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Research article

Longitudinal and abattoir-based surveillance of MERS-CoV in camels in Jordan, 2018–2020

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ABSTRACT

To generate baseline information to help better understand the antibody kinetics and nasal shedding dynamics of MERS-CoV in camels in Jordan, a longitudinal surveillance study was conducted in two phases; phase 1 was between December, 2018 and January, 2019 and phase 2 between August and December 2020. In each phase, two camel herds were studied. These herds were located in Al-azraq and in Al-ramtha area and were named Al-azraq and Al-ramtha herds, respectively. The same camel herd of Al-zarqa area was sampled in both phases while two different camel herds, one in each phase, were sampled in Al-ramtha area. Blood and nasal swabs were collected from same selected animals in all visits to each herd in both phases. Additionally, nasal swabs and retropharyngeal lymph node tissue samples were collected from sixty-one camels slaughtered at Al-ramtha abattoir during phase 2 to enhance virus isolation opportunities and phylogenetic analysis. All sampled animals from Al-azraq camel herd were either borderline or seropositive on spike 1 based ELISA assay and negative on quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) in both phases. In Al-ramtha camel herds, an unsteady pattern prevailed in animals' seropositivity in both phases and viral RNA was detected in all animals in the end of phase 1 and in one animal during phase 2. For the seroconversion, anti-MERS-CoV spike 1 antibodies were detected in two animals in phase 1 in the first collection only. While, in phase 2, intermittent seroconversion pattern was observed in several samples over time of collections that ended with all animals became seropositive in the last collection (after nineteen days from viral RNA detection). In addition, viral RNA was detected in nasal swabs of 3 slaughtered camels. Phylogenetic analysis of a partial fragment of spike 1 gene sequences of all MERS-CoV isolates clustered together with clade B of MERS-CoV. This cluster contains all MERS-CoV sequences obtained either from camels or human sources in the Arabian Peninsula indicating the continuous circulation of this clade also in Jordan.

1. Introduction

Coronaviruses (CoVs) represent a significant global public health threat. The ongoing worldwide COVID-19 pandemic (caused by the SARS-CoV-2 virus) demonstrates the urgent need for continuous active surveillance, rapid diagnosis, and real-time tracking of zoonotic coronaviruses as well as other emerging pathogens in humans and animals. Coronaviruses are known to cause important respiratory and gastrointestinal diseases in animals and humans (Banerjee et al., 2019). Coronaviruses are enveloped viruses with a positive-sense single stranded RNA genome. The Orthocoronavirinae subfamily is one of two subfamilies within the Coronaviridae family (Fehr and Perlman 2015). The Orthocoronavirinae is classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronaviruses and Deltacoronavirus (Han et al.,

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2019). The alphacoronaviruses and betacoronavirus harbor the mammalian coronaviruses. While gammacoronaviruses and deltacoronaviruses contain the avian coronaviruses (Chan et al., 2013). There are four human coronaviruses; two belong to the alphacoronaviruses and the other two are betacoronaviruses (Owusu et al., 2014).

The zoonotic human coronaviruses are all betacoronaviruses: Middle East respiratory syndrome coronavirus (MERS-CoV), as well as severe acute respiratory syndrome coronavirus 1 and 2 (SARS-CoV, and SARS-CoV-2 (Ye et al., 2020). Those zoonotic coronaviruses likely originated in bats, then transmitted to intermediate animals before jumping to humans, causing variable degrees of mortality (2–35/%) (Wu et al., 2020). Of these zoonotic coronaviruses, MERS-CoV causes an endemic respiratory infection in dromedary camels, ranging from sub-clinical to mild rhinitis. MERS-CoV has shown the highest fatality rate in human (almost 33%) (Zhang et al., 2021).

Camels have been identified as intermediate hosts for MERS-CoV Reusken et al., 2013; Reusken et al., 2014) and the MERS-CoV receptor (the DPP-4) is found in camels' nasal turbinates, trachea, lung, and kidneys (Alnaeem et al., 2020). MERS-CoV infection entails loss and disorientation of cilia, as well as hyperplasia of goblet cells and interstitial pneumonia, are the main changes at the cellular level. The degradation of glomerular capillaries was observed in the renal cortex, as well as the complete disappearance of glomerular tufts, which were replaced with fibrinous exudate (Alnaeem et al., 2021). MERS-CoV transmission dynamics in camel herds have shown that the virus can spread quickly, and seroprevalence studies conducted in camel herds across the globe revealed a prevalence rate of 71–100 % in most countries, with younger camels being the most affected (Dighe et al., 2019). Many longitudinal studies have been carried out to investigate the dynamics of active MERS-CoV infection and the detection of seropositivity. In one of these studies, two herds in Saudi Arabia were sampled between September 2014 and May 2015, and active MERS-CoV infection was detected, with both herds having 100 % seropositivity in most sampling time points (Hemida et al., 2017). MERS-CoV genomic RNA was found in a 1-month-old calf, and MERS-CoV seroprevalence positivity rates increased from 75% to 90% in the same herd with age (Wernery et al., 2015). According to a study conducted at an abattoir, 41% of sloughed camels tested positive for viral RNA (Alnaeem et al., 2020).

In Jordan, previous studies reported a 100% seroprevalence in 2013 and an 82% prevalence in 2016 (Reusken et al., 2013; van Doremalen et al., 2017). The specific objective is to generate baseline information to help better understand antibody kinetics and MERS-CoV shedding dynamics and to enhance virus isolation and phylogenetic analysis in camels in Jordan.

