

Biphasic MERS-CoV Incidence in Nomadic Dromedaries with Putative Transmission to Humans, Kenya, 2022–2023

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Middle East respiratory syndrome coronavirus (MERS-CoV) is endemic in dromedaries in Africa, but camel-to-human transmission is limited. Sustained 12-month sampling of dromedaries in a Kenya abattoir hub showed biphasic MERS-CoV incidence; peak detections occurred in October 2022 and February 2023. Dromedary-exposed abattoir workers (7/48) had serologic signs of previous MERS-CoV exposure.

Middle East respiratory syndrome coronavirus (MERS-CoV) is endemic in dromedary camels from the Arabian Peninsula and Africa; seroprevalence is >75% (1–3). Zoonotic transmission to humans has occurred sporadically, mainly on the Arabian Peninsula; >2,400 MERS cases and >800 deaths have occurred (4). Despite Kenya being a major camel-breeding country, only 3 potentially autochthonous camel-exposed humans with subclinical MERS-CoV infections were identified in 2019 (5). The apparent regional epidemiologic differences might be linked to factors such as limited diagnostics, local risk factors

(e.g., human comorbidities, camel herding practices, seasonality), or MERS-CoV strain-specific features (6).

In farmed dromedary camels, MERS-CoV outbreaks were associated with annually synchronized camel parturition (7). In particular, camel calves tested MERS-CoV RNA-positive upon the loss of maternal antibodies 4–6 months after birth. Because of seasonality and changing food availability, most camels in Africa are nomadic and have variable population density. High population density is correlated with MERS-CoV seropositivity in camels in Kenya (1), but detailed insights into MERS-CoV circulation are missing.

Field studies on nomadic camels are hampered by limited infrastructure in remote and resource-restricted regions (8). However, nomadic camels are regularly transported to abattoir hubs, enabling sustained daily testing. We performed a continuous 12-month study at an abattoir hub in northern Kenya to investigate MERS-CoV incidence in nomadic camels and explore potential transmission to slaughterhouse workers.

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The Study

Our sampling site was an abattoir hub in Isiolo, northern Kenya, where camels from Marsabit, Samburu, and Isiolo counties are slaughtered (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/30/3/23-1488-App1.pdf>). During September 2022–September 2023, we took samples from 10–15 dromedary camels 4–5 days per week (Appendix). The camels (n = 2,711) were originally from 12 different administrative wards, mainly from Laisamis in Marsabit County (n = 1,841, 67.9%) and Burat in Isiolo County (n = 578, 21.3%) (Table; Appendix Figure 1).

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Table. Overview of camel samples and MERS-CoV RNA positivity in study of MERS-CoV incidence in nomadic dromedaries with putative transmission to humans, Kenya, 2022–2023*

Region Isiolo	MERS-CoV RNA-positive samples/ total samples (%)	Town of origin	MERS-CoV RNA-positive samples/total samples (%)	
			Female camels	Male camels
	15/859 (1.75)	Burat	1/252 (0.4)	10/326 (3.1)
		Bulla Pesa	0/2	0/0
		Kinna	0/13	1/19 (5.3)
		Oldo/Nyiro	0/2	2/23 (8.7)
		Garbatulla	0/1	0/1
		Ngare Mara	0/106	0/114
Marsabit	26/1,846 (1.41)	Laisamis	1/250 (0.4)	21/1,591 (1.3)
		Marsabit Central	0/0	0/1
		Sololo	0/1	0/0
		North Horr	0/0	0/2
		not defined	0/0	0/1
Samburu	0/6 (0)	Wamba East	0/0	0/6
Total	36/2,711 (1.3)		2/627 (0.3)	34/2,084 (1.6)

*MERS-CoV, Middle East respiratory syndrome coronavirus.

MERS-CoV RNA was detected in 36/2,711 (1.3%) (Table; Figure 1) camels using quantitative reverse transcription PCR, which amplifies the upstream of the envelope E gene, and confirmed by open reading frame (ORF) 1ab quantitative reverse transcription PCR or sequencing (Appendix). The cumulative RNA positivity rate was higher in September–October 2022 at 19/381 (5.0%) compared with 17/727 (2.3%) in January–March 2023 (Figure 1). Incidence was biphasic, showing detection peaks in the first weeks of October 2022 (7/60, 11.7%) and February 2023 (7/58, 12.1%) (Figure 1, panel B). For 9/36 MERS-CoV-positive samples, we obtained ORF1ab sequences and performed phylogenetic analysis. The 9 ORF1ab sequences were highly similar (>99.93% nucleotide identity) and had 99.75%–99.78% nucleotide identity with the closest MERS-CoV relative

identified in Akaki, Ethiopia, in 2019 (9). Phylogenetic analysis showed that the 9 sequences clustered as a monophyletic group within clade C2.2, which encompasses East Africa strains initially detected in Kenya in 2018 (10) (Appendix Figure 2). Those sequences represent 3 putative MERS-CoV outbreaks occurring contemporarily in camels in Kenya (Appendix Table 1).

