Epidemiological study of Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia, April–May 2015

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Summary

A cross-sectional study was conducted in five regions in Saudi Arabia (SA) to investigate the epidemiology of Middle East respiratory syndrome coronavirus (MERS–CoV) infection in dromedary camels (*Camelus dromedarius*) during April and May 2015. Serum and nasal swab samples were tested for MERS–CoV antibodies and ribonucleic acid (RNA) using a recombinant enzyme-linked immunosorbent assay (rELISA) and real-time reverse-transcription–polymerase chain reaction (rRT–PCR), respectively. The overall MERS–CoV antibody seroprevalence was 80.5%, whereas the overall viral RNA prevalence was 2.4%. The associations of risk factors with each prevalence were quantified using univariate and multivariate analyses. The multivariate models identified region, age, grazing system, exposure to wild animals and dung removal as factors significantly associated with seroprevalence ($p \leq 0.05$). A higher seroprevalence was more likely to occur in: camels from the Riyadh, Eastern, Northern and Makkah regions than those from the Jazan region; camels ≥4 and 1–3 years of age (marginally significant) than calves <1 year; and camels raised in zero grazing and semi-open grazing systems than those raised in an open grazing system. However, the presence of wild animals and daily dung removal were negatively associated with seroprevalence. On the other hand, region and sex were significantly associated with MERS–CoV RNA prevalence ($p \leq 0.05$). A higher viral RNA prevalence was more likely to occur in camels from the Riyadh region and Eastern region (marginally significant) than in those from the Makkah region, and in male camels than female camels. In conclusion, the risk factors identified in this study can be considered to be predictors of MERS–CoV infection in camels and should be taken into account when developing an efficient and cost-effective control strategy.

Keywords

Dromedary camel – Epidemiology – MERS–CoV – Middle East respiratory syndrome coronavirus – Prevalence – Risk factor – Saudi Arabia.

Introduction

Middle East respiratory syndrome coronavirus (MERS–CoV) has been identified as the cause of severe respiratory disease in humans in Saudi Arabia (SA) since 2012 (1, 2, 3, 4). Many Gulf, Middle East and African countries have also been affected by MERS–CoV infection (4, 5, 6, 7, 8). Several months after the diagnosis of the index case in SA, dromedary camels (*Camelus dromedarius*) were implicated as a possible source of human infection on the basis of the presence of MERS–CoV neutralising antibodies and ribonucleic acid (RNA) in the blood and nasal swab samples of camels in SA (9, 10), the United Arab Emirates (11), Oman (12, 13), Egypt (14, 15) and Jordan (16), and in milk samples in Qatar (17). Furthermore, evidence of camel-to-human transmission of MERS–CoV was reported (18), as well as of a human infection following direct exposure to an infected camel (3). Also, the investigation of the genetic characteristics and sequence of the MERS–CoV isolated from infected camels indicated a close similarity to the virus isolated from human cases (13, 19). These studies have provided evidence that camels play a primary role in the transmission of MERS–CoV infection to humans. In addition to the Gulf and Middle East countries, serological investigations identified the presence of MERS–CoV-specific antibodies in camels in several countries in Africa (20). Apart from camels, bats are thought to be a potential source of the MERS–CoV. A molecular investigation has shown that bats in SA were infected with several alphacoronaviruses and betacoronaviruses, with nucleotide identity in some of the latter betacoronaviruses related to the virus isolated from the index human case (21).

Several studies were conducted to investigate and diagnose MERS–CoV infection in camels. An enzyme-linked immunosorbent assay

(ELISA) based on Vero cells infected with MERS–CoV was initially used to detect neutralising antibodies in camels in SA and positive samples were confirmed by Western blot assays techniques (10). Also, a MERS–CoV antibody ELISA for camels (immunoglobulin G [IgG]) was used to detect specific antibodies in the sera of camels from the Canary Islands (22), as well as from Eastern Africa (23). This latter study reported that the recombinant enzyme-linked immunosorbent assay (rELISA) based on MERS–CoV S1 was 99% specific when correlated with the results of a microneutralisation confirmatory test (23). In human cases in SA, a commercial anti-MERS–CoV rELISA was used to detect antibodies in human sera, and seropositivity was confirmed by MERS–CoV recombinant immunofluorescence and plaque reduction neutralisation tests (PRNTs) (24). Furthermore, several studies detected MERS–CoV RNA in nasal swab samples of camels in SA by reverse-transcription–polymerase chain reaction (RT–PCR) (10, 25, 26). The detection of RNA by RT–PCR was reported to be highly sensitive and specific (27).