2. Materials and methods

2.1. Study site and sampling

This study was conducted from December, 2018 to December, 2020 in commercial private camel farms. All the procedures implemented in this work complied and locally approved by the ethical standards of the Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology (Proposal No. 470/2018). In a previously reported cross-sectional study (van Doremalen et al., 2017) conducted in Jordan 2016 revealed a high prevalence of MERS-CoV in young dromedary camels. In order to follow up on the MERS-CoV status in Jordan, a longitudinal surveillance of MERS-CoV in camels was conducted in two phases; Phase 1 was between December, 2018 and January, 2019, and phase 2 between August and December 2020. In each phase, two camel herds were studied. These herds were located in Al-azraq and in Al-ramtha area and were named Al-azraq and Al-ramtha herds. Al-azraq herd is located in Al-Zarqa governorate and Al-ramtha herds located in Irbid governorate. These two governorates contained the camel herds that were tested positive for MERS-CoV RNA by qRT-PCR as per the results of the 2017 cross-sectional study (van Doremalen et al., 2017). Al-zarqa camel herd was used in both phases while two different camel herds (one each) were used in Al-ramtha area. Al-azraq herd consisted of 105 animals of different age groups. This was a closed herd in which camels were allowed to graze freely, veterinary services and vaccination programs were supervised by two veterinary medical officers. This herd belongs to the Jordan Police Department (Al-Hajaneh Section) and were used for breeding, police parades and police control activities. All animals of this herd were identified using Microchip numbering system.

Al-ramtha herds used in phase 1 and phase 2 were open herds and the total number of animals varied in each visit. At the start of phase 1 and phase 2, herds consisted of 30–35 juvenile camels < one-year-old, 5-7 adult females and 2 bull camels each. These herds were a stationery farm setting type where camels are kept in a large pen and water and feed were provided. Veterinary services were provided upon the owner's request by the Jordan Ministry of Agriculture veterinary services and no vaccination program was applied. These herds were used for meat and milk production and were owned by local butchers (2–5 animals of each herd were slaughtered weekly) who purchased the animals from different parts of Jordan.

Three different questionnaires/animal modules were developed by the Food and Agriculture Organization of the United Nations (FAO) to gather information about date of the visit, animal age, sex, identification number, collected samples, previous health history and vaccinations program applied. During each visit in both phases, general examination and all health-related events, herd practices including nutrition, management, production and reproduction programs used on the farm were recorded. Initially, the questionnaires were completed on papers by a trained veterinary medical officer (a veterinarian employed by Jordan Ministry of Agriculture) during an interview with the owners of the herds on the first visit.

In both phases, in Al-azraq herd, a total of 105 camels were present. Eighteen (n = 18; all females, age ranged 3–12 years old) animals of different age groups were selected randomly using systematic random sampling method. The sample size required was determined based on the formula of Martin (Martin et al., 1987). This herd was visited once every 11 days for three consecutive visits (visits' dates: 12/12/2018; 23/12/2018; 3/1/2019). Using the same methods selection, twelve (n = 12; 8 males age ranged 6–10 months and 4 females age ranged 7–14 months) animals of different age groups were selected randomly from Al-ramtha camel herd. Selected animals from this herd were identified and separated and kept in a small pen. This herd was visited on a weekly basis for three consecutive weeks (visits' dates: 20/12/2018; 27/12/2018; 3/1/2019).

For phase 2, from Al-azraq and Al-ramtha herds, ten animals each (Al-Azraq, n = 10; 8 females age ranged 2–4 years old and 2 males both were 10 years old; Al-ramtha, n = 10, 7 females age ranged 5 months to 9 years old and 3 males age ranged 6 months to 7 years old) of different age groups were selected randomly. Al-azraq herd was visited biweekly for five consecutive visits and Al-ramtha herd was visited also biweekly for the first five consecutive visits, then every ten days for the following five consecutive visits.

Each phase included the collection of two nasal swabs; one was stored in viral transport media and the other in a TRI Reagent (Zymo research, USA). Blood was also collected in plain tubes. Blood and nasal swabs were taken from each animal sampled in each herd. Additionally, in the phase 2, between August 31 and December 2, 2020, Al-ramtha abattoir (Al-ramtha slaughtering camel facility) was visited twelve times. Nasal swabs and retropharyngeal lymph nodes tissue samples were collected and put in aplastic container from sixty-one slaughtered camels in Al-ramtha slaughterhouse (56 males age range 6 months to 5 years old and 6 females, age ranged 1–2years old). During each visit all slaughtered animals ware sampled by a trained veterinarian employed by Jordan Ministry of Agriculture.

Collected blood, nasal and tissue samples were kept in an ice box and transferred within 24hr to the diagnostic laboratories at Faculty of Veterinary Medicine/Jordan University of Science and Technology or to

Jordan Ministry of Agriculture laboratories (blood or serum). Serum samples were stored in – 20 °C while nasal swabs and tissue samples were stored in – 80 °C until analysis.

2.2. Serological assays

Whole blood samples were collected by jugular venipuncture into 10 ml red top serum vacutainer sterile plain tubes. Sample centrifugation occurred at Jordan University of Science and Technology, Faculty of Veterinary Medicine laboratories or at Jordan Ministry of Agriculture Laboratories. Samples were centrifuged for 10 min at 3000 rpm and serum was obtained and stored at –20 °C until testing. Serology testing was performed by the Anti-MERS-CoV ELISA (S1 Protein) Camel (IgG) kit (Euroimmun, Germany). ELISA test results were interpreted according to the kit's manufacturer's guidelines (ratio <0.8 negative; ratio ≥0.8 to <1.1 borderline and ratio >1.1 positive).

