

## Risk Factors for Primary Middle East Respiratory Syndrome Coronavirus Infection in Camel Workers in Qatar During 2013–2014: A Case-Control Study

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The transmission routes and risk factors for zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) infections are still unknown. We used the World Health Organization questionnaire for MERS-CoV case-control studies to assess risk factors for human MERS-CoV seropositivity at a farm complex in Qatar. Nine camel workers with MERS-CoV antibodies and 43 workers without antibodies were included. Some camel-related activities may pose a higher risk of MERS-CoV infection, as may cross-border movements of camels, poor hand hygiene, and overnight hospital stays with respiratory complaints. The risk factors identified in this study can be used to develop infection prevention and control measures for human MERS-CoV infections.

**Keywords.** MERS-CoV; risk factors; transmission; zoonotic; coronavirus.

In 2012, a novel coronavirus, later named “Middle East respiratory syndrome coronavirus” (MERS-CoV), was isolated from a patient with pneumonia in Saudi Arabia [1]. In 2013, the first serological evidence of dromedary camels as reservoir host species for MERS-CoV was published [2], followed by detection of highly similar viruses in dromedary camels and symptomatic humans in contact with these animals. Further support for zoonotic MERS-CoV infection was provided by the detection of MERS-CoV antibodies in camel-exposed persons, but the transmission routes and risk factors for primary, zoonotic MERS-CoV infections are still not elucidated [3].

Therefore, using the World Health Organization (WHO) questionnaire for case-control studies, we assessed risk

factors for the presence of MERS-CoV antibodies in camel workers at a farm complex with circulation of MERS-CoV in camels in Qatar [4]. Previously, a cross-sectional MERS-CoV serosurvey at this farm complex revealed a 5.1% seropositivity rate among the camel workers [5]. The outcomes of this study can be used to further establish evidence-based infection prevention and control measures for primary human MERS-CoV infections.

### METHODS

#### Study Cohort

The camel farm complex in the Dukhan area of West Qatar consists of 5 barns with approximately 6000 racing and milking camels. Each barn includes a communal dormitory for all personnel. The results of MERS-CoV-specific serologic tests of the camel workers were described in a broader Qatar-wide seroprevalence study by Reusken et al [5]. Nine camel workers with antibodies specific for MERS-CoV and 43 workers without such antibodies as previously determined by S1-based protein microarray testing were included in this study [5]. A total of 3–4 seronegative workers per seropositive worker were randomly selected, based on the proximity of their bed to a seropositive worker, their age, their sex, and the date they joined the farm. Exclusion criteria were hospital admission within 14 days before serum sampling or recent contact (ie, within 14 days) with a person confirmed to be infected with MERS-CoV or with a hospitalized patient with a respiratory illness of unknown cause. None of the selected workers refused to participate or met the exclusion criteria.

#### Data Collection

All study participants were subjected to the WHO questionnaire, which was slightly adapted to the local situation (Supplementary Materials). The interviews took place in April 2014 and were completed within 1 week. The questionnaires were conducted in Arabic or Urdu by trained staff from the Qatar Ministry of Health.

#### Data Analysis

We compared the questionnaire results of seropositive and seronegative workers by using the Welch *t* test and the Fisher exact test, performed in Stata/SE 14.1 for Windows. The Mantel-Haenszel test was used for multivariate testing, performed in R-3.3.2 for Windows, with a maximum of 2 variables and with a minimum of 4 events each. When a question was left unanswered, we assumed a negative (ie, “no” or “never”) response. Likert scales were converted to binary answers (“never,” “rarely,” and “monthly” were converted to “rarely”; “weekly” and “daily” were converted to “frequently”).

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## Ethical Approval

The investigation was part of an official public health outbreak investigation. The joint investigation team obtained written informed consent from all participants, as well as written approvals from the Public Health Department of the Qatar Ministry of Health.

## RESULTS

### General Cohort Characteristics

The study subjects were all male, with a mean age of 28 years (Table 1). They originated from Bangladesh, Pakistan, Sudan, Nepal, and India. On average, they lived in Qatar for 3 years. Four of 52 (8%) smoked shisha, and 16 of 52 respondents (31%) smoked tobacco, either now or in the past. No subjects reported preexisting disease.

