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Middle East respiratory syndrome coronavirus specific antibodies in naturally exposed Israeli llamas, alpacas and camels

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ABSTRACT

Thus far, no human MERS-CoV infections have been reported from Israel. Evidence for the circulation of MERS-CoV in dromedaries has been reported from almost all the countries of the Middle East, except Israel. Therefore, we aimed to analyze MERS-CoV infection in Israeli camelids, sampled between 2012 and 2017. A total of 411 camels, 102 alpacas and 19 llamas' sera were tested for the presence of antibodies to MERS-CoV. Our findings indicate a lower MERS-CoV seropositivity among Israeli dromedaries than in the surrounding countries, and for the first time naturally infected llamas were identified. In addition, nasal swabs of 661 camels, alpacas and lamas, obtained from January 2015 to December 2017, were tested for the presence of MERS-CoV RNA. All nasal swabs were negative, indicating no evidence for MERS-CoV active circulation in these camelids during that time period.

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV), a lineage 2C-betacoronavirus, was first identified in 2012 [1]. Serologic surveys identified > 90% MERS-CoV–specific antibody seroprevalence in adult dromedary camels (*Camelus dromedarius*) in many countries in the Middle East and Africa. Moreover MERS-CoV viral RNA was detected in the nasal swabs of dromedaries in Qatar, Oman, Saudi Arabia, Egypt and United Arab Emirates (UAE) [2].

Confirmed MERS human cases in Iran, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, Qatar, UAE and Yemen, were epidemiologically linked to camels, indicating camels as a potential source of human infections [3]. Alpacas (*Vicugna pacos*) and llamas (*Lama glama*) are also susceptible to experimental MERS-CoV infections [4–6] but only alpacas were found to be naturally infected until now.

Presently, the highest MERS-CoV prevalence in humans has been documented in Saudi Arabia, as reported by the World Health Organization, whereas no cases have been reported from Israel despite the occurrence human-camel contact.

Approximately 155,000 Bedouins, traditionally a semi-nomadic pastoralist population, inhabit the Negev Desert and breed camels, sheep and goats. The estimated dromedary camel population in Israel is 3000–5000. In addition, the largest herd of alpacas and llamas, outside South America, is in the Negev. Since the virus is circulating in the surrounding countries, we aimed to assess the presence of MERS-CoV specific antibodies and viral RNA among camelids in Israel.

2. Material and methods

2.1. Samples

A total of 411 blood samples from dromedary camels, 102 from alpacas and 19 from llamas were collected during 2012–2017 (Table 1, Fig. 1). The sampled camels were from 20 farms, 18 located in the Negev, one in central Israel and one in the north (Fig. 1). The camels included 37 were males and 374 were females, and their ages ranged from 1 month to 12 years. The alpacas and lamas sampled from one farm located in the Negev. The alpacas include 65 females and 37 males and their ages ranged from 6 month to 15 years. The lamas include 8 females and 11 males and their ages ranged from 2 years to 20 years.

A total of 540 nasal swabs from camels, 102 from alpacas and 19 from llama were collected between January 2015 and November 2017.

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Table 1
Total sera collected between 2012 and 2017, by location and species.

No.	Village	Year of Collection						Total
		2012	2013	2014	2015	2016	2017	
		Camels						
1	Mas'udin Al Azazme	4	5	–	11	16	15	51
2	Segev- Shalom	2	–	–	–		–	2
3	En Habesor	12	10	–	31	20	–	73
4	Aroer	9	–	–	–		–	9
5	Tarabin AS-Sani	4		6	39		19	68
6	Sede Boqer	7	2	–	–	1	–	10
7	Abu Qureinat	4	–	–	12		–	16
8	Abu Rubeia	9	–	–	3	22	27	61
9	Qabboa	–	3	–	–		9	12
10	Rahat	–	–	2	2	18	10	32
11	Azem	–	2	–	1	3		6
12	Revivim	–	–	–	14	14	–	28
13	Hura	–	–	–	1			1
14	Shibli	–	2	–	–		–	2
15	Arara	–	7	–	–			7
16	Mitzpe Ramon					2	–	2
17	Ksifea					1	24	25
18	Tel Sheva						1	1
19	Nokdim					3		3
20	Azuz					2		2
	Total camels	51	31	8	114	102	105	411
Alpaca								
	Mitzpe Ramon					102		
	Total					102		102
Llamas								
	Mitzpe Ramon					19		
	Total					19		19

Camel samples were collected from 486 females and 54 males, aged from 1 month to 19 years. The alpacas and lamas were from one farm in the Negev.