2.3. Viral RNA extraction and real time RT-PCR and sequencing

The nasal swabs and the retropharyngeal lymph node tissue were tested using qRT-PCR. Nasal swab samples were collected in TRI reagent (Zymo research, USA). Total RNA was extracted from all nasal swabs and retropharyngeal lymph nodes tissue samples using the Direct-zol RNA extraction kit (Zymo research, USA). Five microliters of RNA were used in a one-step qRT-PCR upE assay for MERS-CoV using the QuantiFast RT-PCR probe kit (Qiagen, Germany). The samples were analyzed using Rotor Gene Q real time Thermal cycler (Qiagen, Germany). Results of the qRT-PCR were expressed as positive or negative based on the Ct-value (threshold cycle value). Positive samples (Ct < 37) in the upE qRT-PCR were further tested for conformation using ORF1a assay qRT-PCR (Corman et al., 2012). Positive samples on ORF1a qRT-PCR assays were subjected to hemi-nested PCR technique using three primers targeting the spike gene. The primers (NM-SPIKE-F3, NM-SPIKE-R1, NM-SPIKE-R2) sequences and PCR conditions were as mentioned (Alagaili et al., 2014). The heminested PCR product of 1142 bp was subjected to bi-directional sanger sequencing using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequences of the spike gene were viewed by the BioEdit software and edited by the Edit sequence interface of the lasergene package. Sequence alignment, calculation of the sequence nucleotide similarities and construction of the phylogenetic tree was done by the MegAlign interface of the DNASTAR software.

The phylogenetic analysis was assembled using Neighbor-Joining method and Kimura 2-parameter method with bootstrap consensus tree inferred from 500 replicates. The analysis was conducted in MEGA X (Kumar et al., 2018). All molecular assays were done at the molecular biology and virology laboratory, Faculty of Veterinary Medicine, Jordan University of Science and Technology.

3. Results

Results of the ELISA test (S1 protein) for Al-azraq herd in phase 1 all tested seropositive for MERS-CoV in the three consecutive sample collections (Table 1). Also, in phase 2, all sampled animals tested seropositive for MERS-CoV except one camel (camel No 4) was borderline in the first, second and third collections then became positive in the following two collections (Table 2).

In phase 1, ELISA (S1 protein) testing for MERS-CoV of the Al-ramtha herd revealed that out of the twelve sampled animals, only 2 animals (camels No 9 and 11) were seropositive for MERS-CoV in the first blood collection and all animals were seronegative in the second and the third collection.

Results of the ELISA test for the Al-ramtha herd in phase 2 indicated that all animals were seronegative for MERS-CoV (S1 protein) on ELISA testing for the first 9 collections except animals' number 5 and 7 that were seropositive in the first 3 collections, and animal number 7 that was positive only in the 6th collection. All animals became seropositive in the 10th collection (Tables 3 and 4; all sera sample of Al-ramtha herd were tested again for confirmation and same results were obtained).

Table 1. Results of ELISA test for MERS-CoV of Al-azraq camel herd (Phase 1: December, 2018 to January, 2019), Jordan.

Camel No	Camel Age	Camel Sex	1st collection (12/12/2018) Ratio[1]	2nd collection (23/12/2018) Ratio	3rd collection (3/1/2019 Ratio
1	3Y	F	4.3798	5.3125	5.7110
2	5Y	F	5.0288	4.8942	4.6284
3	6Y	F	4.8125	3.7307	3.6605
4	4Y	F	3.5336	3.0192	5.8577
5	6Y	F	3.8750	4.8846	5.1009
6	10Y	F	5.8413	4.4711	4.8119
7	7Y	F	4.3269	5.8125	3.7568
8	12Y	F	6.4278	4.4951	5.7247
9	6Y	F	5.3269	5.3846	3.5183
10	4Y	F	4.4855	5.6538	2.7706
11	5Y	F	4.6394	5.3846	5.3440
12	6Y	F	6.1057	6.2740	5.0642
13	8Y	F	3.9423	3.8501	5.2568
14	9Y	F	5.7211	5.7836	4.8944
15	12Y	F	3.0240	4.4951	4.7844
16	7Y	F	5.2163	5.1730	6.8990
17	6Y	F	5.3028	3.7163	5.4633
18	9Y	F	5.3269	6.1875	5.1605

[1] Ratio ≥1.1 is considered positive.

3.1. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

The results of the qRT-PCR were expressed as threshold cycle (Ct) value. Results of qRT-PCR conducted on nasal swabs from the Al-azraq herd were all negative for MERS-CoV in both phases. Results of qRT-PCR conducted on nasal swabs from the Al-ramtha herd for phase 1 are show in (Table 5). In the first collection, all sampled animals were negative on qRT-PCR. In the second collection (day 7), nasal swab samples obtained from animal numbers 1, 2 and 15 were tested positive on upE qRT-PCR, and were negative on ORF1a qRT-PCR. Therefore, these samples were considered negative. As for the third collection (day 14), all collected nasal swabs were positive on upE qRT-PCR, ORF1a qRT-PCR (Ct < 37) and on the specific MERS-CoV spike gene RT-PCR. These positive samples (n = 12) were sent for sequencing to a specialized sequencing facility (Macrogen, South Korea) and 9/12 were successfully sequenced and their results were confirmed to be MERS-CoV. Results of the phylogenetic analysis of partial MERS-CoV spike gene is shown in (Figure 1).

Results of qRT-PCR conducted on nasal swabs from the Al-ramtha herd for phase 2 are shown in (Tables 6 and 7). For the first 7 collections, all sampled animals tested negative on qRT-PCR. As for the 8th collection, camel number 1 tested positive but became negative thereafter (Table 5). Additionally, results of qRT-PCR done on retropharyngeal lymph node tissues for animals slaughtered in the Al-ramtha abattoir were all negative while three nasal swab samples obtained from three slaughtered male juvenile-camels tested positive on qRT-PCR (5m, 9/10/2020; 9m, 2/12/2020; 9m, 2/12/2020). All samples that were confirmed positive on qRT-PCR assay (animal number 1 from Al-ramtha herd and the 3-male juvenile-camels) were subjected to sequencing. Fragment of

Table 2. Results of ELISA test for MERS-CoV of Al-azraq camel herd (Phase 2: August to December, 2020), Jordan.