To test whether biphasic MERS-CoV RNA-positivity is accompanied by increased MERS-CoV IgG levels, we tested randomized camel serum samples ($n = 369/2,711$) by MERS-CoV S1 ELISA (Appendix). MERS-CoV IgG levels showed a median optical density ratio (ODR) of 2.14 (95% CI 0.59–3.48) and a seroprevalence of 80.76% (298/369) (Appendix Figure 3, panel A). Lowest IgG levels were identified in June (median ODR 1.28, 95% CI 0.20–3.31),

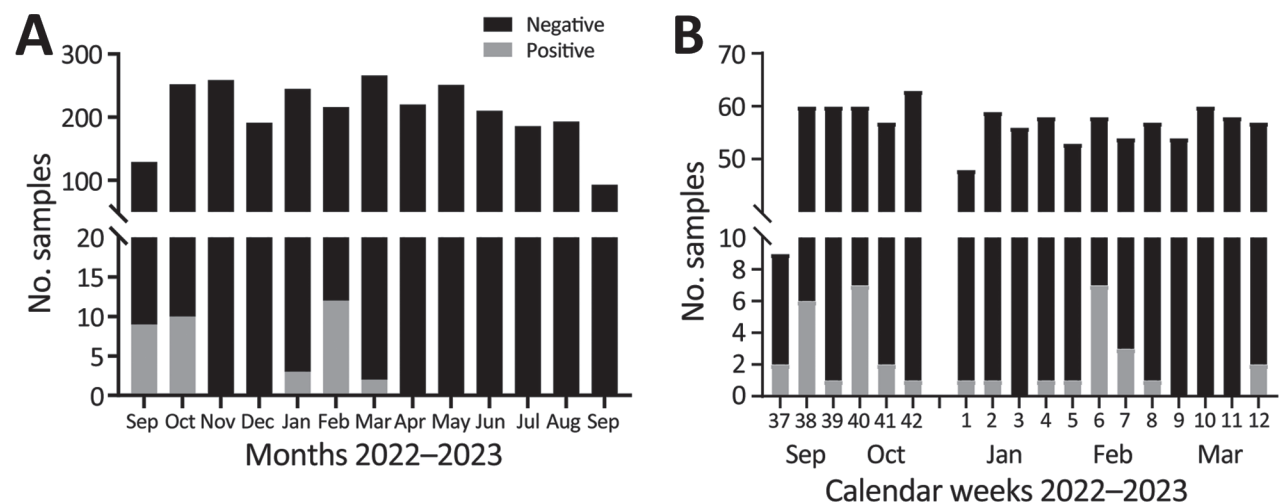


Figure 1. Biphasic Middle East respiratory syndrome coronavirus (MERS-CoV) incidence in dromedaries sampled in an abattoir hub, northern Kenya, 2022–2023. A) MERS-CoV RNA detection rates in nasal swab specimens from dromedary camels tested by MERS-CoV upE quantitative reverse transcription PCR. Continuous 12-month sampling (4–5 days per week) took place in Isiolo abattoir from mid-September 2022 to mid-September 2023. Sampling was suspended for 1 week in December 2022 and 1 week in July 2023. B) Detailed weekly overview of MERS-CoV RNA detections, peaking in October 2022 and February 2023.

whereas the highest levels were seen in March (median ODR 2.72, 95% CI 1.67–3.76). MERS-CoV IgG levels were negatively associated with RNA-positivity (odds ratio [OR] 0.20, 95% CI 0.09–0.44; $p < 0.0001$) (Appendix Figure 3, panel B). RNA-positivity was negatively associated with the season (dry vs. wet, OR 0.14, 95% CI 0.06–0.30; $p < 0.0001$). Male camels were more likely to be RNA positive (OR 3.94, 95% CI 0.86–29.2; $p = 0.11$) and less likely to be seropositive (OR 0.27, 95% CI 0.08–0.77; $p = 0.021$) than were female camels. Older animals (>3 years of age) were more likely to be seropositive (86%) than were animals ≤ 3 years of age (72%), but this difference was not statistically significant.

Seroepidemiologic studies have suggested that abattoir workers in contact with dromedaries are at increased risk for MERS-CoV exposure (11). Seroconversion of subclinical MERS cases might be missed when diagnostically implemented ELISA cutoffs of commercial kits (e.g., ODR = 1.1 for IgG positives) are applied (11,12). We identified MERS-CoV S1 IgG reactivity (ODR >0.2) in 7/48 (14.6%) of Isiolo abattoir workers (Figure 2, panel A). We excluded SARS-CoV-2 infection- or vaccine-induced antibody cross-reactivity with MERS-CoV S1 by comparison of ELISA ODRs of MERS-CoV S1-based with SARS-CoV-2 S1-based ELISA (Appendix Table 2, Figure 4). A control cohort ($n = 12$) with no history of camel exposure showed no MERS-CoV S1 IgG reactivity

(0/12; 0%) despite high SARS-CoV-2 S1 IgG levels (11/12; 92%) (Appendix Table 2).

Neutralization tests (NT) based on GFP-encoding vesicular stomatitis virus pseudoparticles (VSVpp) carrying the MERS-CoV S protein from clade A EMC/2012 or clade C2.2 (Kenya) showed that 1/7 serum samples (1:20 dilution) had a VSVpp-NT 50% reduction of foci-forming units for EMC/2012 and a 90% reduction for Kenya VSVpp-S (Figure 2, panel B). A MERS-CoV EMC/2012-based plaque-reduction neutralization test (PRNT) showed a 50% PRNT at the 1:20 dilution, fulfilling the World Health Organization criteria for a confirmed MERS-CoV seroconversion. None of 6 selected MERS-CoV S1 ELISA-negative abattoir samples showed neutralizing capacity when tested by VSVpp-NT and PRNT (Appendix Table 2).

