Exploring the potential roles of some rodents in the transmission of the Middle East respiratory syndrome coronavirus

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Abstract
Middle East respiratory syndrome coronavirus (MERS-CoV) is one of the recently identified zoonotic coronaviruses. The one-hump camels are believed to play important roles in the evolution and transmission of the virus. The animal-to-animal, as well as the animal-to-human transmission in the context of MERS-CoV infection, were reported. The camels shed the virus in some of their secretions, especially the nasal tract. However, there are many aspects of the transmission cycle of the virus from animals to humans that are still not fully understood. Rodents played important roles in the transmission of many pathogens, including viruses and bacteria. They have been implicated in the evolution of many human coronaviruses, especially HCoV-OC43 and HCoV-HKU1. However, the role of rodents in the transmission of MERS-CoV still requires more exploration. To achieve this goal, we identified MERS-CoV that naturally infected dromedary camel by molecular surveillance. We captured 15 of the common rodents (rats, mice, and jerboa) sharing the habitat with these animals. We collected both oral and rectal swabs from these animals and then tested them by the commercial MERS-CoV real-time-PCR kits using two targets. Despite the detection of the viral shedding in the nasal swabs of some of the dromedary camels, none of the rodents tested positive for the virus during the tenure of this study. We concluded that these species of rodents did not harbor the virus and are most unlikely to contribute to the transmission of the MERS-CoV. However, further large-scale studies are required to confirm the potential roles of rodents in the context of the MERS-CoV transmission cycle, if any.

Keywords
dromedary camels, jerboa, MERS-CoV, mice, nasal swabs, rat, real-time PCR, rectal swabs, rodents, RNA, transmission

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; NA, nucleic acids; RNA, ribonucleic acid; RT-PCR, reverse transcriptase-polymerase chain reaction.
1 | INTRODUCTION

Middle East respiratory syndrome coronavirus (MERS-CoV) is still one of the coronaviruses of major health concerns.1,2 The virus emerged almost 9 years ago3 and still, some cases are reported, especially from the Middle East. MERS-CoV is representing one of the ideal examples of the One Health concept.1,2 The young dromedary camels up to 2 years are more susceptible to MERS-CoV infection. However, older camels seroconverted to MERS-CoV in the Middle East and Africa.4 These animals shed the virus in some of their body secretions (nasal, rectal, and saliva) as well as their breath.5-8 Some studies showed the transmission of MERS-CoV from dromedary camels to humans.9,10 Some other studies showed the occupational risk of some camel handlers and veterinarians who came in close contact with some naturally infected camels.11 However, the exact mechanisms and routes of virus transmission between animals as well as from animals to humans still need further investigation. Some rodents play important roles in the transmission of some viruses, such as Hantavirus and Lassa virus.12 The Akhmeta virus as one of the orthopoxviruses was isolated from some species of the wood mice (Apodemus uralensis and Apodemus flavicollis).13 There are several systems for rearing the dromedary camels. Some animals live in an open system in which they are grazing and kept in the deserts most of the time. Other animals live in a semi-open system. These animals are used to grazing sometimes during the day and return to their owner’s assigned place to spend the night. The other type of camel management is the closed system. In this type of husbandry, the camels are spending all their time indoors. Some of these camel herds are reared for their milk and meat. Usually, some rodents share the habitat with the dromedary camels under all management systems. Identification of the roles of rodents in the transmission of MERS-CoV, if any, may have great impacts on our understanding of the dynamics of MERS-CoV from camels to humans as well as among animals within the same herd and some other far distance herds. Our objective is to conduct a molecular survey to detect MERS-CoV in some species of rodents captured within the pen housing positive MERS-CoV camel herd.

2 | MATERIALS AND METHODS

2.1 | Description of the camel population and its habitat

We conducted molecular surveillance among some camel populations for the MERS-CoV. This camel herd consists of 50 animals reared in a closed housing system. The animals were divided into several pens. Each pen holds several female animals. The adult male animals (over 3 years old) were kept individually in separate pens. Males are allowed to approach females only during the breeding season. These animals usually feed on a mixture of green grasses, dry hay, and concentrated ration. All animals were kept in about 6000 feet space with grooming and playing space connected to the animal pens. Animals receive both dry concentrated ration as well as some dry hay and grasses (Figure 1).