Fifty percent (26 of 52) reported that they regularly cleaned animal housing facilities. Most subjects in this subset also indicated that they handled animal waste (25 of 26;  $P < .001$ ) and cleaned farm equipment (15 of 26;  $P < .001$ ; Table 2). Among all subjects, 25% (13) milked camels more than once per week, and 12% (6) frequently assisted with calvings; all of which were also involved in milking camels ( $P < .001$ ). Thirteen percent of subjects (7) were involved in camel training.

### Univariate Analysis

Regular involvement in training and herding of camels (44% of seropositive participants vs 7% of seronegative participants;  $P = .01$ ), cleaning farm equipment (67% vs 26%;  $P = .05$ ), and milking camels (55% vs 19%;  $P = .03$ ) were associated with MERS-CoV seropositivity (Table 2). Workers involved in milking consumed raw camel milk (55% vs 19%;  $P = .03$ ) and raw milk products (55% vs 19%;  $P = .03$ ) significantly more often than workers not involved in milking, but correcting for these 2 parameters did not change the association between milking and

MERS-CoV seropositivity. MERS-CoV-seropositive workers also seemed to assist with calving more often than seronegative workers, although the difference was not significant (33% vs 7%;  $P = .08$ ).

Handwashing before and after animal handling was more common among seronegative workers (44% vs 86%;  $P = .01$ ), and a greater percentage of seropositive workers indicated that they rarely washed hands, although the difference was not significant (22% vs 2%;  $P = .07$ ). MERS-CoV-seropositive workers were also more likely to be involved in the movement of the camels in their care to other locations (44% vs 14%;  $P = .06$ ). Of the workers who reported such movements, 3 (75%) in the seropositive group reported international movements (Saudi Arabia, United Arab Emirates, and Oman), compared with 0 seronegative workers. Two of these 3 seropositive workers also traveled to Saudi Arabia without animals in the past 6 months.

Workers with MERS-CoV antibodies were significantly more likely to report the presence of animal feces (44% vs 9%;  $P = .02$ ) and dogs (33% vs 5%;  $P = .03$ ) around their living quarters. Four of 5 workers who reported the presence of dogs also reported animal feces around their living quarters ( $P = .001$ ). Other animals frequently seen at the farm complex were cats (reported by 59% of respondents), rats (reported by 55%) and mice (reported by 28%). None of the 52 study subjects indicated the presence of bats. One seropositive respondent reported drinking camel urine, although rarely, and none of the workers reported eating uncooked meat.

Significantly more seropositive workers reported an overnight stay in a hospital with respiratory complaints in the past 12 months (33% vs 2%;  $P = .01$ ). Two seropositive workers indicated that they had fever, cough, vomiting, and headache at the time of the questionnaire, and both had been admitted to the hospital in the last 12 months. Nasal swab specimens from both workers tested negative for MERS-CoV by polymerase chain reaction analysis when they were admitted to the hospital.

## Discussion

We looked at possible correlations between different putative risk factors for MERS-CoV infection and the presence of MERS-CoV antibodies in camel workers. The univariate analysis revealed a correlation between the presence of MERS-CoV neutralizing antibodies in camel farm workers and cleaning farm equipment ( $P = .05$ ), assisting in animal birth ( $P = .08$ ), milking animals ( $P = .03$ ), and training animals ( $P = .01$ ). Cleaning farm equipment might represent an increased risk of MERS-CoV exposure through contact with camel saliva, feces, and/or urine on soiled equipment [6, 7]. All animal workers that were involved in calvings also milked camels more than once per week. The relative high number of workers with MERS-CoV antibodies who assisted in the birth of camels and in milking animals may be explained by intensive contact with young camels. Although newborn calves are still protected by

**Table 1. General Characteristics of the Study Participants With and Those Without Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Infection**

Characteristic	MERS-CoV-Seropositive Workers (n = 9)	MERS-CoV-Seronegative Workers (n = 43)
Male sex	9 (100)	43 (100)
Age, y	30.9 (25.4–36.4)	27.0 (25.2–28.8)
Nationality		
Bangladesh	4 (44)	18 (42)
Pakistan	2 (22)	16 (37)
Sudan	2 (22)	5 (12)
Nepal	1 (11)	3 (7)
India	0 (0)	1 (2)
Tobacco use <sup>a</sup>	5 (56)	11 (26)
Shisha use	1 (11)	3 (7)

Data are no. (%) of subjects or mean value (95% confidence interval).