Camel No	Animal Age (y)	Animal sex	1st collection 31/8/2020 Ratio[1]	2nd collection 14/9/2020 Ratio	3rd collection 28/9/2020 Ratio	4th collection 12/10/2020 Ratio	5th collection 26/10/2020 Ratio
1	4	F	5.48	6.43	4.50	7.04	6.24
2	2	F	1.73	1.26	1.31	1.89	2.51
3	3	F	2.95	2.77	2.40	3.52	3.77
4	3	F	1.00	0.97	1.02	1.24	1.29
5	3	F	2.48	2.78	2.99	4.95	3.83
6	4	F	5.15	4.96	5.19	9.42	6.79
7	4	F	4.91	4.33	5.47	9.53	8.06
8	4	F	5.55	4.18	5.30	9.45	No collection
9	10	M	4.04	5.53	3.96	8.02	5.43
10	10	M	2.40	2.44	3.33	4.84	3.29

[1] Ratio ≥1.1 is considered positive.

Table 3. Results of ELSA test for MERS-CoV of Al-ramtha camel herd (Phase 2: August–December, 2020), Jordan.

Camel No	Animal age	Animal Sex	1st collection 18/8/2020 Ratio[1]	2nd collection 31/8/2020 Ratio	3rd collection 15/9/2020 Ratio	4th collection 29/9/2020 Ratio	5th collection 13/10/2020 Ratio
1	5m	F	0.05	0.02	0.01	0.67	0.03
2	6m	M	0.10	0.11	0.08	0.09	0.01
3	5m	F	0.05	0.09	0.07	0.29	0.06
4	13m	F	0.02	0.01	0.32	0.24	2.89
5	9y	F	2.95	3.57	3.15	0.07	0.09
6	5m	F	0.70	0.62	0.02	0.07	0.04
7	9y	F	4.09	5.41	4.23	0.03	0.13
8	7y	F	0.03	0.17	0.52	0.06	0.33
9	7m	M	0.26	0.21	0.45	0.04	0.35
10	6m	M	0.20	0.14	0.12	0.06	4.77

[1] Ratio ≥1.1 were considered positive.

spike genes of three out of the four positive samples were sequenced (the nasal swab sample of a male juvenile camel slaughtered on 9/10/2020 could not be sequenced due to insufficient amount of RNA). Phylogenetic analysis indicates continued circulation of MERS-CoV clade B in Jordan as shown in (Figure 1).

4. Discussion

Jordan remains an important country to study the dynamics of MERS-CoV since the first human Middle-East respiratory syndrome (MERS) cluster appeared in April 2012, in Al-Zarqa Governorate (Hijawi et al., 2013). This article is a continuation of the two previously published

Table 4. Results of ELSA test for MERS-CoV of Al-ramtha camel herd (Phase 2: August–December, 2020), Jordan.

Camel No	Animal age	Animal sex	6th collection 27/10/2020 Ratio[1]	7th collection 10/11/2020 Ratio	8th collection 19/11/2020 Ratio	9th collection 30/11/2020 Ratio	10th collection 8/12/2020 Ratio
1	5m	F	0.00	0.10	0.21[2]	0.02	4.47
2	6m	M	0.28	0.01	0.01	0.28	4.23
3	5m	F	0.11	0.14	0.16	0.04	4.01
4	13m	F	0.01	0.10	0.02	0.07	4.32
5	9y	F	0.22	0.16	0.07	0.08	4.96
6	5m	F	3.15	0.13	0.18	0.07	4.92
7	9y	F	4.50	0.18	0.11	0.06	4.93
8	7y	F	0.06	0.17	0.24	0.56	4.83
9	7m	M	0.03	0.17	0.23	0.22	4.92
10	6m	M	0.08	0.16	0.21	0.22	4.75

[1] Ratio ≥1.1 were considered positive.
[2] PCR positive.

Table 5. Results of PCR for MERS-CoV for Al-ramtha camel herd (Phase 1: December, 2018–January 2019).

Camel No	Camel Age	Camel Sex	1st collection (20/12/2018) PCR	2nd collection (27/12/2018) PCR	3rd collection (3/1/2019) PCR
1	6M	M	Negative	Negative[1]	Positive
2	8M	M	Negative	Negative[1]	Positive
3	9M	M	Negative	Negative	Positive
4	1Y	M	Negative	Negative	Positive
5	9M	M	Negative	Negative	Positive
6	10M	M	Negative	Negative	Positive
7	7M	M	Negative	Negative	Positive
8	8M	F	Negative	Negative	Positive
9	13M	F	Negative	-	-
10	7M	M	Negative	-	-
11	14M	F	Negative	-	-
12	9M	F	Negative	-	-
13	13M	F	-	Negative	Positive
14	7M	M	-	Negative	Positive
15	14M	F	-	Negative[1]	Positive
16	9M	F	-	Negative	Positive

[1] Samples were tested weak positive with upE real-time RT-PCR, and in ORF1a real-time RT-PCR were negative.

studies on MERS-CoV in camels in Jordan. The first article was published in 2013 (Reusken et al., 2013) and was one of the first papers that investigated the occurrence of MERS-CoV in camels among other livestock animals. All enrolled camels (n = 11) were from Al-Zarqa Governorate and were seropositive for the virus. The study was implemented between June and December 2013. The second study was published in 2017 and reported high incidence of infection with the virus, based on PCR testing, where 42/45 sampled camels tested positive, while only 37/45 samples were seropositive (van Doremalen et al., 2017). Affected camels in the second study were from two different governorates, Al-ramtha and Al-zarqa. The current study was designed to follow up the investigation and further study the dynamics of MERS-CoV transmission and shedding in camels in the two governorates where exposure had been documented. In Al-Zarqa Governorate, the same camel herd was enrolled where the previous incidence of the disease was reported, but in Al-ramtha, different camel herds were enrolled because the herd in phase 2 was slaughtered since camels were raised for meat consumption.

