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Cross-sectional study of MERS-CoV-specific RNA and antibodies in animals that have had contact with MERS patients in Saudi Arabia

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ABSTRACT

Background: Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly emerged coronavirus that is associated with a severe respiratory disease in humans in the Middle East. The epidemiological profiles of the MERS-CoV infections suggest zoonotic transmission from an animal reservoir to humans.
Methods: This study was designed to investigate animal herds associated with Middle East respiratory syndrome (MERS)-infected patients in Saudi Arabia, during the last three years (2014–2016). Nasal swabs and serum samples from 584 dromedary camels, 39 sheep, 51 goats, and 2 cattle were collected. Nasal samples from camels, sheep, goats, and cattle were examined by real-time reverse-transcription PCR (RT-PCR) to detect MERS-CoV RNA, and the Anti-MERS ELISA assay was performed to detect camel humeral immune response (IgG) to MERS-CoV S1 antigen infection. The complete genome sequencing of ten MERS-CoV camel isolates and phylogenetic analysis was performed.
Results: The data indicated that seventy-five dromedary camels were positive for MERS-CoV RNA; the virus was not detected in sheep, goats, and cattle. MERS-CoV RNA from infected camels was not detected beyond 2 weeks after the first positive result was detected in nasal swabs obtained from infected camels. Anti-MERS ELISA assays showed that 70.9% of camels related to human cases had antibodies to MERS-CoV. The full genome sequences of the ten MERS-CoV camel isolates were identical to their corresponding patients and were grouped together within the larger MERS-CoV sequences cluster for human and camel isolates reported form the Arabian Peninsula.
Conclusions: These findings indicate that camels are a significant reservoir for the maintenance of MERS-CoVs, and they are an important source of human infection with MERS.

Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel betacoronavirus that recently emerged. MERS is considered a life-threatening disease with an extensive health impact. The clinical manifestations vary from subclinical manifestations to rapid progressive acute pneumonia. MERS-CoV-infected patients often present fever, sore throat, myalgia, cough, and shortness of breath, and occasionally hemoptysis [1–3]. MERS-CoV was first identified in 2012 in Jeddah, Saudi Arabia [4]. Since its identification, MERS has been responsible for 2040 cases and 712 deaths in 27 countries worldwide by July 21, 2017 [5]. The majority of cases and deaths

Abbreviations: MERS, Middle East respiratory syndrome; MERS-CoV, Middle East respiratory syndrome coronavirus; TUU, Tabuk; AJF, Jouf; HAS, Hail; RAH, Northern Boundaries; ELQ, El-Qassim; MED, Al-Madina; RUH, Riyadh; DWD, El-Dowadmi; SHG, Shagraa; AKH, Alkharj; WAE, Wadi El-Dwasir; ZUL, Zulfi; MJH, Majmaa; TIF, Taif; MAK, Makkah; ABT, Bahaa; AHB, Asir; GIZ, Gizan; EAM, Najran; DMM, Shargia; HOF, Ihsaa.

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occurred in Saudi Arabia, most of which were sporadic infections, sometimes leading to family or hospital clusters [6].

The exact source of MERS-CoV and how it is transmitted to humans is unknown. Initial investigations have indicated that MERS-CoV originated from bats; sequences related to MERS-CoV have been found in several bat species [6,7]. Several other animal species in the Arabian Peninsula have been serologically assessed for MERS-CoV infection [8]. Viral and serological surveillance revealed a high rate of seropositivity to MERS-CoV in dromedary camels, including detection of antibodies to MERS-CoV in sera of camels in different countries (i.e., Saudi Arabia, UAE, Oman, Egypt, Jordan, Nigeria, Tunisia, Ethiopia, Kenya, Canary islands, Pakistan, and Mali) [10–18]. Recovery of MERS-CoV genome sequences from camels with a high degree of homology to their counterparts in humans and isolation of MERS-like CoV from camels have been reported [9,10,14,15,19,20,21]. Together, these results suggest that the MERS-CoV detected in dromedary camels is the most likely source of the human infection [8,9,15,20,21]. The questions that need to be addressed are why the majority of human infections occur in Saudi Arabia, and whether interspecies transmission of the MERS-CoV to humans has only recently occurred or has only been recently recognized. The epidemiological factors related to these questions need to be investigated.

Current epidemiological data on MERS infections refers to zoonotic transmission from an animal reservoir to humans. In this study, animals (camels, sheep, goats, and cattle) related to human cases were examined for MERS-CoV RNA and antibodies. Furthermore, complete genome sequences of MERS-CoV isolated from camels and patients were compared to assess the potential zoonosis of MERS infection.