<sup>a</sup>Three of 5 seropositive workers and 1 of 11 seronegative workers reported that they were former tobacco smokers.

**Table 2. Selection of Possible Risk Factors for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Antibodies: Univariate Analysis**

Risk Factor	MERS-CoV–Seropositive Workers, No. (%) (n = 9)	MERS-CoV–Seronegative Workers, No. (%) (n = 43)	P <sup>a</sup>
<b>Primary job</b>			
Animal care	7 (78)	28 (65)	.70
Animal training	4 (44)	3 (7)	.01
Housework	0 (0)	2 (5)	>.99
Other	3 (33)	7 (16)	.35
<b>Frequently<sup>b</sup> performed activities in past 12 mo</b>			
Touch animals	7 (78)	27 (62)	>.99
Kiss animals	0 (0)	2 (5)	.47
Clean animal housing	6 (67)	20 (47)	.46
Handle animal waste	6 (67)	19 (45)	.28
Clean farm equipment	6 (67)	11 (26)	.05
Assist in birth of animals	3 (33)	3 (7)	.08
Milk animals	5 (55)	8 (19)	.03
Slaughter animals	0 (0)	0 (0)	>.99
Administer vaccines and/or medicines	0 (0)	1 (2)	>.99
<b>Other animals at the farm complex</b>			
Dogs	3 (33)	2 (5)	.03
Cats	3 (33)	23 (53)	.47
Rats	3 (33)	26 (61)	.16
Mice	3 (33)	12 (28)	.70
Chickens	0 (0)	1 (2)	>.99
Pigeons	0 (0)	1 (2)	>.99
<b>Contact with animal waste</b>			
Present around subjects' living quarters	4 (44)	4 (9)	.02
Touched animal waste <sup>c</sup>	6 (67)	21 (49)	.47
<b>Contact with sick or dead camels</b>			
Present around sick camels	5 (58)	17 (40)	.47
Present around dead camels	0 (0)	9 (21)	.33
Touched sick/dead camels	1 (11)	9 (21)	.67
<b>Participation in animal transport</b>			
New camel at the barn	5 (55)	25 (58)	.71
Animal taken to another location <sup>d</sup>	4 (44)	6 (14)	.06
<b>Personal protective equipment</b>			
None	5 (58)	30 (70)	.45
Gloves	2 (22)	10 (23)	>.99
Coveralls	0 (0)	5 (12)	.57
Dust masks	2 (22)	6 (14)	.61
Boots or boot covers	0 (0)	4 (9)	>.99
Eye protection	1 (11)	5 (12)	>.99
<b>Hand washing</b>			
At mealtime	5 (56)	22 (51)	>.99
Before and after animal task	4 (44)	37 (86)	.01
Beginning and end of the day	1 (11)	16 (37)	.21
Bathroom time	5 (56)	16 (37)	.46
Rarely	2 (22)	1 (2)	.07
<b>Consumption of animal products</b>			
Any raw milk product <sup>e</sup>	6 (67)	26 (60)	>.99
Raw camel milk	6 (67)	20 (47)	.47
Raw cow milk	0 (0)	5 (12)	.57
Uncooked meat	0 (0)	0 (0)	>.99
Travel outside Qatar in past 6 mo <sup>f</sup>	2 (22)	2 (5)	.13

Risk Factor	MERS-CoV–Seropositive Workers, No. (%) (n = 9)	MERS-CoV–Seronegative Workers, No. (%) (n = 43)	P <sup>a</sup>
<b>Respiratory complaints in past 12 mo</b>			
Required physician visit	3 (33)	5 (12)	.13
Required overnight hospital stay	3 (33)	1 (2)	.01
<b>Current complaints</b>			
Fever and cough	2 (22)	2 (5)	.13
Shortness of breath	0 (0)	0 (0)	>.99
Vomiting	2 (22)	0 (0)	.03
Diarrhea	0 (0)	0 (0)	>.99
Headache	2 (22)	0 (0)	.03

<sup>a</sup>By the Fisher exact test.

<sup>b</sup>Daily or weekly.

<sup>c</sup>All contact was with camel waste.

<sup>d</sup>Oman, United Arab Emirates, and Saudi Arabia.

