Spatial association between primary Middle East respiratory syndrome coronavirus infection and exposure to dromedary camels in Saudi Arabia

Khalid Al-Ahmadi¹ | Mohammed Alahmadi¹ | Ali Al-Zahrani²

¹King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia
²King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Correspondence
Ali Saeed Al-Zahrani, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.
Email: alisaz@kfshrc.edu.sa

Abstract
Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging zoonotic disease. Exposure to dromedary camels (Camelus dromedaries) has been consistently considered the main source of primary human infection. Although Saudi Arabia reports the highest rate of human MERS-CoV infection and has one of the largest populations of dromedary camels worldwide, their spatial association has not yet been investigated. Thus, this study aimed to examine the correlation between the spatial distribution of primary MERS-CoV cases with or without a history of camel exposure reported between 2012 and 2019 and dromedary camels at the provincial level in Saudi Arabia. In most provinces, a high proportion of older men develop infections after exposure to camels. Primary human infections during spring and winter were highest in provinces characterized by seasonal breeding and calving, increased camel mobilization and camel–human interactions. A strong and significant association was found between the total number of dromedary camels and the numbers of primary camel-exposed and non-exposed MERS-CoV cases. Furthermore, spatial correlations between MERS-CoV cases and camel sex, age and dairy status were significant. Via a cluster analysis, we identified Riyadh, Makkah and Eastern provinces as having the most primary MERS-CoV cases and the highest number of camels. Transmission of MERS-CoV from camels to humans occurs in most primary cases, but there is still a high proportion of primary infections with an ambiguous link to camels. The results from this study include significant correlations between primary MERS-CoV cases and camel populations in all provinces, regardless of camel exposure history. This supports the hypothesis of the role of an asymptomatic human carrier or, less likely, an unknown animal host that has direct contact with both infected camels and humans. In this study, we performed a preliminary risk assessment of prioritization measures to control the transmission of infection from camels to humans.

Keywords
dromedary camels, epidemiology, geographic information system, MERS-CoV, Saudi Arabia, zoonosis
1 INTRODUCTION

Middle East respiratory syndrome coronavirus (MERS-CoV) is endemic to dromedary camels (Camelus dromedaries) in the Arabian Peninsula and is the cause of zoonotic infections and restricted outbreaks in humans (Dudas, Carvalho, Rambaut, & Bedford, 2018). First reported in Saudi Arabia in 2012 (Zaki, van Boheemen, Bestebroer, Osterhaus, & Fouchier, 2012), this infectious viral respiratory disease causes high mortality and morbidity in humans. Worldwide, there have been 2,399 cases, with a 34.47% mortality rate (World Health Organization, 2019a). In Saudi Arabia, the most affected country, there have been 2,008 cases, with a 37.30% mortality rate. Currently, human MERS-CoV infections are reported monthly in Saudi Arabia.

Although MERS-CoV infection and outbreaks occur in healthcare settings, the specific source of MERS-CoV, its ecology and epidemiology, and how it is spread to humans remain unclear (Al-Tawfiq & Memish, 2014). Dromedaries are natural hosts of MERS-CoV, and transmission to humans can occur through sporadic zoonotic infection after direct or indirect exposure to infected dromedary camels or their products (Azhar et al., 2014; Kayali & Peiris, 2015; Memish et al., 2014; Omrani, Al-Tawfiq, & Memish, 2015; Sikkema et al., 2019). In a case-control study, Alraddadi et al. (2016) evaluated variances in environmental exposures among 30 primary laboratory-confirmed MERS-CoV cases and 116 controls; they observed that direct exposure to dromedary camels was independently associated with primary MERS-CoV infection.

The incidence of primary MERS-CoV cases is of particular importance for recognizing possible animal sources and zoonotic infections (Gossner et al., 2016; Kasem et al., 2018). Although the primary source of MERS-CoV human infection in Saudi Arabia appears to be dromedary camels (Alagaili et al., 2014; Memish et al., 2014), most primary MERS-CoV patients report no exposure to camels (Kayali & Peiris, 2015), making the source of the infection unclear (Hemida, Elmoslemany, et al., 2017).