To date, there have been few epidemiological studies of MERS–CoV infection in camels. Furthermore, the role of risk factors in the occurrence, distribution and severity of infection has not been investigated and analysed statistically. Hence, the purpose of the current study was to:

a) determine the prevalence and geographical distribution of MERS–CoV antibodies and RNA in camels in SA using rELISA and real-time reverse-transcription–polymerase chain reaction (rRT–PCR), respectively

b) quantify the associations between MERS–CoV infection and risk factors using univariate and multivariate statistical analyses.

Materials and methods

Study design and sample collection

A cross-sectional study was conducted in five regions in SA during April and May 2015, following a multi-stage sampling method. The

primary sampling unit (regions) was purposely selected to represent the whole country: north (Northern region), south (Jazan region), east (Eastern region), west (Makkah region) and centre (Riyadh region). The secondary sampling unit (camel herd) was chosen in accordance with the verbal consent of the herd's owner and their willingness to participate in the study. The tertiary sampling unit (individual camel) was selected, as convenient, from the recruited herd. A total of 1,674 blood samples were collected from the jugular vein of the selected camels in 10 ml vacutainer tubes without anti-coagulants; these were subsequently transported, within 24 h, to the Riyadh Veterinary Diagnostic Laboratory in cold containers for ELISA testing. Also, a total of 1,673 nasal swab samples (COPAN flocked swab, COPAN Italia, Brescia, Italy) were collected from the nostrils of the same camels and were transported in virus transport media (COPAN Italia, 1 ml/vial) to the same laboratory in Riyadh for polymerase chain reaction (PCR) testing.

Information about the sampled herds and camels was obtained through a pre-structured questionnaire administered by the investigators on a single visit at the time of sampling. The questionnaire was designed to comprise, in as much as possible, concise easily understandable questions, to improve the precision of responses from the camel owners and herders, and to facilitate coding and data processing.

Laboratory tests

Pre-treatment of the sera

The serum samples were heat inactivated at 56°C for 30 min in a water bath, and gently agitated using a vortex before being transferred to microplates.

Recombinant enzyme-linked immunosorbent assay

The presence of MERS–CoV IgG antibodies in the tested sera was detected using a highly sensitive and specific rELISA, which is based on purified S1 antigen from MERS–CoV, with which microtitre strips were coated. The ELISA was processed in a fully automated analyser

(Analyser 1, Euroimmun-AG, Lübeck, Germany), according to the manufacturer's instructions. In brief, the inactivated sera were diluted 1:100, placed into the antigen-coated wells and the plates were then incubated. In the case of positive sera, the specific IgG antibodies bound to the antigen. These bound antibodies were detected by a colour reaction that resulted from the catalysis of the chromogenic substrate (3,3',5,5'-Tetramethylbenzidine or TMB) by horseradish peroxidase (HRP) which was conjugated to the camel IgG antibodies. The reaction was stopped after 10 to 15 min and the absorbance of the developed colour was measured at a wavelength of 450 nm. In each run, positive and negative controls, which were included in separate wells, were used as calibrators. The result was evaluated by calculating the ratio of the absorbance values of the controls or samples above the absorbance values of the calibrators, and was interpreted as follows: a ratio <0.8 was negative, 0.8–1.1 was borderline and >1.1 was positive.