<sup>e</sup>Among those who consumed any raw milk product, 94% consumed milk, and 3% consumed cheese; the type of raw milk products consumed by 3% was unknown.

<sup>f</sup>Both seropositive subjects traveled to Saudi Arabia, 1 seronegative subject traveled to Pakistan, and 1 seronegative subjects traveled to Bangladesh.

maternal antibodies, workers who assist in animal birth or milking often remain in close contact with mothers and their calves beyond the period of maternal protection [8]. For example, milking requires the presence of suckling young camels who trigger milk flow [9]. Moreover, camel milk can contain MERS-CoV RNA [9]. An association between MERS-CoV illness and milking has been described before [10]. Camel training, which requires close contact between the animal and its trainer, also seemed to increase the risk for MERS-CoV infection in camel workers. This is in agreement with previous reports that indicated that close contact with camels can be a risk factor for MERS-CoV illness or the presence of MERS-CoV antibodies [5, 10, 11].

In our cohort, we found a greater frequency of MERS-CoV seropositivity among workers who indicated that the camels they handled had recently traveled abroad within the Arabian Peninsula. Movement of animals to and from the Dukhan farm area occur with high frequency, owing to races, trade, and breeding activities, and can contribute to continuous local MERS-CoV circulation and human exposure due to both a continuous introduction of naive animals and/or acutely infected camels. Two out of 3 workers who reported international movements of their camels also reported personal travel to Saudi Arabia in the past 6 months. It is possible that these workers were exposed to MERS-CoV during their personal travel, rather than via the camels they worked with.

Another association that was found was between the presence of MERS-CoV antibodies and overnight hospitalization because of respiratory complaints. It is possible that the admitted workers had MERS-CoV infection but were tested after virus shedding had stopped or that they acquired nosocomial MERS-CoV infection without severe symptoms. Two of 3 seropositive

workers hospitalized with respiratory complaints also reported headache, vomiting, cough, and fever at the time of the survey.

A remarkable finding was that a significantly greater percentage of seropositive workers reported the presence of dogs around their barn. The reported presence of dogs correlated strongly with the presence of animal feces (origin unknown) around the subject's living quarters ( $P = .001$ ). There is currently no evidence of a role for dogs in MERS-CoV epidemiology. A possible explanation for the association between the presence of dogs and MERS-CoV-seropositive humans could be that dogs mechanically spread contaminated camel products (eg, feces and urine) around the farm complex.

The cohort with antibodies against MERS-CoV had a greater percentage of workers who rarely washed their hands, and washing hands before and after animal tasks appeared to have a preventive effect. Prevention of MERS-CoV infection or exposure by handwashing possibly indicates that MERS-CoV can be indirectly transmitted via fomites. Infectious MERS-CoV could still be detected on surfaces after 1 day at 30°C and in milk after 2 days at 22°C in experimental conditions [12, 13]. Contact with camel excretions and subsequent touching of mucous membranes may be an important source of infection. Nasal secretions have been shown more frequently to contain MERS-CoV and have higher viral loads as compared to camel urine, feces, and saliva [6]. Human-to-human transmission may also take place via fomites. This can explain why many individuals with a primary case of MERS-CoV infection have not reported direct camel contact and, in some cases, have reported a household member who recently visited a camel farm [10, 14].

While providing some interesting observations, this study has several limitations. First, owing to the number of respondents, the power of the study is limited. Therefore, we could only perform univariate analyses and very limited multivariate analyses to demonstrate significant associations between possible risk factors and MERS-CoV antibody presence in the respondents. Moreover, the retrospective study design may have resulted in significant recall bias among participants with regard to their and their camels' activities and health in the last 12 months. Because workers share housing and sleeping areas, MERS-CoV may have spread from human to human. This means that not all seropositive workers may have been infected directly by camels, which may affect the analysis. Last, it is possible that the MERS-CoV immunoglobulin G we detected was a result of exposure in the worker's country of origin rather than in Qatar, with MERS-CoV circulation known to exist among camels in some such countries.

A recent MERS-CoV WHO consultation on public health goals and global priority research activities called on researchers to address knowledge gaps related to, among other topics, animal reservoirs and transmission routes to humans of MERS-CoV [15]. This study adds to the understanding of MERS-CoV

transmission on the human-animal interface and informs risk management. On the basis of these initial results, a larger study was initiated with the aim to include different segments of the population in Qatar.

### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

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