2.2. Virus neutralizing antibodies test (VNT)

The virus neutralizing antibodies test (VNT) is the gold standard assay for the serological diagnosis of the MERS-CoV infection. The camel, alpaca and llama sera were tested at a dilution of 1:20–1:2560 for the presence of neutralizing antibodies to MERS-CoV by the virus neutralization test (VNT) [7]. Briefly, sera were heat-inactivated by incubating for 30 min at 56 °C. Two-fold serial dilutions of sera were prepared in 96-well plates, starting at a 1:10 dilution. Sera reacting from a dilution of 1:20 and up were considered positive. Live MERS-CoV was diluted in Iscove's Modified Dulbecco's Medium (IMDM), supplemented with Penicillin, Streptomycin and 1% FBS, to a dilution of 2000 $TCID_{50}$/ml. Subsequently, 50 µl virus suspensions was added to each well of plates and the plates were incubated at 37 °C for 1 h. Next, virus-serum mixtures were incubated on 96 well plates containing Vero cells for 1 h followed by washing with PBS and incubation with IMDM-1% FBS for 5 days, after which the cytopathic effect was scored.

2.3. ELISA

MERS-CoV antibodies were screened by ELISA (Euroimmun AG, Lubeck, Germany), according to the ELISA manufacturer's instructions. Briefly, diluted serum samples (1100) were incubated in ELISA plate wells, coated with MERS-CoV S1 antigen. Positive, negative and calibrator samples were included. Antibodies were detected by adding peroxidase-labeled rabbit anti–camel IgG. Results were reported as the optical density (OD) ratio, which was calculated as the OD value of the sample divided by the calibrator OD value. We used cutoff values recommended by the ELISA kit manufacturer: a ratio of < 0.8 was considered negative, ≥0.8 to < 1.1 was considered borderline, and ≥1.1 was considered positive [8]. Borderline reacting sera were not included in the comparison.

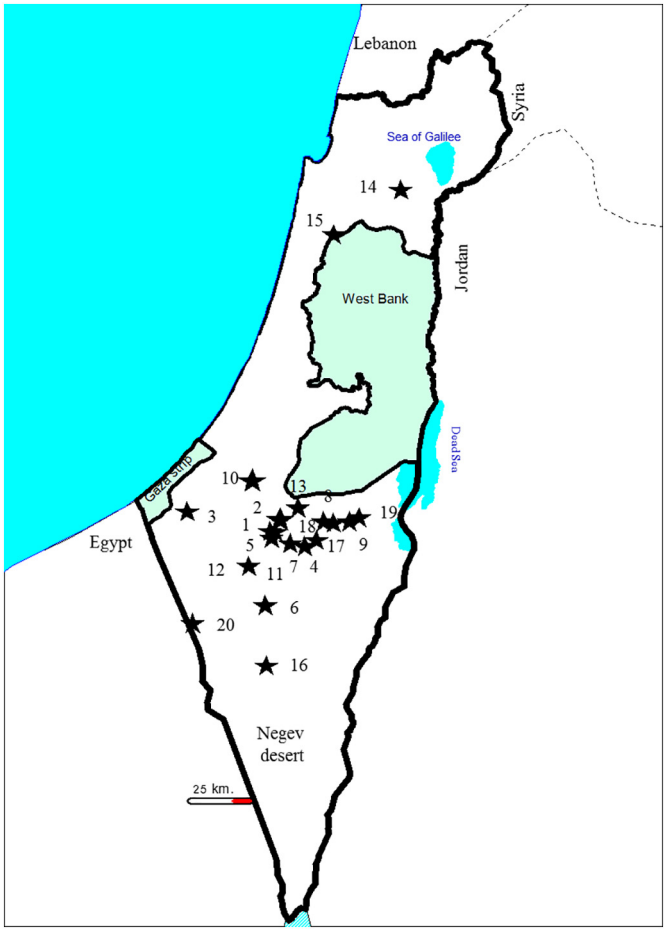

Fig. 1. Locations of camelids sampled for MERS CoV monitoring.

2.4. RNA extraction and detection by real-time RT-PCR

The swab specimens were suspended in 2 ml PBS, incubated for 1 h at room temperature and then clarified by centrifugation at 1000 rpm for 10 min. The supernatants were recovered for extraction and were stored at −80 °C until analysis. Total nucleic acid was extracted from 200 µl swab samples using Invisorb Spin virus RNA mini kit (STRATEC, molecular GmbH, Berlin, Germany), according to the manufacturer's instructions. Extracted RNA was tested for the presence of MERS-CoV RNA by real-time reverse transcription-quantitative polymerase chain reaction (qrt RT-PCR) hydrolysis probe assay using Bio Rad CFX 96 Real Time detection system (Bio Rad, Hercules, CA, USA). The primers and probe encompassed upstream the envelope gene (UpE) [9].

2.5. Statistical method

Serological data were entered into an Excel worksheet and analyzed using Excel functions. Results from Excel were verified using public access statistical software (https://www.medcalc.org/calc/diagnostic_test.php, http://vassarstats.net/kappa.html, http://vassarstats.net/prop1.html

3. Results

MERS-CoV specific antibodies in Israeli camels were analyzed using