Sample processing and ribonucleic acid extraction

The collected nasal swab samples which were either in RNA*later* RNA Stabilization Reagent (QIAGEN, Hilden, Germany, 1 ml/vial) or transport media (COPAN, 1 ml/vial) were thoroughly mixed and centrifuged at 2,000 rpm for 5 min and then the supernatants were used for RNA extraction. Total RNA was extracted from 200 µl of each swab sample in a fully automated MagNA Pure 96 Instrument (Roche Diagnostics, Manheim, Germany), using MagNA Pure 96 DNA and Viral NA [nucleic acid] Small Volume Kit (Roche Diagnostics, Germany), according to the manufacturer's instructions. The concentration and purity of eluted NA was assessed using a nano-spectrophotometer. A ratio of approximately 2.0 was accepted as pure RNA yield and considered suitable for RT–PCR processing.

Real-time reverse-transcription–polymerase chain reaction

MERS–CoV RNA was detected by applying two different one-step rRT–PCR assays that were produced by TIB MOLBIOL GmbH, Berlin, Germany, as previously described (27). One assay was used for screening targets in a region upstream of the envelope protein (*E*)

gene (upE assay) in which the following sets of primers and probes were used: upE-Fwd (GCAACGCGCGATTCAGTT), upE-Rev (GCCTCTACACGGGA – CCCATA), and upE-Probe (6-carboxyfluorescein [FAM])–CTCTTCACATAATCGCCCCGAGCTCG- 6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA]). The second assay was used for confirming targets in the open reading frame *1a* gene (ORF*1a* assay) in which the following primers and probes were used: *Orf1a*-Fwd (CCACTACTCCCATTTCGTCAG), *Orf1a*-Rev (CAGT ATGTGTAGTGCGCATATAAGCA) and *Orf1a*-Probe (FAM – TTGCAAATTGGCTTGCCCCCACT-TAMRA).

The amplification process was run in a LightCycler® 480 Instrument (Roche Diagnostics Ltd, Rotkreuz, Switzerland) (11). Data analysis was performed as described in the LightCycler® 480 Instrument operator's manual. The second derivative maximum method was used. The cycle number of the crossing point (CP) of each positive sample was calculated automatically.

Data management and statistical analyses

All collected data were coded, entered and stored electronically in a Microsoft® Excel for Windows spreadsheet. After verification, data were transferred to the Statistical Package for Social Sciences (SPSS) for Windows Version 18 (SPSS, Inc., Chicago, Illinois, United States of America) which was used for all appropriate statistical analyses. Each test result was reported as a binary outcome (positive or negative) and all risk factors were considered as categorical independent variables, which required further analysis of association. The MERS–CoV antibody seroprevalence and viral RNA prevalence were calculated as the number of positive samples in the corresponding test divided by the total number of samples tested in each risk-factor category, and were reported as a percentage. Descriptive analysis was performed to show the frequency and stratification of results by risk factor.

The associations between the independent variables and the dependent variable were first assessed by univariate analysis using the Pearson

Chi-square (χ^2) test. A risk factor was considered to be significantly associated with MERS–CoV antibodies or RNA prevalence in the univariate analysis if it had $p \leq 0.20$. This level of significance was chosen to allow for more risk factors to enter the multivariate analysis. Firstly, the χ^2 results were interpreted by observing the prevalence and p value in each risk-factor category. Secondly, and to control for potential confounding effects of various risk factors, all significantly associated risk factors at $p \leq 0.20$ in the univariate analysis were further evaluated by the multivariate analysis using logistic regression. The statistical significance level in the multivariate model was set at $p \leq 0.05$. Hence, only those risk factors statistically significant at the stated level ($p \leq 0.05$) were reported in the final multivariate models. Furthermore, the strength and direction of association between either MERS–CoV antibodies or viral RNA prevalence and a specific risk factor were assessed using the odds ratio (OR), considering the category with the least prevalence and/or exposure as a reference. Hence, an OR > 1.0 means positive or putative association, <1.0 means negative or protective association and 1.0 means no association. The 95% confidence interval (95% CI) for the OR was considered statistically significant if the entire CI range did not include 1.0.

Results

Statistical analyses

Prevalence and univariate association

Tables I and II present the descriptive characteristics and categories of the risk factors, number of samples tested in each category, number of positive samples, prevalence (%) and χ^2 results.