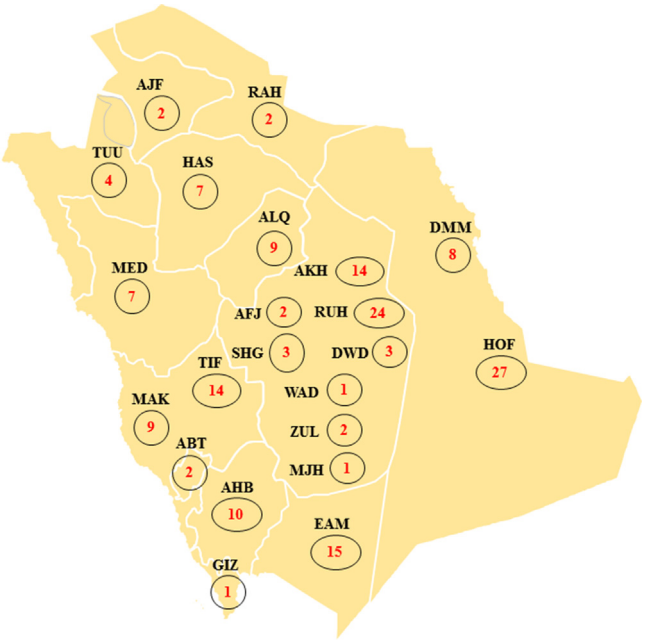

Fig. 1. Distribution of confirmed human cases of MERS with a history of contact with animals in different regions of Saudi Arabia, notifications issued by the Ministry of Health (MOH).

Materials and methods

Samples

This study was carried out during 2014, 2015, and 2016 at the Ministry of Environment, Water and Agriculture (MEWA), Riyadh, Saudi Arabia. This was part of a cooperative epidemiological response to the confirmed human cases of MERS with a history of animal contact. Notifications were issued by the Ministry of Health (MOH), Riyadh, Saudi Arabia. We received 167 notifications from the MOH, distributed throughout all regions of Saudi Arabia. Sixty-eight of these notifications were associated with camels, while 14 were related to other animals. Seventy-two notifications had no relationship with animals, and in the remaining 13 notifications, the owners did not allow us to investigate their animals (Fig. 1).

A total of 780 nasal swabs were collected from all animals with a history of contact with MERS-patients (595 dromedary camels, 93 sheep, 90 goats, and 2 cattle) of different ages and sex in different regions of Saudi Arabia. Nasal swabs from animals were transferred to the Riyadh veterinary laboratory in transport medium (COPAN Italia, Italy) within 24–72 h after collection. All animals positive for MERS-CoV RNA were isolated, quarantined, and examined weekly until two consecutive negative samples were obtained to determine the maximum period of viral shedding.

Blood samples were collected from dromedary camels, centrifuged to separate the sera, and then frozen at −20 °C. Serum samples were tested for the presence of IgG antibodies reacting with MERS-CoV.

Nucleic acid extraction, real-time reverse transcription-PCR, and sequencing

RNA was extracted from nasal swabs samples, using a Qiagen viral RNA extraction kit in accordance with the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). Real-time reverse transcription-PCR (rtRT-PCR) targeting the envelope protein gene upstream (UpE) of MERS-CoV was conducted for screening [22,23]. Open reading frame (ORF) 1a was used to confirm the MERS-CoV diagnosis, based on the recommendation of the World Health Organization (WHO) [24]. Briefly, 5 μL of extracted RNA was subjected to rtRT-PCR using UpE primers, as described elsewhere [22]. The rtRT-PCR was performed using LightMix Molecular Dx MERS-CoV upE kits (Roche) according to the manufacturer's protocol. All positive samples from the UpE screening assay were confirmed by ORF1a as previously described [22,23].

Ten positive RT-PCR samples {Riyadh (3), Jeddah (3), Unayzah (1), Quwaiyah (1), Artawiyah (1), and Taif (1)} were subjected to full genome sequencing. Phylogenetic analyses based on full genome sequencing of MERS-CoV were carried out with MEGA7 [25]. The evolutionary distances were estimated by means of the neighbor-joining method [26] based on the Tajima-Nei method [27]. Bootstrap analyses were performed with 1000 repeat samples of the data sets [28].

Anti-MERS-CoV ELISA

Camel serum samples were collected and assayed for MERS-CoV specific antibodies, using Anti-MERS-CoV ELISA Camel (IgG) (EUROIMMUN, Lübeck, Germany). The ELISA test kit allows a semi-quantitative assay for IgG antibodies against MERS coronavirus in plasma or serum from camels. The ELISA assay was carried out according to the manufacturer's protocol. In summary, the serum samples were diluted 1:101 in the sample buffer; the diluted samples were incubated in wells coated with the purified S1 antigen of MERS coronavirus (MERS-CoV S1). In the case of positive samples, the specific antibodies will bind to MERS antigens. A second incubation is carried out using an enzyme-labeled anti-camel IgG (enzyme conjugate) stimulating the reaction to detect the bound antibodies [12,29].

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