in 2015 and 2017 for evidence of Middle East respiratory syndrome coronavirus (MERS-CoV) infection. MERS-CoV antibodies were abundant in Sudan camels, but we found no evidence of MERS-CoV infection in camel workers, other livestock, or bats.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic virus from camels that can cause serious respiratory disease and death in humans (1). Camel populations across the Middle East and Africa are highly seropositive. However, the only known human cases of clinical MERS-CoV infection in Africa were related to travel from Qatar and Saudi Arabia (https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/RRA\_MERS-CoV\_7th\_update.pdf), and serologic evidence for infections in humans resulting from camel exposure in Africa is limited (2).

The only published report of MERS-CoV circulation in camels in Sudan involved the testing of camel samples from 1983; that study found a seroprevalence of 82% (49/60) (3). Two publications from Egypt describe evidence of possible MERS-CoV circulation in Sudan, reporting a seroprevalence of 91% (543/594) in camels originating from Sudan and a seroprevalence of 92% (48/52), combined with a reverse transcription PCR positivity rate of 5.6%, in camels originating from Ethiopia and Sudan (3). Neither study presented conclusive evidence for MERS-CoV circulation in Sudan. Here, we provide the results of a study conducted in the Butana region of Al Gezira, Sudan, to investigate the local point prevalence of MERS-CoV and MERS-CoV antibodies among camel handlers, camels, and other animals in 2015 and 2017. We also report the results of a MERS-CoV screening in camels from Sudan sampled in Qatar directly upon importation.

We collected samples from humans and animals at a live animal market, an outdoor slaughter area adjacent to that market, and the Tamboul Camel Research Centre (TCRC), all located in Tamboul, Sudan. Overall, ≈1,660 camels and additional other livestock are usually present at the animal market; these camels come from individual small farms, where they are largely kept under free-roaming conditions. At the TCRC,  $\approx 100$  camels are generally present and kept out of contact with other camels. Before their arrival at the TCRC, they were herded on the Butana Plain. We also collected samples from 90 Sudan camels that were imported into Qatar in 2015. After arriving at the Hamad International Airport in Doha, Qatar, these camels were directly transported to the Al Shahaniya animal market in Doha. We sampled them immediately after their arrival. We stored all samples locally (1-1.5 years in Sudan, 1 month in Qatar) and tested them after shipment to the Netherlands.

We tested 56 human, 190 camel, 3 bat, 14 donkey, 15 cow, 15 sheep, and 15 goat serum samples for antibodies against MERS-CoV spike S1 using the protein microarray technique (4). We performed a virus neutralization test and a spike S1 protein-based ELISA (human serum samples only) to confirm the detection of MERS-CoV antibodies by protein microarray (5). In confirmatory tests, we included equal numbers of negative serum samples of the same species, when available. We considered samples positive if results of all tests were positive (protein microarray cutoff 1:20, 50% plaque-reduction neutralization titer cutoff 1:20, ELISA cutoff optical density 0.5). To resolve problems with possible mislabeling, we tested all animal serum samples collected in 2017 with a cytochrome B gene PCR to confirm species origins (6). We tested camel nasal (n = 168), nasopharyngeal (n = 24), and rectal (n = 61)swab specimens and milk (n = 33), urine (n = 30), and fecal (n = 42) samples for MERS-CoV RNA using a reverse