Regarding MERS–CoV antibodies, the overall seroprevalence in SA was 80.5% (1348/1674). As for regions, a high seroprevalence was reported in Makkah (88.1%), Riyadh (88.0%), Eastern (87.8%) and Northern (83.8%); whereas a lower seroprevalence (75.9%) was reported in Jazan. The seroprevalence stratification by risk factor is presented in Table I.

Ten risk factors were investigated for associations at the $p \leq 0.20$ level with MERS–CoV antibody seroprevalence in the univariate analysis. The following risk factors were significantly associated at $p \leq 0.001$: region, grazing system, exposure to wild animals, dung removal and breed. In addition, age, herd size, adding new animals and nasal discharge were significantly associated at *p* values within the range >0.001–0.05, and sex was significantly associated at $p = 0.183$. Hence, all risk factors were significantly associated at $p \leq 0.20$ and were eligible for further multivariate analysis (Table I).

As for MERS–CoV RNA, the overall prevalence in SA was 2.4% (40/1673). The highest prevalence was in the Riyadh region (12.0%) followed by the Eastern region (2.8%), and 0.5% in both the Northern and Makkah regions. No virus RNA was detected in camels from the Jazan region. The stratification of viral RNA prevalence by risk factor is presented in Table II.

For the associations between MERS–CoV RNA prevalence and the ten investigated risk factors in the univariate analysis, the following risk factors were significantly associated at *p* values within the range ≤0.001–≤0.05: region, sex, grazing system, herd size, dung removal and nasal discharge. There was no evidence of an association with age, breed, exposure to wild animals and adding new animals ($p > 0.20$) (Table II).

Multivariate association

The multivariate analysis identified significant associations ($p \leq 0.05$) between MERS–CoV antibody seroprevalence and region, age, grazing system, exposure to wild animals and dung removal. Camels from the Riyadh region (OR = 3.55), the Eastern region (OR = 5.16), the Northern region (OR = 1.69) and the Makkah region (OR = 1.74) were more likely to test positive than those from the Jazan region. Camels ≥ 4 years of age (OR = 1.86) and 1–3 years (OR = 1.96, $p = 0.064$, i.e. marginally significant) were more likely to test positive than calves < 1 year. Camels raised in a zero grazing system (OR = 19.96) and a semi-open grazing system (OR = 2.31) were more likely to test positive than those raised in an open-grazing system.

Interestingly, the presence of wild animals (OR = 0.34) and daily dung removal (OR = 0.06) were negatively associated (protective) with seroprevalence (Table III).

Moreover, the multivariate analysis identified region and sex as significantly associated ($p \leq 0.05$) with MERS–CoV RNA prevalence. Camels from the Riyadh region (OR = 47.71) and the Eastern region (OR = 4.36, $p = 0.077$, i.e. marginally significant) were more likely to test positive than those from the Makkah region. Also, male camels were more likely (OR = 3.22) to test positive than female camels (Table IV).

Discussion

Middle East respiratory syndrome coronavirus antibody seroprevalence

The overall MERS–CoV antibody seroprevalence in camels in SA was high (80.5%) and widespread in camels throughout the investigated regions. The antibody seroprevalence was highest (range: 83.8–88.1%) in the Makkah, Riyadh, Northern and Eastern regions; whereas it was lowest (75.9%) in Jazan. These results are within the range of reported seroprevalences in SA and other Gulf countries. An overall seroprevalence of 74% was reported in SA (10), 93.2% in Dubai (28), 97.1% in the United Arab Emirates (29), and 100% in Oman (12). The differences in the antibody seroprevalences among these studies could be attributed to different methods of diagnosis, and variability in climatic conditions, seasons, management practices and environmental factors between these countries.