The two governorates were chosen for the study to increase the chance of finding camels with active infection and viral shedding. MERS-CoV in camels is known to be associated with high seropositivity, while no viral RNA is usually detected in seropositive camels. This is in particular reported in studies from the Asian countries (David et al., 2018; Islam et al., 2018). This agrees with the findings of this study in Al-azraq herd in both phases, although one camel seroconverted during the second phase (Tables 1 and 2). It is known that seroconversion is considered an evidence of current and recent infection and one might expect to find camels with viral shedding in the same period. Unfortunately, this was not the case; all sampled camels tested negative on qRT-PCR in both phases. The reason why this individual camel seroconverted while no other camels in the herd showed evidence of active infection remains unknown, although a false positive result cannot be excluded.

The dynamics and behavior of MERS-CoV in the Al-ramtha herds was different and perhaps indicated a recent infection and viral activity. In phase 1, 2 animals were seropositive at the beginning of the study (at first collection) and unfortunately were slaughtered and replaced by other animals (Table 8). These 2 animals might have been exposed to the virus before they were enrolled in the study. However, it is difficult to know the time when they were exposed since the half-life of MERS-CoV antibodies in camels is not well documented. On the other hand, all camels had the viral RNA in their nasal swabs and tested positive on PCR a week later (the third collection, Table 5). It is likely that these 2 seropositive camels (slaughtered) were the source of infection for the herd in phase 1 of the study.

In phase 2, in camels of the Al-ramtha herd, the seroconversion pattern was not steady (Tables 3 and 4) and viral RNA was found in the nasal secretions in one camel on the eighth collection (Ct value = 29.22, Table 7). However, the same camel tested negative afterwards. Given the inability to determine the time onset or the duration of the presence of the virus in the nasal secretion prior and after the sampling time (8th collection), it is not surprising that the result appeared negative on PCR after 11 days (Table 7) and this is in line with a number of previously published studies (Al Hammadi et al., 2015; Wernery et al., 2015; Muhairi et al., 2016; Yusof et al., 2017).

Although Al-Zarqa Governorate was the main focus for the MERS-CoV study and investigations in the past, this study shed light on the Al-ramtha area as being an important focus for viral activity. In both phases, camel herds in Al-ramtha had evidence of viral RNA shedding and seroconversion at the same time which indicates recent exposure and activity of the virus, while this was not evident in the Al-zarqa herd. Furthermore, nasal swabs collected from 3 camel cadavers in the Al-ramtha abattoir had viral RNA detected, and this indicates that the virus is actively circulating in other camel herds in the area. Future studies should focus on camel herds in the Al-ramtha area and perhaps with higher numbers of camel herds and individual camels enrolled.

In the literature, MERS-CoV occurrence in camels doesn't appear to follow a seasonal pattern. When reviewing the previously published studies from Jordan, seropositivity and viral RNA shedding in camels were detected in different months of the year where no clear trend was observed in the different seasons. Studies were done during 10 months of the year, starting from May till February. However, in this study the positive PCR nasal swabs obtained from Al-ramtha herds and from 3

Table 6. Results of PCR testing for MERS-CoV for Al-ramtha camel herd (Phase 2: August–December 2020), Jordan.

Camel No	Animal age	Animal Sex	1st collection 18/8/2020	2nd collection 31/8/2020	3rd collection 15/9/2020	4th collection 29/9/2020	5th collection 13/10/2020
1	5m	F	Negative	Negative	Negative	Negative	Negative
2	6m	M	Negative	Negative	Negative	Negative	Negative
3	5m	F	Negative	Negative	Negative	Negative	Negative
4	13m	F	Negative	Negative	Negative	Negative	Negative
5	9y	F	Negative	Negative	Negative	Negative	Negative
6	5m	F	Negative	Negative	Negative	Negative	Negative
7	9y	F	Negative	Negative	Negative	Negative	Negative
8	7y	F	Negative	Negative	Negative	Negative	Negative
9	7m	M	Negative	Negative	Negative	Negative	Negative
10	6m	M	Negative	Negative	Negative	Negative	Negative