A Saudi Arabian nationwide cross-sectional serological study confirmed human MERS-CoV seropositivity in 0.15% of 10,009 individuals with asymptomatic or mild infections, who could be the source of infection for confirmed primary MERS-CoV cases without camel exposure (Muller et al., 2015). They also found that in camel-exposed individuals, seroprevalence was significantly higher (15 and 23 times) in shepherds and slaughterhouse workers, respectively, compared with the general population in the study. However, only 2.4% of the 87 samples from shepherds and 3.6% of the 140 samples from slaughterhouse workers had serological evidence of infection. Another study conducted in Saudi Arabia reported higher MERS-CoV seroprevalence (53%) in camel workers using a cohort of 30 camel workers from the Riyadh New Camel Market and 44 controls from Saudi Arabia and the United States (Alishukairi et al., 2018). These findings suggest that camel workers are probable intermediaries for human infection due to some humans’ prevalent and continued contact with camels.

Kandeil et al. (2019) demonstrated, for the first time, evidence of MERS-CoV infection among domestic livestock in close and prolonged contact with camels and advised that spillover infection to other livestock may take place, which can be a risk factor for human infection. However, scientists have reported no evidence of MERS-CoV infection in domestic livestock in several other studies (Hemida, Chu, et al., 2017; Hemida et al., 2013; Kasem et al., 2018; Meyer et al., 2014; Reusken et al., 2013).

The global camel population is estimated to be 35 million (Food & Agriculture Organization, 2014), of which 95% are dromedary camels (Faye, 2013). The wild dromedary camel was likely domesticated in the Arabian Peninsula 5,000–6,000 years ago (Uerpmann & Agriculture Organization, 2014), of which 95% are dromedary camels. The wild dromedary camel was likely domesticated in the Arabian Peninsula 5,000–6,000 years ago (Uerpmann & Agriculture Organization, 2014). Raising camels is part of the Saudi Arabian sociocultural heritage; they are also used for traditional races and festivals (Abdallah & Faye, 2013; Auty, 2001). Increasing demand for camel milk and meat has led to the establishment of peri-urban dromedary camel husbandry, and these increased interactions between camels and humans introduce a risk of transmission of zoonotic diseases, such as MERS-CoV, to humans (Gikonyo et al., 2018).

According to historical serological records of dromedary specimens and serum, MERS-CoV has been circulating among camels for at least two decades in Saudi Arabia (Alagaili et al., 2014). In Saudi Arabia, a higher viral MERS-CoV RNA load was found in camels from Riyadh and Eastern provinces, including male camels and camels younger than 2 years old compared with those from Makkah province, female camels and older camels (Alagaili et al., 2014; Elfdail et al., 2018; Hemida et al., 2013; Kasem et al., 2018). Additionally, there is a higher risk of human MERS-CoV infection during winter than in summer (Kasem et al., 2018; Khalafalla et al., 2015), driven by many seasonal activities, such as breeding, calving and racing (Abbas, Al Qarawi, & Al Hawas, 2000; Gossner et al., 2016; Padalino, Monaco, & Lacalandra, 2015).
The spread of disease, particularly infectious disease, is inevitably affected by spatial factors. Using ecological modelling in a geographic information system (GIS) environment, Reeves, Samy, and Peterson (2015) suggested that camel exposure plays a major role in the transmission of MERS-CoV. Additionally, Gikonyo et al. (2018) found that areas of high camel density, camel convergence points, previous MERS-CoV cases and high seroprevalence were potential risk factors for spatial MERS-CoV hotspots.

Although scientific evidence indicates that direct or indirect exposure to camels is a risk factor for primary MERS-CoV infection, few studies have addressed the spatial association between the distribution of MERS-CoV and camels in Saudi Arabia. Therefore, in this study, we aimed to examine the correlation between the spatial distribution of primary MERS-CoV cases in Saudi Arabia and the dromedary camel population at national and provincial levels.

2 | MATERIALS AND METHODS

2.1 | MERS-CoV data

The analysis was performed on all primary, laboratory-confirmed MERS-CoV cases reported between June 2012 and March 2019 (Ministry of Health in Saudi Arabia, 2019; World Health Organization, 2019b). The MERS-CoV data were checked for consistency, completeness and suitability. The data set included the date of diagnosis, age, sex, nationality, source of infection and place of residence (i.e. city, governorate and province).

A laboratory-confirmed primary MERS-CoV case with camel exposure was defined as any person who did not have contact with infected individuals but was known or believed to have had direct or indirect exposure to camels or camel habitats and exhibited a positive real-time reverse-transcription polymerase chain reaction for at least two specific genomic targets or a region upstream and open reading frame 1a (Corman et al., 2012). Primary MERS-CoV cases with no camel exposure included laboratory-confirmed MERS-CoV with no positive history of direct or indirect camel exposure. Direct camel exposure included direct physical contact with camels and their surroundings, consumption of raw milk or other unpasteurized camel products, and handling of raw camel meat. Indirect exposure included unintentional visits to camel markets or farms or living with a household member who was in direct contact with camels (Ministry of Health in Saudi Arabia, 2018).

