



Dromedary camels in northern Mali have high seropositivity to MERS-CoV



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ABSTRACT

A high percentage (up to 90%) of dromedary camels in the Middle East as well as eastern and central Africa have antibodies to Middle East respiratory syndrome coronavirus (MERS-CoV). Here we report comparably high positivity of MERS-CoV antibodies in dromedary camels from northern Mali. This extends the range of MERS-CoV further west in Africa than reported to date and cautions that MERS-CoV should be considered in cases of severe respiratory disease in the region.

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1. Introduction

Dromedary camels (*Camelus dromedarius*) appear to play an important role in the maintenance of MERS-CoV in the environment and its subsequent transmission to humans [1,2]. While it is suspected that MERS-CoV originated in African bats [3], contact with camels is associated with primary human infections. Serological evidence demonstrates that MERS-CoV (or serologically related viruses) have been present in camels in the Middle East and parts of Africa for over 30 years. Studies have found that a high proportion of camels from Egypt, Tunisia, Nigeria, Sudan, Somalia, Ethiopia, Kenya, United Arab Emirates, Saudi Arabia, Oman, Qatar and Jordan [4–9] have antibodies against MERS-CoV. This suggests that widespread exposure to MERS-CoV among camels has been ongoing for an extended period of time. In contrast, serum from

other animals that are common in the Middle East including sheep, goats, cattle and horses has not been found to contain antibodies that are reactive to MERS-CoV [7,8]. Infectious virus has also been isolated from nasal swabs of camels in some of these regions [10,11].

Mali is one of the largest producers of camel milk in the world, and concomitantly has a large population of camels – estimated at just over 1 million [12]. While MERS-CoV seropositivity has been well established in the Middle East and eastern and central Africa, there are few data on camels in western Africa, with the exception of Nigeria. To determine whether Malian camels have been exposed to MERS-CoV, serum from camels in northern and central Mali was assayed for reactivity to MERS-CoV.

2. Materials and methods

Serum from 562 dromedary camels from 16 different locations in Kidal region (north-eastern Mali) and from 9 dromedary camels on a single farm in Nara (central Mali) were collected as part of a survey for peste des petits ruminants virus between November 2009 and February 2010 according to local regulations. Samples were stored at –25 °C at Laboratoire Central Vétérinaire (LCV) in Bamako, Mali. For

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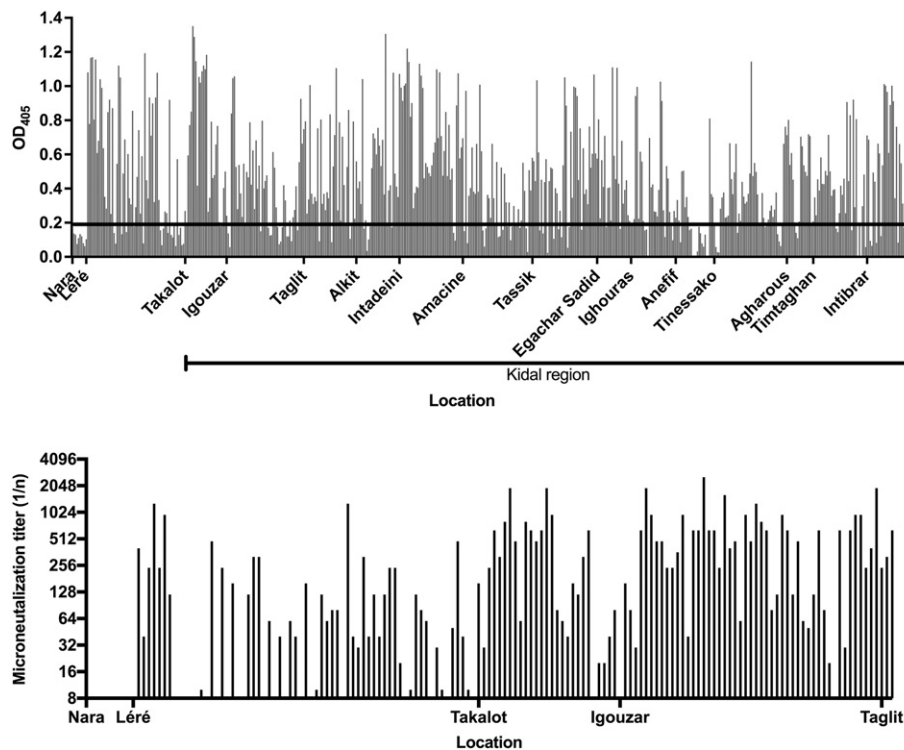


Fig. 1. Antibodies against MERS-CoV in dromedary camels from Mali. (A) Optical density at a reciprocal dilution of 1600 in the MERS-CoV spike (S1) ELISA. The cut-off value is indicated by the black bar. Locations of the camels sampled is indicated below. (B) Neutralizing titers (\log_2) from a microneutralization assay against MERS-CoV/EMC/2012.

the MERS spike protein (S1) ELISA, serum obtained from LCV was diluted 1:1 in 0.2% Triton X – 100 (Sigma), heat inactivated for 15 min. at 65 °C and subsequently stored at –20 °C. NUNC MaxiSorp plates were coated with recombinant S1 antigen (1 µg/ml) (Sino Biological) in PBS overnight at 4 °C. Unbound antigen was removed and plates were blocked with blocking solution (PBS + 5% non-fat skim milk, 0.05% Tween20). Serum was diluted in blocking solution and added to the plates for 1 h at 37 °C. Plates were washed with PBS + 0.05% Tween20. A rabbit anti-llama IgG (H + L) HRP conjugated antibody (AgriSera) was used as the secondary antibody at 1:2000 in blocking buffer and incubated for 1 h at 37 °C. Plates were washed, ABTS Peroxidase substrate (KPL) was added and plates were incubated in the dark for 30 min. Plates were subsequently read at 405 nm. It has been determined that this assay does not cross-react with antibodies to bovine coronavirus, OC43 or SARS-CoV.

