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# Middle East Respiratory Syndrome Coronavirus Seropositivity in Camel Handlers and Their Families, Pakistan

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A high percentage of camel handlers in Saudi Arabia are seropositive for Middle East respiratory syndrome coronavirus. We found that 12/100 camel handlers and their family members in Pakistan, a country with extensive camel MERS-CoV infection, were seropositive, indicating that MERS-CoV infection of these populations extends beyond the Arabian Peninsula.

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Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), identified in 2012, causes a highly lethal pneumonia with a 34.5% mortality rate (<https://www.who.int/emergencies/mers-cov>). As of July 31, 2019, a total of 2,458 cases and 848 deaths have been reported to the World Health Organization, with all cases in the Middle East or in travelers from this region or their contacts (1). MERS cases fall into 2 categories, primary and secondary. Secondary cases, which result most commonly from human-to-human transmission in hospitals, were most prominent during the early years of the outbreak. However, as stringent infection control measures have been followed more closely, a greater proportion of cases are classified as primary. Camels are believed to be the zoonotic source for primary infections, but a large proportion of patients describe no camel contact, raising the question of how they acquired the disease (2).

To determine the source of the infection, several studies have focused on a potential role in transmission for

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camel handlers. These reports indicate that the percentage of MERS-CoV-immune camel handlers is much greater than in the general population of Saudi Arabia, the country with the largest number of MERS cases. These studies have reported that 3%–67% of camel handlers in this country are MERS-CoV exposed, compared with 0.15% of the general population (3–5). In Saudi Arabia, much of camel farming is labor intensive, and many camel owners hire camel handlers, generally from outside of the country, to tend to them (6). To determine the generalizability of these observations, we tested blood samples from 100 camel handlers and their families in the Cholistan desert in Punjab, Pakistan, a country with no reported human MERS (7).

## The Study

We chose Cholistan as the study site because it is the most important region of Pakistan for the camel industry, and handlers and their families are in close contact with dromedaries. We engaged study participants in the Bahawalnagar and Bahawalpur districts, located in southern Punjab Province, Pakistan. The Institutional Ethical Review Board (IERB) of the Institute of Public Health, Government of Punjab, Lahore, Pakistan, approved the study. We obtained written informed consent from all study participants.

Camel handlers in Cholistan differ from those in Saudi Arabia in that they own their camels, along with cows, goats, and sheep, and they and their families take care of these animals. Both men and women are responsible for grazing, feeding, milking, and waste disposal. In addition, they live in close proximity to camels and share similar water sources (8–10). Camel handlers in Cholistan are either nomadic, seminomadic, or sedentary, with varying degrees of exposure to camels. Nomads live with their camels in the desert and migrate throughout Cholistan, whereas seminomads tend to live at a base camp and migrate depending on availability of fodder and water. Nomadic camel handlers and their families have the highest exposure to camels, whereas sedentary ones have the least exposure.

During 2017–2018, we obtained blood samples from 100 participants from nomadic, seminomadic, and sedentary populations. The age range was 8–76 years (average

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**Table 1.** Characteristics of participants in study of Middle East respiratory syndrome coronavirus seropositivity in camel handlers and their families, Pakistan

| Characteristic                          | No. (%) participants |
|---|----------------------|
| Lifestyle                               |                      |
| Sedentary                               | 10 (10)              |
| Seminomadic                             | 64 (64)              |
| Nomadic                                 | 26 (26)              |
| Concurrent conditions                   | 21 (21)              |
| Consumption of unpasteurized camel milk | 98 (98)              |
| Tobacco use                             | 38 (38)              |

30.1 years). We obtained demographic and clinical information at sampling by written questionnaire, including participant age, lifestyle (nomadic, seminomadic, sedentary), role in family (husband, wife, child, etc.), underlying medical conditions, numbers of camels owned, history of tobacco use (smoking or chewing), and consumption of camel products (milk) (Table 1; Appendix Table, <https://wwwnc.cdc.gov/EID/article/25/12/19-1169-App1.pdf>). We transported samples to the microbiology department at the University of Veterinary and Animal Sciences (Lahore, Punjab, Pakistan). We prepared serum samples, stored them at  $-80^{\circ}\text{C}$ , and shipped them to the University of Iowa (Iowa City, Iowa, USA) for analysis.

We tested all the samples for MERS-CoV–specific antibodies by ELISA and 50% reduction plaque-reduction neutralization test (PRNT<sub>50</sub>). Of 91 participants examined by a commercially available ELISA, 49 were positive for MERS-CoV–specific antibody. Twelve had PRNT<sub>50</sub> titers  $>1:20$  and were considered positive; of these, 5 were also positive by ELISA. In addition, 10/12 were positive by immunofluorescence assay. Of the 12 PRNT<sub>50</sub>-positive participants, 3 were women and 1 was an 8-year-old child (Table 2).

All but 2 of the study participants were exposed to camels. There was no significant correlation ( $p>0.5$ ) between MERS-CoV seropositivity and lifestyle, presence of concurrent conditions, drinking unpasteurized camel milk, or tobacco use, with the caveat that the sample size was small.

## Conclusions

In general, nomads had the most and sedentary populations had the least camel contact, although nearly all family members were exposed to and took care of camels. Of 100 participants, we identified 12 who were MERS-CoV seropositive, as measured by the presence of PRNT<sub>50</sub> antibody. Of note, several PRNT<sub>50</sub>-positive samples were negative by ELISA, but most were positive by immunofluorescence assay. This lack of concordance between ELISA and PRNT<sub>50</sub> titers was observed previously (3,11) and may reflect lower sensitivity of the commercial ELISA kit (12). Other coronaviruses circulate in camel populations (13), and it is conceivable that the high rate of ELISA seropositivity resulted from immune responses to other, possibly MERS-like, coronaviruses present in Pakistan. Thus, it will be important to assess camel (and human) populations for other coronaviruses that might elicit a cross-reactive response.