2.2 | Dromedary camel populations

Camel populations at the provincial level were obtained from a national agricultural census (General Authority for Statistics, 2015). The data included the distribution of camels by sex, age group, and breed and the number of barns and the total number of camels in each barn. We used 12 camel population clusters: total camels, total camels <1 year (old), total camels >1 year, male camels, female camels, male camels <1 year, male camels >1 year, female camels <1 year, female camels >1 year, dairy camels, camel barns and the number of camels in barns.

2.3 | Excess risk map

Excess risk was determined by a comparison of the observed number of events with the number of events that would be expected in a region or country had a reference risk been applied. A reference risk was estimated from the aggregate of all observations under consideration. Excess risk maps of primary MERS-CoV incidence were produced using GeoDa software (GeoDa™ v1.12, 2018). The number of primary MERS-CoV cases in each province was used as the event variable, and the provincial population from a 2010 census (General Authority for Statistics, 2010) was used as the base variable.

2.4 | Statistical analysis

We compared the demographic and seasonal characteristics of patients with primary MERS-CoV infection using the chi-squared test or Fisher’s exact test as appropriate. We also used Pearson’s correlation coefficient to measure the statistical significance (p-values of <.05 and <.01) of the correlation between primary MERS-CoV cases and the distribution of camel populations in Saudi provinces. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22.

2.5 | Cluster analysis

Clustering is a machine learning technique for identifying natural groupings among data (Duque, Ramos, & Suriñach, 2007), and K-means clustering is a popular, unsupervised machine learning algorithm (Pimpler, 2017). We applied the K-means algorithm using Esri ArcGIS version 10.5 software to identify clusters of Saudi provinces based on primary MERS-CoV infection and camel distribution. The resulting maps identified distinct groups of data (i.e. MERS-CoV and camel clusters).

3 | RESULTS

3.1 | Primary MERS-CoV incidence by demographic characteristics and seasonality

Primary MERS-CoV cases accounted for 24.15% of the total number of confirmed cases in Saudi Arabia (n = 2,008). A total of 485 laboratory-confirmed human primary MERS-CoV cases were reported between 2 June 2012 and 31 March 2019, of which 49.69% (n = 241) reported positive camel exposure and 50.31% (n = 244) did not. Table S1 shows the demographic characteristics and seasonality of the primary incidence of MERS-CoV. Confirmed primary
MERS-CoV infection was more frequent among males (n = 413, 85.16%) and elderly patients aged 60 and older (n = 241, 49.69%). Most of the reported primary MERS-CoV cases occurred by year in 2014 (n = 164, 33.81%), by season during spring (n = 182, 37.53%) and by month in April (n = 73, 15.04%). Overall, significant differences were observed between primary MERS-CoV cases with camel exposure compared with those without in terms of sex, age group, year, season and month.

3.2 | Spatial pattern of primary MERS-CoV cases

The highest number of primary MERS-CoV infections was reported in Riyadh province (n = 189, 38.96%), followed by Makkah province (n = 116, 23.91%; Figure 1). Najran province had the highest incidence rate of primary MERS-CoV infection in the country, with 6.50 cases per 100,000 people (Figure 1). The excess risk map showed that the risk was higher than the national average (excess risk ratio > 1) in Najran, Riyadh and Qassim provinces, ranging from 1.41 to 2.42 (Figure S1a). Most provinces had higher proportions of infection due to camel exposure, although the Riyadh and Makkah provinces showed higher proportions of infections not due to camel exposure (Figure S1b). Confirmed cases of primary MERS-CoV infection were more frequent among males than females in all provinces, especially among individuals older than 60 years (Figure S1c,d).

3.3 | Spatial distribution of camel populations

The total number of camels in Saudi Arabia was estimated to be 1.39 million in 2015 (General Authority for Statistics, 2015), and the distribution of camel populations is presented in Figure 2, Table S1 and Figure S2. Riyadh and Eastern provinces had the largest number of camels (23.21% and 18.42% of the national total, respectively). There were many more female camels than males, with a ratio of 4:1, and more than 70% of camels were older than 1 year. The greatest camel density in relation to the human population was found in Hail, Najran, Qassim and Jouf provinces (16,831–22,584 camels per 100,000 people). The highest camel densities in relation to land area were observed in Qassim, Assir, Jazan and Makkah provinces (16,391–23,960 camels per km²). The spatial distribution of dairy camels showed a different pattern than that of all camels, with the highest distributions in Makkah, Qassim, Riyadh and Eastern provinces (13.60%–17.66%). Furthermore, the
distribution patterns for camels in barns were different than those for all camels, with most found in Riyadh, Qassim and Eastern provinces (13.78%–27.70%).