Middle East respiratory syndrome coronavirus ribonucleic acid prevalence

The overall MERS–CoV RNA prevalence in camels in SA was 2.4%. This prevalence is higher than that reported in a countrywide survey (1.6%) in Abu Dhabi Emirate (30), but lower than that reported in Oman (6.6%) (13), and also less than that reported in slaughter camels in Egypt (3.6%) (15). Moreover, the overall viral RNA prevalence in

the current study is much lower than that reported in the previous studies conducted in SA. One study reported an overall viral RNA prevalence of 25% (10). The same study also reported a prevalence of 66% in camels from Taif (Makkah region), 35% in camels from Tabuk (Northern region), 5% in camels from Hofuf (Eastern region) and 14% in camels from the Riyadh region. It is difficult to explain this great variation in viral RNA prevalences compared with the current study since both studies tested nasal swab samples from the same regions by RT–PCR. However, the difference in the period of sampling may explain the variation. In the current study, camels were sampled during the warmer spring/summer months (April and May), while the comparable study sampled camels during the colder winter months (November and December) (10). Also using RT–PCR, another study conducted in the Al-Ahsa province (Eastern region) reported RNA prevalence of 29.2% in nasal swabs from 96 camels sampled at three sites (a livestock market, an abattoir and a veterinary hospital) (25). In Qatar, a higher viral RNA prevalence of 60% was reported in nasal swabs of camels sampled at a slaughterhouse (31).

It is interesting to note the low MERS–CoV RNA prevalences in countrywide surveys (13, 30) in which camels were sampled from pastoral areas compared with higher viral RNA prevalences in camels sampled from livestock markets and slaughterhouses (25). It is also notable that the viral RNA prevalence in certain groups of camels could be related to the seroprevalence of MERS–CoV antibodies in humans who had been in contact with these camel groups. Previous studies in SA have reported significantly higher MERS–CoV antibody seroprevalences in livestock-market and slaughterhouse workers than the general population (24, 32).

Risk factors associations

Association with Middle East respiratory syndrome coronavirus antibody seroprevalence

The multivariate model (Table III) identified region, age, grazing system, exposure to wild animals and dung removal as significantly associated ($p \leq 0.05$) with MERS–CoV antibody seroprevalence.

Regarding region, the significantly positive association with camels from the colder regions (Riyadh, Eastern, Northern and Makkah) compared with the warmer region (Jazan) could be attributed to lower temperatures during the winter season, which may exert considerable stress on camels. Another possible explanation is that the serum samples in the current study were collected during April and May, which coincides with the end of the calving season and the start of lactation during which camels undergo considerable physiological stress. In addition, the overall camel population as well as the herd size in each of the Riyadh, Eastern, Northern and Makkah regions are higher than that in Jazan. The association of camel population and herd size with MERS–CoV infection in camels has been previously reported (33, 34). Nevertheless, management and husbandry practices in these regions together with the environmental and geographical characteristics that were not investigated in the current study might have contributed to the observed significant association.

In the current study, MERS–CoV antibody seroprevalence in camels grew consistently with the increase in age. A similar pattern has been reported in previous studies conducted in SA (9, 10), Dubai (28) and Africa (20). The multivariate analysis further documents that camels ≥ 4 years of age (OR = 1.86) and 1–3 years (OR = 1.96, $p = 0.064$, i.e. marginally significant) were almost twice as likely to be seropositive when compared with camels < 1 year. The significant association of adult camels with a higher seroprevalence was probably due to greater exposure to the virus with increasing age.

Furthermore, the multivariate results revealed that camels raised using zero-grazing and semi-open grazing systems were more likely to be significantly associated ($p \leq 0.05$) with MERS–CoV antibody seroprevalence than those raised in an open grazing system. The seroprevalence in camels was highest in the zero grazing system (97.9%) followed by semi-open grazing (90.4%) and was lowest in open grazing (77.3%). This result is in accordance with previous studies which have highlighted that close contact between infected and non-infected camels in the intensive and semi-intensive systems

increases the chances of airborne transmission of MERS–CoV (10, 33).

It has been suggested that bats might play a role in MERS–CoV infection or act as likely natural reservoirs for MERS–CoV or an ancestral MERS-like–CoV (21, 35). Since the current study included the presence of bats within the grouping wild animals as a risk factor, it was not possible to identify the separate role of bats. In contrast, our results indicated a significant negative (protective) association (OR = 0.34) between the presence of wild animals and seroprevalence. However, the authors note that the number of camels in herds that were not exposed to wild animals is much higher than the number of camels in those herds that were exposed (1,496 vs 178). Therefore, further investigation is required to elucidate the role of bats as well as that of other wild animals in MERS–CoV infection in camels.