We conducted a longitudinal follow-up study in these animals for almost 2 months (Feb 20th–April 5th). We sampled these animals at weekly intervals to make sure that the virus is still circulating in this herd. Meanwhile, we set up the rodent wire traps to be in very close contact with animals. They were placed inside and around the camel pens as well as very close to their food supply. Rodents used to share some of the camel’s food, particularly the concentrated pellets. Meanwhile, these rodents were burrowing in different places close to these animals and their source of food. This environment allowed a close interaction between rodents in this study and the positive MERS-CoV animals.

2.2 | Procedures of nasal swab collection and processing from dromedary camels

We collected nasal swabs from the dromedary camel population for five time points biweekly during the duration of this study. Swabs are introduced deep into the nasal orifices of each animal.
to touch the inner mucous membranes. Special attention was paid that each cotton swab is fully soaked with the nasal secretions per each animal. Each swab was transferred into tubes containing the viral transport medium and processed as previously described.5,6

2.3 | Handling and sample collection from rodents sharing habitat with MERS-CoV dromedary camels

We captured 15 rodents (rat, jerboa, and mice); five animals per species during the duration of this study (Figure 2). Briefly, we set up some rodent traps close to the animal feeders and drinkers. We added some bites inside each trap, such as pieces of tuna or tomato (Figure 1). On the following day, we inspected each trap for the presence of any rodents. As soon as we found a rodent inside these live traps, we immediately transferred it to the laboratory for further sampling. We secured the animals by the proper restrain methods for the rodents as described earlier.14 Nasal and rectal swabs were collected for each animal on a viral transport media. The collected swabs were stored at (-80°C) for further processing.

2.4 | Extraction of the total viral RNAs

The total viral RNAs were extracted from the nasal and rectal swabs of dromedary camels and rodents using the Qiagen viral RNA kits (RNeasy Mini Kit; Qiagen). The process of these extractions was carried out as previously described.7,15 Simply, 140µl per sample was used to extract the total viral RNAs. The extracted RNAs were eluted in 50µl of the elution buffer and then were kept at (-80°C) for further testing.

2.5 | The real-time PCR, the reverse transcriptase-polymerase chain reaction (RT-PCR)

We tested the nasal swabs collected from dromedary camels as well oral and rectal swabs collected from various species of rodents as explained elsewhere (Alnaeem, 2020,7,8,15). Briefly, we used the commercially available real-time PCR MERS-CoV kits (RealStar® MERS-CoV RT-PCR Kit 1.0; altona Diagnostics GmbH) to test various samples for the presence of the viral nucleic acids. Samples were considered positive only when both targets (ORF1a and UpE) showed positive results. Meanwhile, we considered positive samples when (Ct ≤ 30).

3 | RESULTS

3.1 | Molecular surveillance of MERS-CoV in dromedary camel population

We conducted a molecular surveillance longitudinal study among the camel population for up to 2 months. Initially, we screened 40 animals (80%) of the total population. Fifteen animals out of 40 were positive by real-time PCR (37.5%). We selected nine positive animals with a minimum Ct value of 30 for the follow-up study. Those nine animals were sampled at the biweekly interval. The nasal swabs of seven out of the nine animals remained positive until the end of the study (Figure 2).

FIGURE 2  Schematic representation of the animal experiment and mapping the timeline of the longitudinal study in dromedary camels and the molecular surveillance in rodents. Schematic illustration showing the timeline for the longitudinal study among dromedary camel herd. The top line shows the timescale for sample collection at five time points biweekly. The middle line shows the results of the MERS-CoV molecular surveillance among the dromedary camel population. The bottom line shows the timeline of capturing various species of rodents that shared the habitat with the positive MERS-CoV dromedary camel population. The abbreviations are as follows (R = rats, M = mice, and J = jerboa). MERS-CoV, Middle East respiratory syndrome coronavirus
3.2 Testing some rodents sharing the habitat with some positive MERS-CoV dromedary camel population

Our molecular MERS-CoV surveillance on the collected rodents’ samples (rat, mice, and jerboa) from 15 animals representing those three species revealed that all the collected oral and rectal swabs were negative throughout the tenure of this study (Figure 2).