### 3.4 Association between primary MERS-CoV infection and camel distribution

The relationship between the distribution of primary MERS-CoV cases and the Saudi camel population was examined at the provincial level (Table 1). We examined the correlations between the 12 camel clusters and primary MERS-CoV cases with or without camel exposure. The total number of camels was significantly and highly correlated with the distribution of primary MERS-CoV cases ($r = 0.90$ and $0.79$ for cases with and without camel exposure, respectively). The association of camel sex and age was higher for primary MERS-CoV cases with camel exposure ($r = 0.78$–$0.91$) than without ($r = 0.72$–$0.80$). Clusters of dairy camels were moderately correlated with primary MERS-CoV cases ($r = 0.70$ and $0.66$ for cases with and without camel exposure, respectively). The distributions of camel barns and total camels within barns were more correlated with primary MERS-CoV cases with camel exposure ($r = 0.84$ and $0.83$, respectively) compared with cases without camel exposure ($r = 0.74$ and $0.66$, respectively).

### 3.5 Spatial cluster analysis

Primary MERS-CoV cases were clustered in provinces with large numbers of camels (Figure 3). The provincial spatial clusters were differentiated based on exposure. Riyadh province had the highest numbers of camels and primary MERS-CoV cases with camel exposure (Figure S3a). Riyadh, Makkah and Eastern provinces had the highest numbers of camels and primary MERS-CoV cases without camel exposure (Figure S3b).

### 4 DISCUSSION

MERS-CoV is a zoonotic virus, and dromedary camels are the likely source of primary human infection (Memish et al., 2014). Results from this study show statistically significant correlations between primary MERS-CoV cases (with and without camel exposure) and camel clusters at the provincial level, which is consistent with
Hemida, Elmoslemany, et al. (2017). This correlation was more significant for camel-exposed groups compared with non-exposed groups, consistent with Alraddadi et al. (2016). Asymptomatic carriers and mild infections that are less likely to be detected by a public health surveillance system might explain infection from humans for MERS-CoV primary cases without exposure to camels. This is supported by a nationwide survey in Saudi Arabia that estimated more than 44,000 individuals older than 15 years might be seropositive for MERS-CoV (Muller et al., 2015). Primary MERS-CoV infection without camel exposure might also be transmitted by workers with occupational exposure to camels who have high MERS-CoV seropositivity rates with mildly developed disease (Alshukairi et al., 2018).

Several studies of domestic animals have shown no evidence for MERS-CoV infection in screened cattle, goats or sheep from Saudi Arabia (Hemida et al., 2013; Kasem et al., 2018) or Jordan (Reusken et al., 2013). Serology for horses from Saudi Arabia and Oman (Hemida, Chu, et al., 2017) and equids from the United Arab Emirates (Meyer et al., 2014) were also negative. In contrast to this, a limited number of more recent studies reported that domestic livestock, such as sheep in Senegal, Tunisia and Egypt and goats in Egypt, naturally acquired MERS-CoV infection after prolonged contact with infected dromedary camels (Ali et al., 2017; Kandeil et al., 2019). This raises the possibility that other types of livestock can be a potential risk for the transmission of MERS-CoV to humans.

In the current study, more than half of the laboratory-confirmed primary MERS-CoV cases had no history of direct or indirect contact with camels. Incidental contact with asymptomatic or unrecognized mild disease might have contributed to human-to-human transmission; however, it is also possible that there are other contributing factors not yet investigated, such as undiscovered transient hosts in the surrounding environment that are in close and prolonged contact with infected dromedary camels (Kandeil et al., 2019).