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#### RESEARCH LETTERS

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		2015			2017		
			MERS-CoV	MERS-CoV		MERS-CoV	MERS-CoV
			RNA, no.	antibody, no.		RNA, no.	antibody, no
	Location of sample	No.	positive/total	positive/total	No.	positive/total	positive/tota
Sample type	collection	samples	no. (%)	no. (%)	samples	no. (%)	no. (%)
Camel worker serum	Tamboul slaughter area	3	NT	ND	35	NT	ND
	Tamboul market	8	NT	ND	NA		
	TCRC	7	NT	ND	3	NT	ND
Camel serum†	Tamboul slaughter area‡§	4	ND	4/4 (100)	13	ND	13/13 (100)
	Tamboul marketद	27	ND	26/27 (96)	NA		. ,
	TCRC¶	31	ND	27/31 (87)	25	ND	25/25 (100)
	Qatar§¶#	90	ND	89/90 (99)	NA		. ,
Camel nasopharyngeal	TCRC¶	NA		• •	24	ND	NT
swabs†							
Camel nasal swabs†	Tamboul slaughter area‡§	11	ND	NT	NA		
	Tamboul marketद	11	ND	NT	NA		
	TCRC¶	31	ND	NT	25	ND	NT
	Qatar§¶#**	90	3/90 (3)	NT	NA		
Camel ticks (Hyalomma	TCRC	155	ND	NT	NA		
dromedarii) from 25							
camels							
Other animal serum							
Cattle	Tamboul slaughter area	6	ND	ND	9	NT	ND
Goat	Tamboul slaughter area	5	ND	ND	10	NT	ND
Sheep	Tamboul slaughter area	5	ND	ND	10	NT	ND
Donkey	Tamboul slaughter area	5	ND	ND	9	NT	ND
Bat††	TCRČ	3	ND	NT	NA		
Bat tissue‡‡	TCRC	13	ND	NT	NA		

Table. MERS-CoV RNA and antibody positivity among humans, bats, camels, and other livestock, Sudan, 2015 and 2017\*

\*MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not available; ND, not detected; NT, not tested; TCRC, Tamboul Camel Research Centre.

+From camels >2 years of age.

‡Serum and swab samples received from the slaughter field and live animal market were not matched.

§Sample set included meat camels.

¶Sample set included milk camels.

#Sample set included race camels.

\*\*Just imported into Qatar from Sudan.

ttGenus unknown.

##Tadarida spp.; lung, intestine, and brain tissues stored in formalin.

transcription PCR targeting the upstream of envelope and nucleocapsid genes, as described previously (7,8). In addition, we tested legs of camel ticks (*Hyalomma dromedarii*) and bat (*Tadarida* spp.) tissues collected at the TCRC in 2015 for MERS-CoV RNA.

In 2015, a total of 92% of camels in Sudan and 99% of camels exported to Qatar from Sudan were MERS-CoV seropositive (Table). In 2017, all camels tested in Sudan were seropositive. No MERS-CoV antibodies were found in human or bat serum samples or serum samples from live-stock other than camels. MERS-CoV RNA was detected in the nasal swabs from 3 camels imported into Qatar in 2015 but in no other samples.

The results of this study are in agreement with other seroepidemiologic studies performed in Africa. The camel population was highly seropositive for MERS-CoV, and none or a low percentage of nasal or nasopharyngeal swabs from camels were positive for MERS-CoV RNA. As shown before in other countries in Africa, human serum samples did not show neutralizing activity against MERS-CoV (2). In 1 study in Kenya, 2 of 1,122 livestock handlers were found positive for MERS-CoV neutralizing antibodies (9). Other livestock were also seronegative for MERS-CoV in our study, a finding in agreement with most serosurveys, although some sheep, goats, and donkeys and 1 cow have been reported to have MERS-CoV antibodies (3,10).

The number of human and livestock samples tested was low in this investigation. Therefore, the results of this study are not conclusive. However, this study provides preliminary insight into MERS-CoV circulation in Sudan, the country with the third largest dromedary camel population in the world (http://www.fao.org/faostat/en/#data/QA). We show evidence of extensive MERS-CoV circulation in camels but no evidence of circulation in other livestock, bats, and humans.