In addition, the risk factor of dung removal was significantly associated ($p \leq 0.05$) with MERS–CoV antibody seroprevalence. Daily dung removal was negatively associated (OR = 0.06) with seroprevalence, indicating a protective effect. This protective association may have reflected the overall hygiene practices related to the transmission of infection in camel barns. Consistent with the current study's results, the effect of poor hygiene on the occurrence of increased MERS–CoV infection in camels has been previously reported (33).

Association with Middle East respiratory syndrome coronavirus ribonucleic acid prevalence

The multivariate model (Table IV) revealed significant associations ($p \leq 0.05$) between region and sex and MERS–CoV RNA prevalence. Camels from the Riyadh region (OR = 47.71) and the Eastern region (OR = 4.36, $p = 0.077$, i.e. marginally significant) were more likely to test positive than those from the Makkah region. This association could be attributed to the difference in temperature and climatic conditions, as well as to different management practices. A previous study reported that virus shedding peaked in November to January and declined in March to May (25). However, the current study's results

differ from another study which reported a higher RNA prevalence in Makkah (66%) than in Riyadh (14%) (10).

Regarding sex, male camels were more likely (OR = 3.22) to test positive than female camels. This result is consistent with a previous study which identified a higher viral RNA prevalence in a pooled group of male and female calves than in their mothers (28). However, the association with sex in the same study should be considered with caution because it could be confounded by age. The authors note that most of the male camels in the investigated herds in the current study were < 1 year, and that is why age is forced in the final multivariate model to control for confounding between sex and age (Table IV). As for age, it is well documented that MERS–CoV RNA prevalence in young calves is higher than in adult camels (10, 25, 28). This situation contrasts with the results of the current study which indicated a higher prevalence of MERS–CoV RNA in camels > 1 year (Table IV). However, the current study's results could be related to the immunologically naive status of the animals following the waning of passive acquired immunity in camels > 1 year.

Some statistically significant ($p \leq 0.20$) potential risk factors in the univariate analysis (Tables I and II) did not remain statistically significant ($p \leq 0.05$) in the final multivariate models (Tables III and IV). However, such results may either reflect the low importance of these risk factors or the limitation of the statistical analysis method used in the current study, including the lack of control for collinearity.

In the current study, data and information related to the risk factors were obtained through pre-structured questionnaires. Information regarding camel herd environment, management practices and camel characteristics were provided by herders and recorded by the investigators. This information may not have reflected the exact situation, resulting in either information, recall or observer biases. Since it was not possible to verify this information, owing to no records being available, the results are prone to these biases. Therefore, this is considered to be a potential limitation of the current study. Another potential limitation could be in the current statistical

analysis, including the lack of control for collinearity between the risk factors prior to the analysis of data through univariate and multivariate methods. Control for collinearity, if performed, might have resulted in more accurate results on the associations between MERS–CoV infection and the investigated potential risk factors. Overall, this study has provided valuable insights on the epidemiology of MERS–CoV infection in dromedary camels in SA and has identified important risk factors.

Conclusions

The authors conclude that MERS–CoV antibody seroprevalence is high and widespread in camels throughout the investigated regions in SA; and significantly positively associated with region, age and grazing system, and negatively associated with the presence of wild animals and daily dung removal. In contrast, MERS–CoV RNA prevalence is low in camels, and significantly positively associated with region and sex. These identified risk factors can be considered predictors for MERS–CoV infection in camels and should be considered when developing an efficient and cost-effective control strategy in SA. Further long-term prospective studies are warranted to improve the current understanding of the dynamics of MERS–CoV transmission cycles between camels, as well as those between camels and humans.