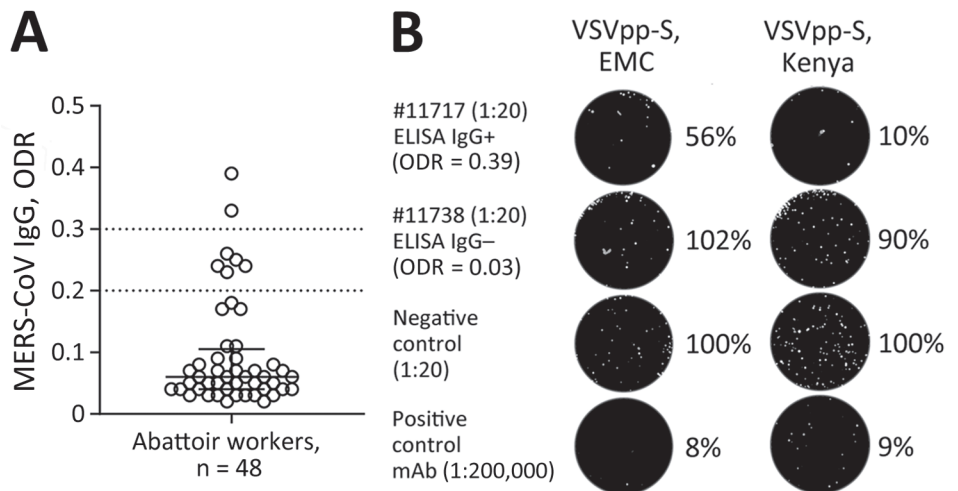
Conclusions

Our sustained sampling of dromedary camels showed a biphasic MERS-CoV incidence in northern Kenya not observed in previous studies (1,10,13). One explanation might be the short time of virus excretion in MERS-CoV-infected dromedaries (14), making viral RNA detection difficult without daily surveillance. Phylogenetic analysis suggests that we identified ≥ 3 MERS-CoV clusters over 3 different weeks in dromedaries originating from different wards. The first potential factor likely influencing the outbreaks is increased animal-to-animal interactions, because

Figure 2. MERS-CoV immune responses in camel-exposed abattoir workers in Isiolo, Kenya.

A) Results of commercial MERS-CoV S1-protein ELISA to detect IgG responses in 48 serum samples (diluted 1:100) from Isiolo abattoir workers. Samples with ODR >0.2 were considered ELISA-reactive, suggesting that 7/48 persons had MERS-CoV-reactive IgGs. Of note, all persons tested negative by MERS-CoV quantitative reverse transcription PCR. B) GFP-VSVpp-MERS-CoV S protein-based neutralization test (VSVpp-NT). VSVpp-S (EMC) and VSVpp-S (Kenya) contained human codon-optimized Spikes

from prototypic MERS-CoV EMC/2012 clade A and Kenya clade C2.2 (#L00009980). All 7 ELISA-reactive human serum samples were mixed with 200 foci-forming units VSVpp in final serum dilutions 1:20–1:160. Out of the 7 ELISA-reactive persons, 1 showed a VSVpp-NT 50% foci-forming units reduction titer of 1:20 (EMC) and 1:40 (Kenya). The picture shows an example of the 1:20 dilution of an ELISA-reactive (#11717) and ELISA-nonreactive (#11738) abattoir worker. Negative control = ELISA-negative human serum (1:20) was used as reference and set to 100%. Positive control = monoclonal anti-MERS-CoV Spike receptor-binding domain binding antibody (mAb 7.7G6) previously shown to neutralize MERS-CoV at the tested dilution ($1:2 \times 10^5$). For better graphical visibility, all pictures were enhanced in contrast and brightness identically. mAb, monoclonal antibody; MERS-CoV, Middle East respiratory syndrome coronavirus; ODR, optical density ratio; VSVpp, vesicular stomatitis virus pseudoparticles.



camels from different herds are transported to Isiolo and kept in holding pens together before slaughter, which could enhance MERS-CoV outbreaks. Second, increased interactions between immunologically naive and infected animals during transport and in holding pens increases the probability of transmitting MERS-CoV. That hypothesis is supported by the high percentage of IgG-negative adult camels (19.24%, ODR<0.3) (1,7). Although identifying the exact MERS-CoV transmission scenario between camels is logistically difficult, rapid point-of-care tests might help trace infections even in resource-limited conditions.

The overall biphasic MERS-CoV incidence might be linked to seasonal factors, such as the biannual alternating wet and dry seasons in northern Kenya. During dry seasons, herds congregate using limited forage, then migrate back to the point of origin in wet seasons. Because calves are mainly born during the 2 wet seasons, the loss of protection by maternal antibodies coincides with the dry seasons. Of note, the 2 dry seasons during July–October 2022 and January–February 2023 matched the peaks of MERS-CoV RNA-positivity in October 2022 and February 2023. The combination of immunologically naive, possibly infected camel calves and the dry season-specific increased population density and probability of contact at limited waterholes might encourage MERS-CoV infections and transmissions among camels.