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#### References

- Reusken CB, Raj VS, Koopmans MP, Haagmans BL. Cross host transmission in the emergence of MERS coronavirus. Curr Opin Virol. 2016;16:55–62. https://doi.org/10.1016/ j.coviro.2016.01.004
- So RT, Perera RA, Oladipo JO, Chu DK, Kuranga SA, Chan KH, et al. Lack of serological evidence of Middle East respiratory syndrome coronavirus infection in virus exposed camel abattoir workers in Nigeria, 2016. Euro Surveill. 2018;23. https://doi.org/10.2807/1560-7917.ES.2018.23.32.1800175
- Sikkema RS, Farag EABA, Islam M, Atta M, Reusken CBEM, Al-Hajri MM, et al. Global status of Middle East respiratory syndrome coronavirus in dromedary camels: a systematic review. Epidemiol Infect. 2019;147:e84. http://dx.doi.org/10.1017/ S095026881800345X
- Reusken CBEM, Haagmans BL, Müller MA, Gutierrez C, Godeke G-J, Meyer B, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis. 2013;13:859–66. https://doi.org/10.1016/ S1473-3099(13)70164-6
- Okba NMA, Raj VS, Widjaja I, Geurts van Kessel CH, de Bruin E, Chandler FD, et al. Sensitive and specific detection of low-level antibody responses in mild Middle East respiratory. Emerg Infect Dis. 2019;25:1868–77. http://dx.doi.org/10.3201/ eid2510.190051
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, et al. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A. 1989;86:6196–200. https://doi.org/10.1073/pnas.86.16.6196
- Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17:20285. https://doi.org/10.2807/ ese.17.39.20285-en
- Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 2012;17:20334. https://doi.org/10.2807/ese.17.49.20334-en
- Liljander A, Meyer B, Jores J, Müller MA, Lattwein E, Njeru I, et al. MERS-CoV antibodies in humans, Africa, 2013–2014. Emerg Infect Dis. 2016;22:1086–9. https://doi.org/10.3201/ eid2206.160064
- Kandeil A, Gomaa M, Shehata M, El-Taweel A, Kayed AE, Abiadh A, et al. Middle East respiratory syndrome coronavirus infection in non-camelid domestic mammals. Emerg Microbes Infect. 2019;8:103–8. https://doi.org/10.1080/22221751.2018. 1560235

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## **Recombination between** Vaccine and Field Strains of Porcine Reproductive and Respiratory Syndrome Virus

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We isolated and plaque-purified IA76950-WT and IA70388-R, 2 porcine reproductive and respiratory syndrome viruses from pigs in the same herd in Iowa, USA, that exhibited coughing and had interstitial pneumonia. Phylogenetic and molecular evolutionary analysis indicated that IA70388-R is a natural recombinant from Fostera PRRSV vaccine and field strain IA76950-WT.

Porcine reproductive and respiratory syndrome (PRRS) characterized (PRRS), characterized by reproductive failure in sows and respiratory distress in pigs of all ages, causes substantial economic loss to the worldwide swine industry. PRRS virus (PRRSV) is an enveloped, single-stranded, and positive-sense RNA virus belonging to the family Arteriviridae (1). Historically, PRRSV comprises type 1 (PRRSV-1) and type 2 (PRRSV-2); recently, PRRSV-1 was taxonomically classified into the species Betaarterivirus suid 1 and PRRSV-2 into the species Betaarterivirus suid 2. PRRS has remained the most important disease of swine throughout the world, and live attenuated vaccines are used to reduce the clinical impact of PRRSV infection. Several studies have reported that recombinant PRRSV strains emerged in China, Korea, and France because of recombination between wild-type and vaccine strains (2-6). Nevertheless, recombination between a live attenuated vaccine strain and a circulating strain has not been reported in the United States.

In October 2018, a farm with a history of using Fostera PRRSV vaccine had been experiencing an ongoing problem with porcine respiratory disease. Histopathologic examination of 2 samples (lungs A and B) revealed the lungs of both pigs demonstrated significant interstitial pneumonia. Open reading frame (ORF) 5 Sanger sequencing identified a wild-type PRRSV from sample A