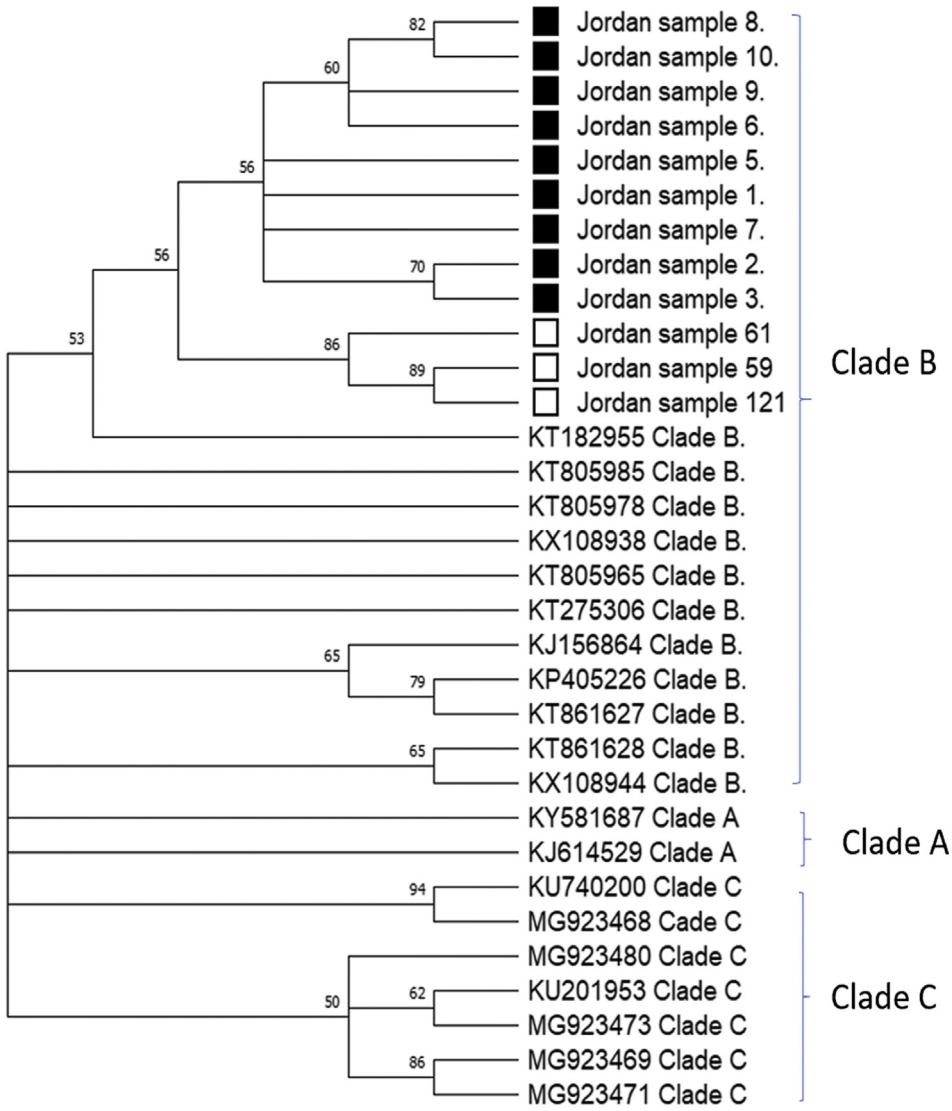

Figure 1. Phylogenetic analysis of the partial spike gene sequence of the Jordanian isolates collected in two cohort longitudinal studies (November 2018–February 2019; 9 samples with solid squares) and (August–December, 2020; 3 samples with open squares) in comparing to reference MERS-CoV sequences representing the excited clades of this virus. All Jordanian samples are within Clade B. The phylogenetic analysis was done using Neighbor-Joining method and Kimura 2-parameter method with bootstrap consensus tree inferred from 500 replicates. The analysis was were conducted in MEGA X.

slaughtered animals occurred in the fall and winter seasons (October to December) when the temperature decreases. Perhaps this is expected since the transmission of the MERS-CoV is direct in nature, does not appear to need a vector and the virus activity, shedding and transmission might increase during cold seasons.

In dromedaries, it is reported that viral shedding lasts for 2 weeks and RNA positivity could persist even for an extended period of time (Hemida et al., 2014; Reusken et al., 2014; Al Hammadi et al., 2015; Wernery et al., 2015; Muhairi et al., 2016; Yusof et al., 2017; Ali et al., 2017). In phase 1 of this study in the Al-ramtha camel herd, it was unfortunate that camels that were positive on PCR testing on the last sampling day could not have been followed for a longer period of time to observe the continuity of the viral shedding and serum antibody titer changes (Table 3), while in phase 2, one camel tested PCR positive in the eighth collection

Table 7. Results of PCR testing for MERS-CoV for Al-ramtha camel herd (August–December, 2020), Jordan.

Camel No	Animal age	Animal sex	6th collection 27/10/2020	7th collection 10/11/2020	8th collection 19/11/2020	9th collection 30/11/2020	10th Collection 8/12/2020
1	5m	F	Negative	Negative	Positive	Negative	Negative
2	6m	M	Negative	Negative	Negative	Negative	Negative
3	5m	F	Negative	Negative	Negative	Negative	Negative
4	13m	F	Negative	Negative	Negative	Negative	Negative
5	9y	F	Negative	Negative	Negative	Negative	Negative
6	5m	F	Negative	Negative	Negative	Negative	Negative
7	9y	F	Negative	Negative	Negative	Negative	Negative
8	7y	F	Negative	Negative	Negative	Negative	Negative
9	7m	M	Negative	Negative	Negative	Negative	Negative
10	6m	M	Negative	Negative	Negative	Negative	Negative

Table 8. Results of ELSA test for MERS-CoV of Al-ramtha camel herd (Phase 1: December, 2018 to January, 2019), Jordan.

Camel No	Camel Age	Camel Sex	1st collection (20/12/2018) ELISA Ratio[1]	2nd collection (27/12/2018) ELISA Ratio	3rd collection (3/1/2019) ELISA Ratio
1	6M	M	0.0865	0.0408	0.1005
2	8M	M	0.0432	0.0357	0.1269
3	9M	M	0.0432	0.0357	0.0952
4	1Y	M	0.0096	0.0510	0.1005
5	9M	M	0.0288	0.0459	0.0264
6	10M	M	0.1153	0.0306	0.0476
7	7M	M	0.2307	0.0000	0.0846
8	8M	F	0.0384	0.0153	0.1587
9	13M	F	2.9134	-	-
10	7M	M	0.0625	-	-
11	14M	F	1.4230	-	-
12	9M	F	0.0048	-	-
13	13 M	F	-	0.0357[2]	0.1216[2]
14	7m	M	-	0.0357[2]	0.1058[2]
15	14m	F	-	0.0051[2]	0.0793[2]
16	7m	F	-	0.0306[2]	0.0740[2]

[1] Ratio ≥1.1 is considered positive.
[2] New animals replaced slaughtered animals of 1st collection.

(Table 8) and the virus was not dedicated 11 days later. Camels in Jordan are of high sale value and the herd owner buys and sells camels all the time and it was extremely hard to keep those camels for a longer period of time without significant compensation for the owner. This fact makes clinical camel research difficult and limits the accessibility to higher numbers of camels which is a prerequisite for well-designed clinical research.