We identified 7/48 abattoir workers with putative MERS-CoV exposure or past subclinical infection by implementing ELISA ODR cutoffs previously shown to be suitable for seroepidemiologic studies outside clinical settings. In 1/7 cases, we confirmed MERS-CoV neutralizing antibodies by VSVpp-based NT and PRNT. None of the abattoir workers experienced severe symptoms in recent years, supporting the hypothesis that clade C strains might have limited pathogenicity and transmissibility (15). Identifying defined factors that drive MERS-CoV outbreaks will assist in predictive epidemiology, risk assessment, and timely precautionary interventions for public and occupational health.

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M.A.M and V.M.C. are named on patents regarding SARS-CoV-2 serologic testing and monoclonal antibodies.

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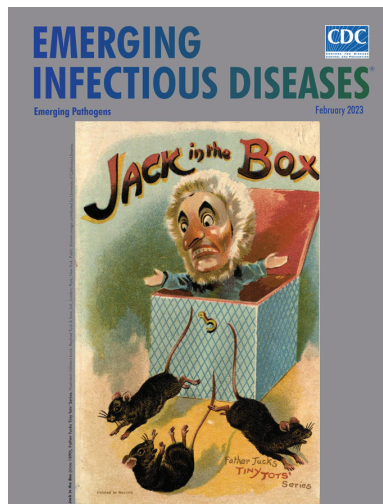
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February 2023

Emerging Pathogens

- Sentinel Surveillance System Implementation and Evaluation for SARS-CoV-2 Genomic Data, Washington, USA, 2020–2021
- Crimean-Congo Hemorrhagic Fever, Spain, 2013–2021
- *Streptococcus dysgalactiae* Bloodstream Infections, Norway, 1999–2021
- Changing Disease Course of Crimean-Congo Hemorrhagic Fever in Children, Turkey
- Relationship between Telework Experience and Presenteeism during COVID-19 Pandemic, United States, March–November 2020
- Circovirus Hepatitis Infection in Heart-Lung Transplant Patient, France
- Incidence and Transmission Dynamics of *Bordetella pertussis* Infection in Rural and Urban Communities, South Africa, 2016–2018
- Influence of Landscape Patterns on Exposure to Lassa Fever Virus, Guinea
- Increased Multidrug-Resistant *Salmonella enterica* I Serotype 4,[5],12:i:- Infections Associated with Pork, United States, 2009–2018
- Novel Prion Strain as Cause of Chronic Wasting Disease in a Moose, Finland
- Novel Species of *Brucella* Causing Human Brucellosis, French Guiana



- Penicillin and Cefotaxime Resistance of Quinolone-Resistant *Neisseria meningitidis* Clonal Complex 4821, Shanghai, China, 1965–2020
- Combined Phylogeographic Analyses and Epidemiologic Contact Tracing to Characterize Atypically Pathogenic Avian Influenza (H3N1) Epidemic, Belgium, 2019
- Age-Stratified Model to Assess Health Outcomes of COVID-19 Vaccination Strategies, Ghana
- Neoehrlichiosis in Symptomatic Immunocompetent Child, South Africa

- Early Introduction and Community Transmission of SARS-CoV-2 Omicron Variant, New York, New York, USA
- Correlates of Protection, Thresholds of Protection, and Immunobridging among Persons with SARS-CoV-2 Infection
- Longitudinal Analysis of Electronic Health Information to Identify Possible COVID-19 Sequelae
- Nipah Virus Exposure in Domestic and Peridomestic Animals Living in Human Outbreak Sites, Bangladesh, 2013–2015
- (Mis)perception and Use of Unsterile Water in Home Medical Devices, PN View 360+ Survey, United States, August 2021
- Molecular Detection of *Candidatus Orientia chuto* in Wildlife, Saudi Arabia
- Powassan Virus Lineage I in Field-Collected *Dermacentor variabilis* Ticks, New York, USA
- *Bartonella* spp. and Typhus Group Rickettsiae among Persons Experiencing Homelessness, São Paulo, Brazil
- *Candida auris* Discovery through Community Wastewater Surveillance during Healthcare Outbreak, Nevada, USA, 2022
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Biphasic MERS-CoV Incidence in Nomadic Dromedaries with Putative Transmission to Humans, Kenya, 2022–2023

Appendix

Material and Methods

Four-to-five days per week, 10–15 dromedary camels were sampled post-mortem from mid-September 2022 to mid-September 2023 (total $n = 2,711$). The majority of sampled camels were male (77%, 2,084/2,711) with a median of 3.5 years (range 2–8 years). The median age of females was 6 years (range 2–9 years). The post-mortem swabbing was performed on the caudal turbinate of the nose using Copan FLOQSwabs (Mast Diagnostica GmbH, Reinfeld, Germany), after making a transverse incision above the nostrils to avoid contaminations from the lower part of the nose. For serologic analyses, blood samples were obtained by jugular vein puncture.

A cross-sectional sampling of camel workers ($n = 48$) at the Isiolo abattoir was conducted in February 2023. Oropharyngeal sampling of humans was also done using Copan FLOQSwabs. For serologic testing, 5 mL blood were drawn in VACUETTE® gold cap tubes (Greiner Bio-One GmbH, Kremsmünster, Austria). In addition, a control cohort ($n = 12$) with no known camel contact was recruited at the University of Nairobi. Ethical approval for the collection of camel and human samples was granted by the Kenyatta National Hospital Ethics and Research Committee (protocol number P534/08/2020), and the Kenya National Commission of Science and Technology (NACOSTI) no. P/22/21987.