4 DISCUSSION

MERS-CoV is still circulating in many camel populations in several Asian and African countries. The dromedary camels infected with MERS-CoV did not show any pathognomonic clinical signs either under the experimental or natural infection. The infected animals showed mild pyrexia with some subtle respiratory signs, such as rhinorrhea, for a short period. However, the detection of various MERS-CoV antigens, especially the MERS-CoV-S and MERS-CoV-N, were reported under both natural and experimental infection. This suggests that MERS-CoV does replicate in some respiratory organs of the infected dromedary camels, such as the turbinate bones, trachea, and lungs. The high seroprevalence of MERS-CoV among dromedary camels’ especially older animals is well-documented. There are some potential reasons behind this continuous circulation of the virus among animals. Repeated exposure of the same animal or group of animals to the virus, presence of other intermediate host or reservoir animals may help in the sustainability of the virus in a certain population of animals. Rodents play important roles in the transmission of many pathogens, including viruses, bacteria, and parasites. The rodents were implicated in the evolution and presumably the origin of some human coronaviruses, especially HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1. However, the roles of rodents in the transmission of MERS-CoV is not explored yet. Several rodents share the habitats with dromedary camels either in the desert as an open system or even in the farms in the closed system. The environment within the camel farm (presence of food, water, and the possibility of having burrows or holes) and around these animals may favor the presence and breeding of different species of rodents. We observed that several species of rodents live close to dromedary camels, such as rats, mice, and jerboa. Some of these rodents live and adapt to living in the deserts, particularly the jerboa (hopping rodents live in the Arabian peninsula and Asia). Some of these animals live in burrows to protect them from high temperatures in summer. We identified some naturally infected MERS-CoV dromedary camels. We captured and collected some samples from these rodents that may share the food and water as well as the habitats with these positive camels. One of the main findings was the continuous circulation of MERS-CoV in this camel population during the sampling period or rodents. It was ensured that these rodents came in very close contact with a positive MERS-CoV camel population. Our results showed that despite some of the dromedary camels being infected with MERS-CoV for the duration of the study, none of the tested rodents was positive for MERS-CoV until the end of this study. We believe that the duration of this longitudinal study was quiet enough to represent enough exposure of rodents to a positive and actively shedding MERS-CoV camel population. This time should be enough to get these captured species of rodents infected with the virus if they were to do so. Thus, the absence of the detection of any MERS-CoV-NA in swabs from these rodents suggests that these rodents are unlikely to play a role in the transmission of MERS-CoV. Based on this data, we assume that rodents, at least the tested species, in this study, are unlikely to play a role in the transmission of MERS-CoV from animal to animal as well as from animals to the environment and then to humans. However, large-scale studies are highly encouraged to study the potential roles of other species of rodents or animals that may share the habitat of the dromedary camels in the transmission cycle of MERS-CoV. Meanwhile, testing various species of rodents living in close proximity of positive MERS-CoV dromedary camel is one of the most important research directions to confirm the potential roles of rodents in the transmission cycle of MERS-CoV, if any.

5 CONCLUSIONS

Our results clearly show the absence of any detectable MERS-CoV-RNA in the oral and rectal swabs of the tested animals. These results strongly suggest that the rodents are unlikely to transmit MERS-CoV among animals as well as from animals to humans.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Maged Gomaa Hemida and Abdelmohsen Alnaeem: conceived the ideas, conducted the fieldwork and laboratory work, analyzed the data, and drafted the manuscript. Mohammed Alhammadi: conducted some fieldwork, helped in the data analysis, and helped in the drafting of the manuscript. Faisal Almathen: helped in field surveillance and writing of the manuscript. All authors wrote the manuscript and agreed to the final version.

ETHICS STATEMENT

The animal experiments conducted in the current study were carried out according to the instructions of the Animal Ethics protocols and the National Committee of Bio-Ethics, King Abdul-Aziz City of Science and Technology, Royal Decree No. M/59 (http://www.kfsh.med.)
The animal experiments and protocols were reviewed and approved by the animal ethics committee of the deanship of scientific research, King Faisal University, Saudi Arabia (Approval No: KFU-REC/2020-09-21). All the necessary paperwork for sample collections was obtained.

**DATA AVAILABILITY STATEMENT**

Data available on request from the authors.

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