Serum for microneutralization assays was aliquoted in Mali and imported into the United States via the USDA (Plum Island, NY) where it was inactivated by gamma irradiation. 100 TCID₅₀ of MERS-CoV were added to two-fold dilutions of serum and incubated for 1 h at 37 °C. The resulting mixture was added to Vero cells and incubated for 5 days when wells were scored for cytopathic effect. The virus neutralization titer was expressed as the reciprocal value of the highest dilution of serum that inhibits virus replication.

3. Results

Camels from Nara showed no reactivity at all dilutions in the MERS-CoV S1 ELISA and as such were used to set up cut-off values for all other

samples (Figs. 1A, 2). Subsequent analysis of neutralizing activity of the sera from the Nara camels revealed no neutralization titers in a microneutralization assay, further supporting their lack of reactivity (Fig. 1B). At the lowest dilution tested in the MERS-CoV S1 ELISA (1:400), nearly all samples assayed (88%) were positive for antibodies that react to S1. While the positivity decreased with increasing dilution, 70% and 67% of samples respectively were positive at 1:1600 and 1:6400 (Table 1). Even at a dilution of 1:25,600, 24% of samples remained positive. There was no variation in seropositivity between different sampling locations in Kidal province (Fig. 1A). In terms of seropositivity and approximate endpoint dilution, there were no notable differences when animals were categorized by age or sex. When camels were grouped by age (1–2, 3–8 and 9–16 years) neither the seropositivity rate (83, 91, 88%) nor the endpoint dilution were significantly different. Similarly, the seropositivity rate in male ($n = 245$) and female ($n = 328$) camels of 86 and 92% respectively was comparable. A small number ($n = 10$) of serum samples from Malian cattle and sheep were also tested in the S1 ELISA but consistent with reports from other countries [8,13] were negative. To confirm data from the S1 ELISA, as subset samples ($n = 147$) were assayed for neutralizing antibodies against MERS-CoV in a microneutralization assay. Of the animals tested, 78% had neutralizing antibodies with a reciprocal titer ≥ 20 , with titers up to 1920 (Fig. 2B).

4. Discussion

The frequency of camels that are positive in the S1 ELISA and have neutralizing antibodies suggests that the majority of camels in

Table 1
Seropositivity of MERS-CoV in dromedary camels in Mali. The percent positive of serum samples at the indicated reciprocal dilution from dromedary camels by ELISA against recombinant MERS-CoV spike protein (number of positive animals over number of samples tested) is shown.

Reciprocal dilution	400	1600	6400	25,600
Seropositivity	88% (502/570)	70% (390/557)	67% (375/557)	24% (136/557)



Fig. 2. A map of Mali indicating the locations where camels were sampled. Samples were obtained from a single farm in the town of Nara, multiple locations in the village of Léré and from various locations including villages, encampments and water points across the region of Kidal (shaded in grey). The number of positive samples over the number of samples collected and percent positivity is shown for each location.

northeastern Mali have been exposed to MERS-CoV or a MERS-CoV-like virus. The camels in Kidal province are used for transport and frequently cover vast distances, interact with other animals, and are actively traded. A subset of these samples was from military camels that provide transport over most of the northern region of Mali. Camels from the Kidal region are also frequently used for transportation to trading posts in Algeria, highlighting the need for further studies in neighbouring countries. In contrast, the dromedary camels in Nara were used exclusively on a farm and it is assumed that they did not leave the farm.

Based on the currently available data, camels in the Middle East and in every country in Africa where sampling has been performed have high rates of exposure to MERS-CoV or MERS-CoV-like viruses. So far only studies of camels in Kenya, Tunisia and the Canary Islands have reported less than half of the camels in the study positive [5,6,14]. Only dromedary camels from central Asia (Kazakhstan) [15] and Australia [16] appear to not have evidence of exposure to MERS-CoV in samples tested to date.

The data presented in this study show that Malian camels have comparable levels of seropositivity to Middle Eastern and east African countries. Thus it seems likely that most regions of Africa with large camel populations that are not geographically isolated will have been exposed to MERS-CoV. This is remarkable given that there has yet to be a confirmed locally acquired human MERS-CoV case identified in Africa, likely due to lack of diagnostic resources and previous lack of general awareness. It is also possible that transmission requires some environmental or activity-based conditions that are more prevalent in the Middle East, especially Saudi Arabia, than in Africa. A serosurvey of human populations in African countries with high numbers of camels would be valuable, as would heightened surveillance for undiagnosed severe respiratory disease. Additionally, sampling of camels for isolation and characterization of viruses would be useful to determine the similarity of viruses circulating in Mali compared to the viruses from the Middle East.

5. Conclusions

Dromedary camels in northern Mali have been exposed to MERS-CoV or a MERS-CoV-like virus, with nearly 90% of animals having reactive antibodies. This expands the distribution of MERS-CoV westward, and when combined with data from others suggests that most regions of Africa with camels likely have a high frequency of exposure to MERS-CoV.

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