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Table I
Univariate analysis of risk factors associated with Middle East respiratory syndrome coronavirus antibody seroprevalence in dromedary camels in Saudi Arabia, April–May 2015, using the Chi-square test

Risk factor	Number of samples tested	Number of positive samples (%)	Degree of freedom	Chi-square	*p*-value
Region			4	29.72	<0.001
Riyadh	250	220 (88.0)			
Eastern	213	187 (87.8)			
Makkah	394	347 (88.1)			
Northern	414	347 (83.8)			
Jazan	403	306 (75.9)			
Total	**1,674**	**1,348 (80.5)**			
Breed			3	29.37	<0.001
Wadah	812	654 (80.5)			
Shaol	365	280 (72.7)			
Homor	249	200 (80.3)			
Majaheem	228	214 (93.9)			
Total	**1,654**	**1,348 (81.5)**			
Age			2	11.18	0.004
<1 year	141	99 (70.2)			
1–3 years	86	67 (77.9)			
≥4 years	1,447	1,182 (81.7)			
Total	**1,674**	**1,348 (80.5)**			
Grazing system [(a)]			2	38.72	<0.001
Open grazing	1,285	993 (77.3)			
Semi-open grazing	342	307 (90.4)			
Zero grazing	47	46 (97.9)			
Total	**1,674**	**1,348 (80.5)**			
Sex			1	1.77	0.183
Male	370	289 (78.1)			
Female	1,304	1,059 (81.2)			
Total	**1,674**	**1,348 (80.5)**			

Herd [(b)] size			2	12.25	0.002
Small (1–50)	25	25 (100)			
Medium (51–100)	1,480	1,186 (80.1)			
Large (>100)	169	137 (81.1)			
Total	**1,674**	**1,348 (80.5)**			
Dung removal			3	298.07	<0.001
Daily	135	128 (94.8)			
Weekly	649	386 (59.6)			
Monthly	730	679 (93.0)			
Never	160	154 (96.3)			
Total	**1,674**	**1,347 (80.6)**			
Adding new animals			1	7.38	0.003
Yes	30	30 (100)			
No	1,430	1,318 (90.3)			
Total	**1,460**	**1,348 (92.3)**			
Nasal discharge			1	3.86	0.049
Yes	33	31 (93.9)			
No	1,641	1,317 (78.1)			
Total	**1,674**	**1,348 (80.5)**			
Exposure to wild animals [(c)]			1	16.57	<0.001
Yes	178	123 (69.1)			
No	1,496	1,225 (81.9)			
Total	**1,674**	**1,348 (80.5)**			

a) Open grazing: most of the day spent on pasture; semi-open grazing: about half of the day spent on pasture; and zero grazing: housed day and night on a farm and fed cut grass

b) Herd refers to a group of camels with shared common grazing sites that are housed together on one farm

c) These include bats, rodents, reptiles, deer, gazelles, foxes, hyenas and wolves

Table II
Univariate analysis of risk factors associated with Middle East respiratory syndrome coronavirus ribonucleic acid prevalence in dromedary camels in Saudi Arabia, April–May 2015, using the Chi-square test

Risk factor	Number of samples tested	Number of positive samples (%)	Degree of freedom	Chi-square	*p*-value
Region			4	121.46	<0.001
Riyadh	250	30 (12.0)			
Eastern	212	6 (2.8)			
Makkah	394	2 (0.5)			
Northern	414	2 (0.5)			
Jazan	403	0 (0)			
Total	**1,673**	**40 (2.4)**			
Breed			3	0.23	0.972
Wadah	812	18 (2.2)			
Shaol	365	10 (2.6)			
Homor	249	6 (2.4)			
Majaheem	228	6 (2.6)			
Total	**1,654**	**40 (2.4)**			
Age			2	2.21	0.334
<1 year	141	1 (0.7)			
1–3 years	86	3 (3.5)			
≥4 years	1,446	36 (2.5)			
Total	**1,673**	**40 (2.4)**			
Grazing system [(a)]			2	26.35	<0.001
Open grazing	1,284	19 (1.5)			
Semi-open grazing	342	21 (6.1)			
Zero grazing	47	0 (0)			
Total	**1,673**	**40 (2.4)**			
Sex			1	7.72	0.005
Male	370	18 (4.9)			
Female	1,303	22 (1.7)			
Total	**1,673**	**40 (2.4)**			