The mechanism of MERS-CoV transmission from camels to humans in Pakistan is not established, but most camel handlers and their families drink fresh camel milk, obtained after young camels have finished nursing. Juvenile camels demonstrate the highest rate of seroconversion and of MERS-CoV positivity (6,14), so it is possible that drinking fresh milk is a source of infection. In this region of Pakistan, camel handlers and their families also share water sources with camels, which probably

**Table 2.** Characteristics of camel handlers and their families positive for Middle East respiratory syndrome coronavirus in study in Pakistan\*

| Patient no. | Family no. | Age, y/<br>sex | Camel contact†  | Smoking | Concurrent conditions                 | Lifestyle   | PRNT <sub>50</sub> | ELISA result/<br>value‡ | IFA result/<br>titer§ |
|-------------|------------|----------------|-----------------|---------|---------------------------------------|-------------|--------------------|-------------------------|-----------------------|
| SH94        | F2         | 20/M           | Direct/daily    | Yes     | None                                  | Nomadic     | 211                | -0.78                   | +1:80                 |
| SH85        | F2         | 21/M           | Direct/daily    | Yes     | None                                  | Nomadic     | 32                 | +1.51                   | +1:40                 |
| SH100       | F1         | 8/M            | Direct/daily    | No      | None                                  | Nomadic     | 72                 | -0.64                   | +1:40                 |
| SH71        | F9         | 35/F           | Indirect        | No      | HPT, renal and<br>respiratory disease | Seminomadic | 33                 | Borderline/<br>0.99     | +1:20                 |
| SH74        | F9         | 40/F           | Indirect        | No      | HPT                                   | Seminomadic | 40                 | +2.18                   | +1:80                 |
| SH63        | F13        | 35/F           | Direct/monthly  | No      | None                                  | Seminomadic | 27                 | Borderline/<br>0.92     | +1:10                 |
| SH57        | F14        | 20/M           | Direct          | No      | None                                  | Seminomadic | 51                 | +3.11                   | +1:80                 |
| SH58        | F16        | 28/M           | Direct          | Yes     | None                                  | Seminomadic | 68                 | +1.74                   | +1:160                |
| SH21        | None       | 17/M           | Direct/seasonal | Yes     | None                                  | Seminomadic | 80                 | -0.36                   | -<1:10                |
| SH65        | None       | 20/M           | D/daily         | No      | None                                  | Seminomadic | 65                 | +1.13                   | +1:80                 |
| SH43        | None       | 34/M           | Direct          | No      | None                                  | Sedentary   | 1,600              | -0.76                   | +1:160                |
| SH44        | None       | 40/M           | Direct          | Yes     | None                                  | Sedentary   | 89                 | -0.48                   | -<1:10                |

\*All 12 patients tested positive by PRNT<sub>50</sub>. IFA, immunofluorescence assay; HPT, hypertension; PRNT<sub>50</sub>, 50% reduction plaque reduction neutralization assay.

†Direct indicates camel herders with direct camel contact but extent of exposure is not known; direct/daily, camel herders with daily direct camel contact; direct/monthly, camel herders with monthly direct camel contact; direct/seasonal, camel herders with seasonal direct camel contact; indirect, family members of camel herders.

‡Positive result is  $>1.1$ ; borderline, 0.8–1.1; negative,  $<0.8$ , as defined by the test manufacturer.

§Negative test result is  $<1:10$ , as defined by the test manufacturer.

contributes to virus transmission. Zohaib et al. identified a 75.6% MERS seroprevalence in camels throughout Pakistan, but 0% seropositivity in humans, including some with camel contact (7).

Medical services in Cholistan and adjacent areas are limited, making MERS diagnosis and transmission studies difficult. Our findings show a need for additional studies to confirm the absence of clinically apparent MERS in this region and to determine whether epidemiologic, technical, or other factors caused differences in seropositivity between our study and that of Zohaib et al.

Our study, by demonstrating a low but detectable rate of MERS-CoV seropositivity in camel handlers and their families, indicates that this population could contribute to MERS-CoV transmission to the broader community in Pakistan. We previously showed that measurement of T cell responses identified additional MERS-CoV-immune persons (3,11), suggesting that our results may underestimate the prevalence of MERS-CoV infection. Our results also illustrate the importance of educating camel herders and their families about proper infection control measures, including handwashing, to diminish the likelihood of MERS-CoV transmission.

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## Appendix

### Virus and Cells

The EMC/2012 strain of MERS-CoV was provided by Bart Haagmans and Ron Fouchier (Erasmus Medical Center, Rotterdam, the Netherlands). We conducted all work with infectious MERS-CoV in the University of Iowa Biosafety Level 3 (BSL3) Laboratory.

### MERS-CoV ELISA

We performed ELISAs, which use the S1 protein as target, as described previously (11), using commercially available kits (Euroimmun Medizinische Labordiagnostika AG; <https://www.euroimmun.com>) and read them as positive ( $>1.1$ ), negative ( $<0.8$ ), or borderline ( $0.8-1.1$ ).

We performed MERS-CoV IFA, which uses MERS-CoV infected cells, as previously described (11). We considered titers  $<1:10$  negative.

### Plaque Reduction Neutralization Assays (PRNT<sub>50</sub>)

We performed PRNT<sub>50</sub> assays as previously described (11). We repeated assays for PRNT<sub>50</sub>  $\geq 2$  times for each serum sample, with nearly identical results. We considered serum samples with PRNT<sub>50</sub>  $>1:20$  positive.

### Statistical Analysis

Fisher exact test was used to compare differences between groups. We considered p values  $<0.05$  statistically significant.