RNA extraction from camel and human samples was done using a MagnaPure 96-well plate nucleic acid extraction kit (Roche, Penzberg, Germany). MERS-CoV RNA was detected

and quantified by MERS-CoV upE RT-qPCR assay, as previously described (1). All samples were additionally confirmed by the MERS-CoV ORF1a RT-qPCR assay or sequencing (2). For 9 samples with MERS-CoV RNA concentrations $>1 \times 10^6$ genome copies/mL, MERS-CoV partial genomes were generated by Illumina next-generation sequencing (NGS). Sequence assembly was done by mapping the reads to the reference MERS-CoV EMC/2012 strain (GenBank accession No. NC_019843.3) using Geneious (version 2023.2.1), Multiple Alignment using Fast Fourier Transform (MAFFT) for sequence alignment, and a Tamura-Nei, neighbor-joining model for phylogenetic inference. All 9 Orf1ab sequences were uploaded to NCBI (GenBank no. OR742168–75).

MERS-CoV-specific IgGs from dromedary and human sera were detected by a commercial MERS-CoV S1 enzyme-linked immunosorbent assay (ELISA, EUROIMMUN AG, Lübeck, Germany), as described previously (3–6). Serum samples were applied at a 1:100 dilution previously shown to be optimal for screening purposes (4). The assay-specific cutoff ranges (OD ratio = 0.2–0.3 for humans, OD ratio = 0.3 for camels) and the MERS-CoV seropositivity rating (stage 1 = ELISA-reactive; stage 2 = ELISA-reactive + virus neutralization test $\geq 1:20$) were chosen based on our previous seroepidemiological surveys (4,7). SARS-CoV-2-specific IgGs from human sera (abattoir workers and control cohort) were detected by a commercial SARS-CoV-2 S1 ELISA (EUROIMMUN AG, Lübeck, Germany) according to manufacturer's instruction using a 1:100 dilution and an OD ratio = 1.1 as cutoff.

For camel sample randomization, 25 camel samples from each month were selected to be representative of the complete sample set after stratification using the *strata* function in R. All MERS-CoV RNA-positive samples were not included in the randomization but were also tested by ELISA.

Human codon-optimized MERS-CoV Spike sequences of clade A EMC/2012 and clade C2.2 Kenya No. L00009980, lacking the last C-terminal 19 aa that contain an ER retention signal (8), were cloned into pCG1 vector using In-Fusion® Snap Assembly (TAKARA, Japan). Spike-carrying Vesicular Stomatitis Virus-pseudotyped particles (VSVpp) with a GFP reporter were generated in HEK293T cells, as previously described (9). Diluted sera (1:20–1:160) or the positive control mAb 7.7G6 (1:20,000–1:400,000) (8), respectively, were tested in triplicates and mixed with 200 foci-forming units (FFUs) of VSVpp in 96 well plate, and incubated at 37°C for

30 minutes. Following incubation, Vero B4 suspension cells (2.5×10^4 cells/well) were added to the plate and incubated at 37°C for 16 hours. Following the final incubation, culture medium was aspirated, and the cells were fixed with ROTI®Histofix (Carl Roth) for 15 minutes succeeded by 5 minutes of staining with DAPI (0.3 µg/mL, Merck) for cell confluency control. The resulting FFUs were imaged using the Autoimmun Diagnostika V Spot Spectrum, with an exposure time of 1 sec for GFP and 0.2 sec for DAPI at high resolution, and FFUs with intensity of ≥ 80 were counted. The same instrumental settings were used for the automatically processed and documented 96-well plates.

MERS-CoV plaque reduction neutralization test (PRNT) was performed following a previously described protocol (10), but using 500 PFU/mL of a recombinant MERS-CoV EMC/2012 strain stock (11) in a 24-well plates seeded with 3.5×10^5 cells/well 1 day before use. Serum samples were first inactivated at 56°C for 30 minutes and dilutions 1:10–1:40 were tested in duplicates. Serum-virus 1:1 dilution mixtures were incubated for 1 hour at 37°C before being applied onto VeroB4 cell monolayers. As a positive control, the same mAb against MERS-CoV Spike (mAb 7.7G6) was included in a dilution series of 1:100,000–1:400,000 (12). Assayed supernatants were discarded after 1 h, DMEM (Lonza, Switzerland) supplemented with 1.2% Avicel (Sigma-Aldrich, USA) was added and the cultures were incubated for 4 days at 37°C and 5% CO₂. Cells were fixed with 6% paraformaldehyde and stained with 1% crystal violet (Carl Roth, Germany). The PRNT titer was calculated based on a 50% (PRNT50) reduction in infected cells counts.

Graphing was done using GraphPad prism version 9.5.1, while statistical analysis was done on GraphPad and R software (R Studio Version 2022.12.0).