Herd [(b)] size			2	23.45	<0.001
Small (1–50)	25	3 (12.0)			
Medium (51–100)	1,479	36 (2.4)			
Large (>100)	169	1 (0.6)			
Total	**1,673**	**40 (2.4)**			
Dung removal			3	18.48	<0.001
Daily	135	0 (0)			
Weekly	648	10 (1.5)			
Monthly	720	30 (4.1)			
Never	370	0 (0)			
Total	**1,673**	**40 (2.4)**			
Adding new animals			1	0.74	0.387
Yes	30	0 (0)			
No	1,643	40 (2.4)			
Total	**1,673**	**40 (2.4)**			
Nasal discharge			1	23.51	<0.001
Yes	33	5 (15.2)			
No	1,640	35 (2.1)			
Total	**1,673**	**40 (2.4)**			
Exposure to wild animals [(c)]			1	0.15	0.349
Yes	178	5 (2.8)			
No	1,495	35 (2.3)			
Total	**1,673**	**40 (2.4)**			

a) Open grazing: most of the day spent on pasture; semi-open grazing: about half of the day spent on pasture; and zero grazing: housed day and night on a farm and fed cut grass

b) Herd refers to a group of camels with shared common grazing sites that are housed together on one farm

c) These include bats, rodents, reptiles, deer, gazelles, foxes, hyenas and wolves

Table III
Multivariate analysis of risk factors associated with Middle East respiratory syndrome coronavirus antibody seroprevalence in dromedary camels in Saudi Arabia, April–May 2015, using logistic regression

Risk factor	Number of samples tested	Number of positive samples (%)	OR	95% CI for OR	*p*-value
Region					
Riyadh	250	220 (88.0)	3.55	2.10–6.00	<0.001
Eastern	213	187 (87.8)	5.16	3.04–8.78	<0.001
Northern	414	347 (83.8)	1.69	1.12–20.57	0.013
Makkah	394	347 (88.1)	1.74	1.18–2.56	0.005
Jazan	403	306 (75.9)	Ref.		
Grazing system [(a)]					
Open grazing	1,285	993 (77.3)	Ref.		
Semi-open grazing	342	307 (90.4)	2.31	1.42–3.63	<0.001
Zero grazing	47	46 (97.9)	19.96	2.63–151.41	0.004
Dung removal					
Daily	135	128 (94.8)	0.06	0.03–0.13	<0.001
Weekly	649	386 (59.6)	Ref.		
Monthly	730	679 (93.0)	0.56	0.24–1.28	0.166
Never	160	154 (96.3)	1.18	0.38–3.65	0.775
Age					
<1 year	141	99 (70.2)	Ref.		
1–3 years	86	67 (77.9)	1.96	0.96–3.99	0.064
≥4 years	1,447	1,182 (81.7)	1.86	1.18–2.95	0.008
Exposure to wild animals [(b)]					
Yes	178	123 (69.1)	0.34	0.22–0.54	<0.001
No	1,496	1,225 (81.9)	Ref.		

CI: confidence interval
OR: odds ratio
Ref.: reference
a) Open grazing: most of the day spent on pasture; semi-open grazing: about half of the day spent on pasture; and zero grazing: housed day and night on a farm and fed cut grass
b) These include bats, rodents, reptiles, deer, gazelles, foxes, hyenas and wolves

Table IV
Multivariate analysis of risk factors associated with Middle East respiratory syndrome coronavirus ribonucleic acid prevalence in dromedary camels in Saudi Arabia, April–May 2015, using logistic regression

Risk factor	Number of samples tested	Number of positive samples (%)	OR	95% CI for OR	*p*-value
Region					
Makkah	414	2 (0.49)	Ref.		
Riyadh	250	30 (12.0)	47.71	8.79–258.21	<0.001
Eastern	212	6 (2.8)	4.36	0.85–22.21	0.077
Northern	394	2 (0.50)	0.80	0.11–5.86	0.833
Jazan	403	0 (0)			
Sex					
Male	370	18 (4.9)	3.22	1.34–7.63	0.010
Female	1,303	22 (1.7)	Ref.		
Age					
<1 year	141	1 (0.7)	Ref.		
1–3 years	86	3 (3.5)	4.43	0.37–43.71	0.249
≥4 years	1,446	36 (2.5)	3.14	0.39–24.35	0.217

CI: confidence interval
OR: odds ratio
Ref.: reference