Our results show significant correlations between both types of primary MERS-CoV cases (exposed to camels or not) and camel barns, the number of camels in barns and the number of dairy camels. Associations were more significant for those who had a history of direct contact with camels. In past decades, peri-urban camel farming and camel festivals have attracted increased public interest in the Arabian Peninsula. In addition, camel dairy production and human consumption of camel products, including unpasteurized camel milk, have also dramatically increased. These changes could have significantly contributed to the massive spread of MERS-CoV within camel herds and the emergence of this zoonotic disease in humans (Faye, 2013; Faye, Madani, & El Rouili, 2014; Gossner et al., 2016; Reusken et al., 2014). The results also reveal that primary MERS-CoV infection and juvenile male camels are significantly linked, which could be due to their higher viral MERS-CoV RNA load compared with female camels (Alagaili et al., 2014; Elfadil et al., 2018; Hemida et al., 2013; Kasem et al., 2018).

The spatial distribution of the disease shows that Riyadh province has the highest number of reported primary MERS-CoV cases in the country, with a higher proportion of infections not due to camel exposure. This is consistent with the projected large proportion of MERS-CoV seropositive individuals (asymptomatic human carriers) reported for Riyadh (46.7%) by Muller et al. (2015). Interestingly, Najran province, most of which is rural, had a very high camel density in relation to the human population and the highest incidence rate and excess risk ratio of primary MERS-CoV infection in the country. Additionally, most of the primary MERS-CoV cases in this province had been previously exposed to camels, which indicates that areas with high camel density are more likely to be hotspots for MERS-CoV transmission (Gikonyo et al., 2018).

In the current study, the majority of primary human MERS-CoV infections occurred in elderly males. The fact that camel-related activities, such as camel trading, shepherding, slaughtering and animal waste sanitation, are primarily carried out by middle-aged and elderly men supports our findings. We also found that in most Saudi Arabian provinces, the largest proportion of human primary MERS-CoV cases occurred during spring and winter, when camel breeding, calving, mobilization and interactions are more frequent (Padalino et al., 2015). Additionally, camel movements throughout the winter for grazing and participation in camel races and beauty festivals might be another contributing factor of camel-to-human MERS-CoV transmission (Hemida, Elmoslemany, et al., 2017).

Due to a lack of information on the distribution of camels and the geographic locations of camel barns in Saudi Arabia at lower spatial levels (e.g. cities and governorates), the spatial analysis in this study

| Variables | Camel exposure ($r$) | No camel exposure ($r$) | $t$ Test $p$-value |
|-----------|----------------------|------------------------|-------------------|
| Total camels | .80** | .79** | .008** |
| Total camels < 1 year | .78** | .77** | .03** |
| Total camels > 1 year | .78** | .77** | .03** |
| Male camels | .78** | .79** | .02** |
| Female camels | .80** | .77** | .05** |
| Male camels < 1 year | .79** | .78** | .07** |
| Female camels < 1 year | .80** | .78** | .04** |
| Female camels > 1 year | .80** | .79** | .03** |
| Female camels > 1 year | .80** | .79** | .03** |
| Dairy camels | .80** | .79** | .02** |
| Camel barns | .80** | .79** | .02** |
| Camels in barns | .80** | .79** | .02** |

*a*Clusters of camels by sex, age, dairy status and barn information in provinces.

*Correlation is significant at the .05 level.

**Correlation is significant at the .01 level.
was limited to the national and provincial levels. Fortunately, in June 2019, the Saudi Ministry of Environment, Water, and Agriculture launched an electronic system for numbering and tracking camels using microchip implants to generate a national database (Ministry of Environment, Water, & Agriculture, 2019). This initiative will help improve the health status of camels through epidemiological mapping of camel-related diseases to support timely, preventive interventions and effective control measures. Data from the new camel tracking system can be used for detailed investigations at lower spatial levels to study the spatiotemporal patterns of camel movements and human interactions.

Camel-to-human MERS-CoV transmission is evident in most primary cases, but there is still a high proportion of primary infections with ambiguous links to camels. Results from this study show significant correlations between primary MERS-CoV cases and camel populations in all Saudi Arabian provinces, regardless of camel exposure. This supports the hypothesis of the role of an asymptomatic human carrier or, less likely, an unknown animal host that has direct contact with both infected camels and humans.

This study provides a preliminary risk assessment of prioritization measures for the control of camel-to-human transmission of MERS-CoV infection. It is necessary to conduct both case–control and cohort studies to explore the cause–effect relationship between MERS-CoV transmission and exposure to camels and other potential animal hosts. Further seroprevalence studies are required to investigate other domestic animals that are in prolonged contact with infected dromedary camels and estimate attributable rates for potential risks of transmission to contain sporadic and unpredicted MERS-CoV epidemics.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

ORCID
Ali Al-Zahrani https://orcid.org/0000-0001-9183-6150

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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