Appendix Table 1. Overview and details of MERS-CoV RNA-positive camels*

Camel ID	Sampling date	County of origin	Place of origin	Sex	MERS-CoV RNA	OD ratio	Proposed subclusters according to phylogeny
					concentration [copies/mL]	IgG ELISA	
L00009821	16.09.2022	Marsabit	Laisamis	M	1.63×10^8	NS	I
L00009820	16.09.2022	Marsabit	Laisamis	M	6.91×10^4	NS	NA
L00009375	19.09.2022	Isiolo	Burat	M	6.41×10^3	4.24	NA
L00009402	20.09.2022	Isiolo	Burat	M	1.02×10^4	3.91	NA
L00009403	20.09.2022	Isiolo	Burat	F	1.87×10^6	0.3	I
L00009560	21.09.2022	Isiolo	Burat	M	8.5×10^2	NS	NA
L00009558	21.09.2022	Marsabit	Laisamis	M	1.3×10^7	3.11	I
L00009662	23.09.2022	Isiolo	Oldo/Nyiro	M	4.34×10^5	0.06	NA
L00009804	29.09.2022	Isiolo	Oldo/Nyiro	M	2.29×10^3	0.06	NA
L00009954	03.10.2022	Marsabit	Laisamis	M	3.15×10^6	0.18	II
L00009966	04.10.2022	Isiolo	Burat	M	5.88×10^8	NS	II
L00009967	04.10.2022	Isiolo	Burat	M	1.82×10^3	0.21	NA
L00009989	06.10.2022	Isiolo	Burat	M	6.08×10^2	0.04	NA
L00009980	06.10.2022	Marsabit	Laisamis	M	7.4×10^7	0.06	I
L00009890	07.10.2022	Marsabit	Laisamis	M	1.67×10^3	3.84	NA
L00009896	07.10.2022	Marsabit	Laisamis	M	6.1×10^8	0.29	II
L00009913	11.10.2022	Isiolo	Burat	M	2.32×10^5	0.19	NA
L00009817	12.10.2022	Isiolo	Kinna	M	4.89×10^5	0.14	NA
H08150251	20.10.2022	Marsabit	Laisamis	M	5.18×10^3	0.31	NA
L00011101	05.01.2023	Marsabit	Laisamis	M	3.77×10^7	0.21	NA
L00011081	10.01.2023	Marsabit	Laisamis	M	2.95×10^4	0.15	NA
L00011418	24.01.2023	Marsabit	Laisamis	F	4.95×10^6	2.07	III
L00011505	03.02.2023	Marsabit	Laisamis	M	1.26×10^6	0.22	III
L00011523	06.02.2023	Isiolo	Burat	M	1.49×10^3	2.46	NA
L00011520	06.02.2023	Marsabit	Laisamis	M	6.22×10^3	0.7	NA
L00011530	07.02.2023	Marsabit	Laisamis	M	3.76×10^4	0.29	NA
L00011542	08.02.2023	Marsabit	Laisamis	M	4.29×10^4	3.81	NA
L00011543	08.02.2023	Marsabit	Laisamis	M	7.85×10^3	1.9	NA
L00011549	09.02.2023	Marsabit	Laisamis	M	1.36×10^5	0.17	NA
L00011551	09.02.2023	Marsabit	Laisamis	M	1.49×10^3	2.23	NA
L00011576	13.02.2023	Marsabit	Laisamis	M	3.64×10^3	0.67	NA
L00011595	14.02.2023	Isiolo	Burat	M	3.49×10^4	0.15	NA
L00011622	17.02.2023	Isiolo	Burat	M	3.26×10^5	0.28	NA
L00011675	23.02.2023	Marsabit	Laisamis	M	6.78×10^4	2.29	NA
L00011894	21.03.2023	Marsabit	Laisamis	M	2.78×10^3	2.51	NA
L00011930	24.03.2023	Marsabit	Laisamis	M	1.49×10^3	2.54	NA

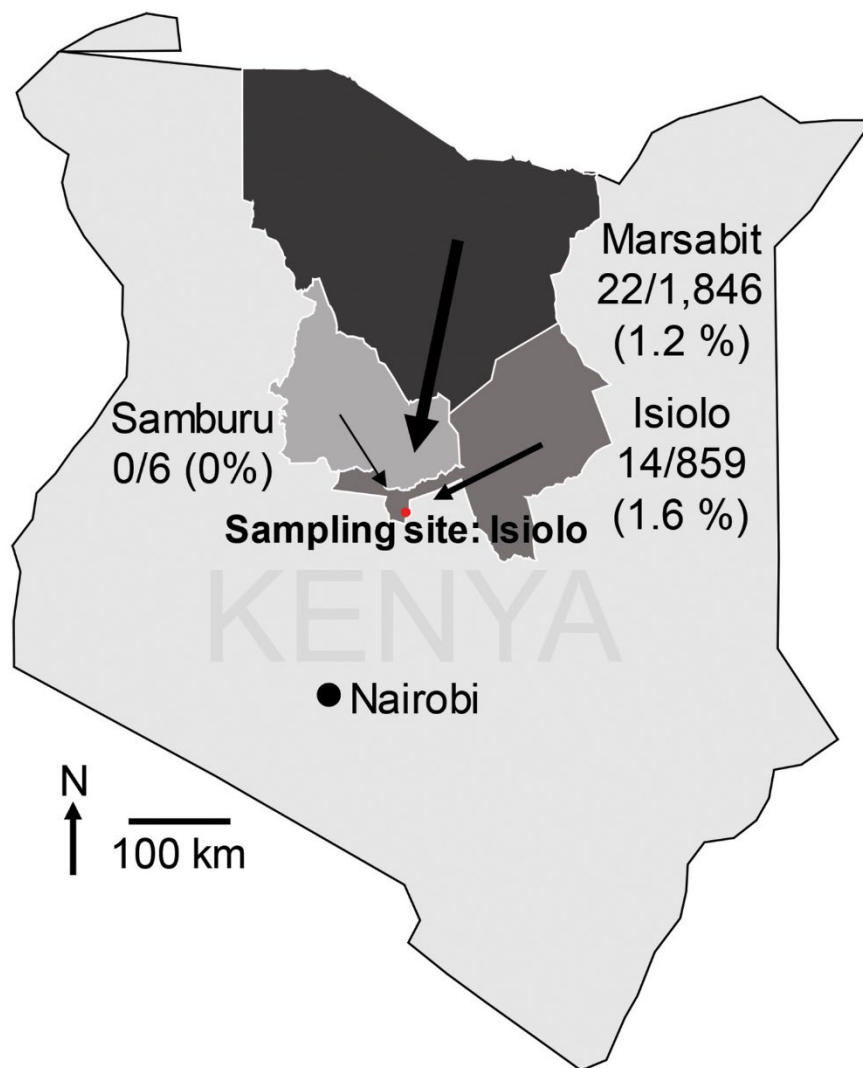
*NS, no serum sample available; NA, not applicable.