Phylogenetic analysis of a partial fragment of spike 1 gene sequences of all Jordanian MERS-CoV isolates from our studies (seasons 2018/2019 and 2020) in camels clustered together with clade B of MERS-CoV. This cluster contains all MERS-CoV sequences obtained either from camels or humans in the Arabian Peninsula, indicating the continuous circulation of this clade in Jordan (Dighe et al., 2019).

5. Conclusions

Results obtained from this research project revealed that MERS-CoV is present in camels in Jordan. Virus shedding was detected using PCR in nasal swabs. Phylogenetic analysis of a partial fragment of spike 1 gene sequences of all Jordanian MERS-CoV in camels clustered together with clade B of MERS-CoV. This is similar to the sequences obtained either from camels or humans sources in the Arabian Peninsula indicating the continuous circulation of this clade in Jordan. Monitoring and surveillance system for MERS-CoV in camels is essential to be implemented regularly in areas where camel populations are present as well as in camel abattoir and slaughter houses facilities. As most coronaviruses, the risk that the virus if given enough opportunities might change into a form that is highly infectious for humans and spreads easily from person to person.

Declarations

Author contribution statement

Mustafa M. Ababneh: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shawkat Q. Lafi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sameeh M. Abutarbush: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamad S. Khalifeh: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zaidoun S. K. Hijazeen: Conceived and designed the experiments; Performed the experiments.

Wafaa A. Ramadneh, Maisa S. Al Ameer, Fadia Y Abukhalifeh, Tamam A. Kutkut, Rachel A. Dodeen: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Ihab El Masry, Sophie von Dobschuetz: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

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Data availability statement

Data will be made available on request.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Alagaili, A.N., Briese, T., Mishra, N., Kapoor, V., Sameroff, S.C., Burbelo, P.D., de Wit, E., Munster, V.J., Hensley, L.E., Zalmout, I.S., Kapoor, A., Epstein, J.H., Karesh, W.B., Daszak, P., Mohammed, O.B., Lipkin, W.I., 2014. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. mBio 5, e00884–14.

Ali, M.A., Shehata, M.M., Gomaa, M.R., Kandeil, A., El-Shesheny, R., Kayed, A.S., El-Taweel, A.N., Atea, M., Hassan, N., Bagato, O., Moatasim, Y., Mahmoud, S.H., Kutkat, O., Maatouq, A.M., Osman, A., McKenzie, P.P., Webby, R.J., Kayali, G., 2017. Systematic, active surveillance for Middle East respiratory syndrome coronavirus in camels in Egypt. Emerg. Microb. Infect. 6, e1.

Alnaeem, A., Kasem, S., Qasim, I., Al-Doweriej, A., Al-Houfufi, A., Alwazan, A., Albadrani, A., Alshaammari, K., Refaat, M., Al-Shabebi, A., Hemida, M.G., 2020. Some pathological observations on the naturally infected dromedary camels (Camelus dromedarius) with the Middle East respiratory syndrome coronavirus (MERS-CoV) in Saudi Arabia 2018-2019. Vet. Q. 40, 190–197.

Alnaeem, A., Kasem, S., Qasim, I., Refaat, M., Alhufufi, A.N., Al-Doweriej, A., Al-Shabebi, A., Hereba, A.R.T., Hemida, M.G., 2021. Scanning electron microscopic findings on respiratory organs of some naturally infected dromedary camels with the lineage-B of the Middle East respiratory syndrome coronavirus (MERS-CoV) in Saudi arabia-2018. Pathogens 10.

Banerjee, A., Kulcsar, K., Misra, V., Frieman, M., Mossman, K., 2019. Bats and coronaviruses. Viruses 11.

Chan, J.F., To, K.K., Tse, H., Jin, D.Y., Yuen, K.Y., 2013. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. 21, 544–555.

Corman, V.M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., van Boheemen, S., Gopal, R., Ballhause, M., Bestebroer, T.M., Muth, D., Muller, M.A., Drexler, J.F., Zambon, M., Osterhaus, A.D., Fouchier, R.M., Drosten, C., 2012. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 17.

David, D., Rotenberg, D., Khinich, E., Erster, O., Bardenstein, S., van Straten, M., Okba, N.M.A., Raj, S.V., Haagmans, B.L., Miculitzki, M., Davidson, I., 2018. Middle East respiratory syndrome coronavirus specific antibodies in naturally exposed Israeli llamas, alpacas and camels. One Health 5, 65–68.

Dighe, A., Jombart, T., Van Kerkhove, M.D., Ferguson, N., 2019. A systematic review of MERS-CoV seroprevalence and RNA prevalence in dromedary camels: implications for animal vaccination. Epidemics 29, 100350.

Fehr, A.R., Perlman, S., 2015. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol. Biol. 1282, 1–23.

Hammadi, Al, M, Z., Chu, D.K., Eltahir, Y.M., Al Hosani, F., Al Mulla, M., Tarnini, W., Hall, A.J., Perera, R.A., Abdelkhalek, M.M., Peiris, J.S., Al Muhairi, S.S., Poon, L.L., May 2015. 2015. Asymptomatic MERS-CoV infection in humans possibly linked to infected dromedaries imported from Oman to United Arab Emirates. Emerg. Infect. Dis. 21, 2197–2200.

Han, Y., Du, J., Su, H., Zhang, J., Zhu, G., Zhang, S., Wu, Z., Jin, Q., 2019. Identification of diverse bat alphacoronaviruses and betacoronaviruses in China provides New insights into the evolution and origin of coronavirus-related diseases. Front. Microbiol. 10, 1900.