Appendix Table 2. Serologic assessment of human abattoir workers and a control cohort*

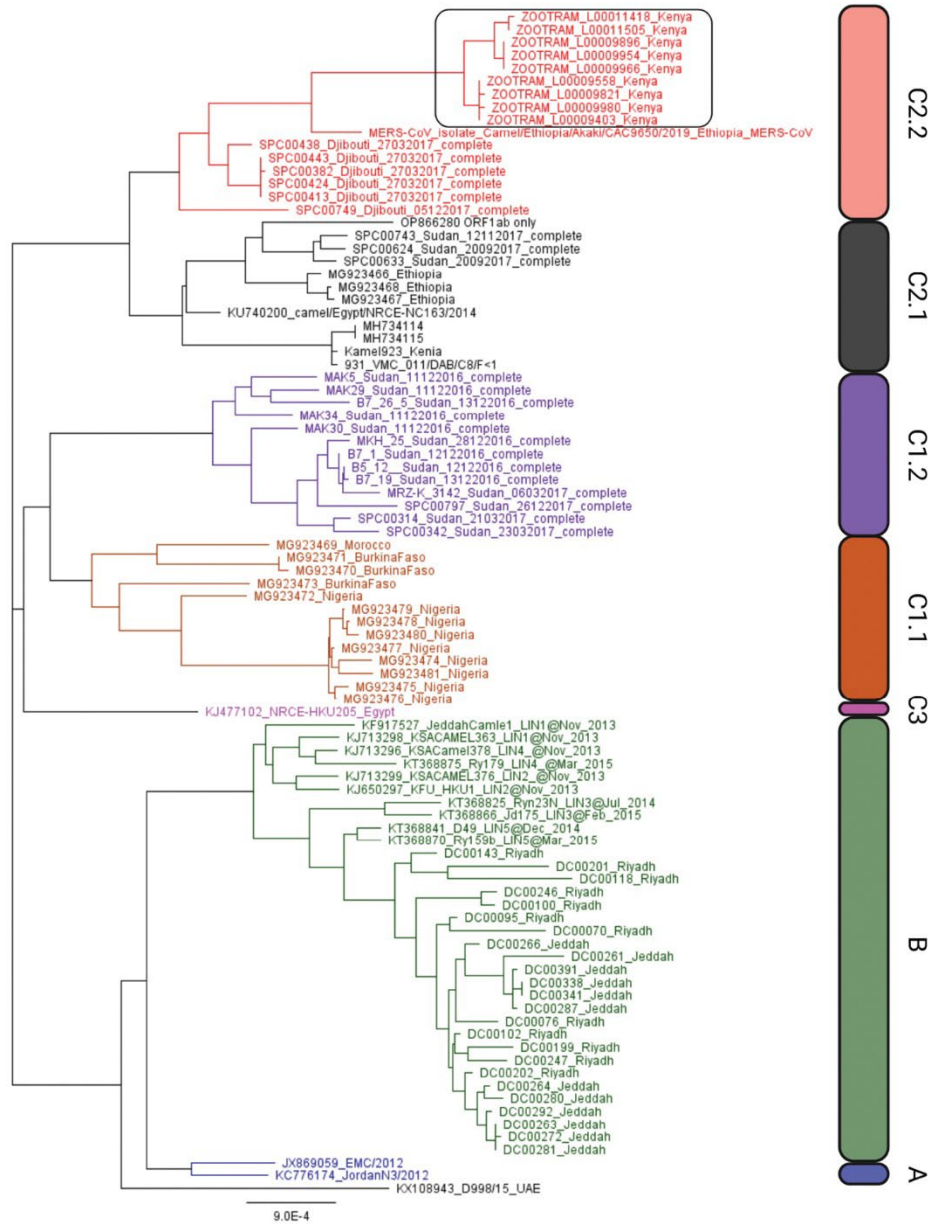
Cohort	Sample ID	MERS-CoV (ELISA OD ratio)	SARS-CoV-2 (ELISA OD ratio)	Reciprocal	Reciprocal	Reciprocal	Rating for MERS-CoV seropositivity
				VSVpp-NT titer 50% (EMC/Kenya Spike)	VSVpp-NT titer 90% (EMC/Kenya Spike)	PRNT50/90 based on MERS-CoV EMC	
Abattoir	11706	0.23	4.48	<20/<20	<20/<20	<10/<10	stage 1 pos.
Abattoir	11717	0.39	6.83	20/40	<20/20	20/10	stage 2 pos.
Abattoir	11722	0.33	0.62	<20/<20	<20/<20	<10/<10	stage 1 pos.
Abattoir	11727	0.25	2.63	<20/<20	<20/<20	<10/<10	stage 1 pos.
Abattoir	11732	0.24	1.28	<20/<20	<20/<20	<10/<10	stage 1 pos.
Abattoir	11741	0.26	5.13	<20/<20	<20/<20	<10/<10	stage 1 pos.
Abattoir	11746	0.24	3.02	<20/<20	<20/<20	<10/<10	stage 1 pos.
Abattoir	11712	0.03	7.61	<20/<20	<20/<20	<10/<10	negative
Abattoir	11738	0.03	4.48	<20/<20	<20/<20	<10/<10	negative
Abattoir	11753	0.03	1.89	<20/<20	<20/<20	<10/<10	negative
Abattoir	11728	0.05	6.34	<20/<20	<20/<20	<10/<10	negative
Abattoir	11734	0.08	5.30	<20/<20	<20/<20	<10/<10	negative
Abattoir	11755	0.11	2.61	<20/<20	<20/<20	<10/<10	negative
Control	83319	0.09	3.12	n.d.	n.d.	n.d.	negative
Control	83320	0.09	7.41	n.d.	n.d.	n.d.	negative
Control	83321	0.15	6.44	n.d.	n.d.	n.d.	negative
Control	83322	0.13	0.47	n.d.	n.d.	n.d.	negative
Control	83323	0.08	5.10	n.d.	n.d.	n.d.	negative