Hemida, M.G., Chu, D.K., Poon, L.L., Perera, R.A., Alhammadi, M.A., Ng, H.Y., Siu, L.Y., Guan, Y., Alnaeem, A., Peiris, M., 2014. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg. Infect. Dis. 20, 1231–1234.

Hemida, M.G., Alnaeem, A., Chu, D.K., Perera, R.A., Chan, S.M., Almathen, F., Yau, E., Ng, B.C., Webby, R.J., Poon, L.L., Peiris, M., 2017. Longitudinal study of Middle East Respiratory Syndrome coronavirus infection in dromedary camel herds in Saudi Arabia, 2014-2015. Emerg. Microb. Infect. 6, e56.

Hijawi, B., Abdallat, M., Sayaydeh, A., Alqasrawi, S., Haddadin, A., Jaarour, N., Alsheikh, S., Alsanouri, T., 2013. Novel coronavirus infections in Jordan, April 2012: epidemiological findings from a retrospective investigation. East. Mediterr. Health J. 1 (19 Suppl), S12–S18.

Islam, A., Epstein, J.H., Rostal, M.K., Islam, S., Rahman, M.Z., Hossain, M.E., Uzzaman, M.S., Munster, V.J., Peiris, M., Flora, M.S., Rahman, M., Daszak, P., 2018. Middle East respiratory syndrome coronavirus antibodies in dromedary camels, Bangladesh, 2015. Emerg. Infect. Dis. 24, 926–928.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

Martin, S. Wayne, Alan, H. Meek, Preben, Willeberg, 1987. Veterinary Epidemiology : Principles and Methods. Iowa State University Press, Ames.

Muhairi, S.A., Hosani, F.A., Eltahir, Y.M., Mulla, M.A., Yusof, M.F., Serhan, W.S., Hashem, F.M., Elsayed, E.A., Marzoug, B.A., Abdelazim, A.S., 2016. Epidemiological investigation of Middle East respiratory syndrome coronavirus in dromedary camel farms linked with human infection in Abu Dhabi Emirate, United Arab Emirates. Virus Gene. 52, 848–854.

Owusu, M., Annan, A., Corman, V.M., Larbi, R., Anti, P., Drexler, J.F., Agbenyega, O., Adu-Sarkodie, Y., Drosten, C., 2014. Human coronaviruses associated with upper respiratory tract infections in three rural areas of Ghana. PLoS One 9, e99782.

Reusken, C.B., Ababneh, M., Raj, V.S., Meyer, B., Eljarah, A., Abutarbush, S., Godeke, G.J., Bestebroer, T.M., Zutt, I., Muller, M.A., Bosch, B.J., Rottier, P.J., Osterhaus, A.D., Drosten, C., Haagmans, B.L., Koopmans, M.P., 2013. Middle East Respiratory Syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan. Euro Surveill. 18, 20662.

Reusken, C.B., Farag, E.A., Jonges, M., Godeke, G.J., El-Sayed, A.M., Pas, S.D., Raj, V.S., Mohran, K.A., Moussa, H.A., Ghobashy, H., Alhajri, F., Ibrahim, A.K., Bosch, B.J., Pasha, S.K., Al-Romaihi, H.E., Al-Thani, M., Al-Marri, S.A., AlHajri, M.M., Haagmans, B.L., Koopmans, M.P., 2014. Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar. Euro Surveill. 19.

van Doremalen, N., Hijazeen, Z.S., Holloway, P., Al Omari, B., McDowell, C., Adney, D., Talafha, H.A., Guitian, J., Steel, J., Amarin, N., Tibbo, M., Abu-Basha, E., Al-Majali, A.M., Munster, V.J., Richt, J.A., 2017. High prevalence of Middle East respiratory coronavirus in young dromedary camels in Jordan. Vector Borne Zoonotic Dis. 17, 155–159.

Wernery, U., Corman, V.M., Wong, E.Y., Tsang, A.K., Muth, D., Lau, S.K., Khazanehdari, K., Zirkel, F., Ali, M., Nagy, P., Juhasz, J., Wernery, R., Joseph, S., Syriac, G., Elizabeth, S.K., Patteril, N.A., Woo, P.C., Drosten, C., 2015. Acute middle East respiratory syndrome coronavirus infection in livestock Dromedaries, Dubai, 2014. Emerg. Infect. Dis. 21, 1019–1022.

Wu, D., Wu, T., Liu, Q., Yang, Z., 2020. The SARS-CoV-2 outbreak: what we know. Int. J. Infect. Dis.

Ye, Z.W., Yuan, S., Yuen, K.S., Fung, S.Y., Chan, C.P., Jin, D.Y., 2020. Zoonotic origins of human coronaviruses. Int. J. Biol. Sci. 16, 1686–1697.

Yusof, M.F., Queen, K., Eltahir, Y.M., Paden, C.R., Al Hammadi, Zmah, Tao, Y., Li, Y., Khalafalla, A.I., Shi, M., Zhang, J., Mohamed, Msae, Abd Elaal Ahmed, M.H., Azeez, I.A., Bensalah, O.K., Eldahab, Z.S., Al Hosani, F.I., Gerber, S.I., Hall, A.J., Tong, S., Al Muhairi, S.S., 2017. Diversity of Middle East respiratory syndrome coronaviruses in 109 dromedary camels based on full-genome sequencing, Abu Dhabi, United Arab Emirates. Emerg. Microb. Infect. 6, e101.

Zhang, A.R., Shi, W.Q., Liu, K., Li, X.L., Liu, M.J., Zhang, W.H., Zhao, G.P., Chen, J.J., Zhang, X.A., Miao, D., Ma, W., Liu, W., Yang, Y., Fang, L.Q., 2021. Epidemiology and evolution of Middle East respiratory syndrome coronavirus, 2012-2020. Infect Dis. Povert. 10, 66.