Cohort	Sample ID	MERS-CoV (ELISA OD ratio)	SARS-CoV-2 (ELISA OD ratio)	Reciprocal VSVpp-NT titer 50% (EMC/Kenya Spike)	Reciprocal VSVpp-NT titer 90% (EMC/Kenya Spike)	Reciprocal PRNT50/90 based on MERS-CoV EMC	Rating for MERS-CoV seropositivity
Control	83324	0.09	8.91	n.d.	n.d.	n.d.	negative
Control	83325	0.08	6.01	n.d.	n.d.	n.d.	negative
Control	83326	0.09	7.95	n.d.	n.d.	n.d.	negative
Control	83327	0.10	7.51	n.d.	n.d.	n.d.	negative
Control	83328	0.12	2.53	n.d.	n.d.	n.d.	negative
Control	83329	0.09	6.95	n.d.	n.d.	n.d.	negative
Control	83330	0.17	8.87	n.d.	n.d.	n.d.	negative

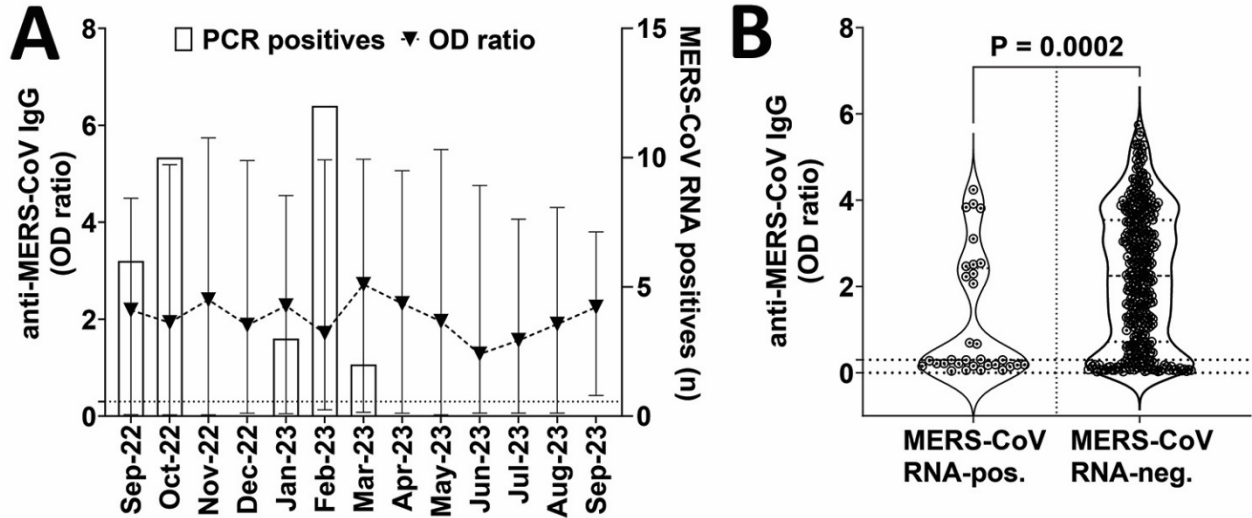
*Stage 1 pos., ELISA-reactive; stage 2 pos., ELISA-reactive and virus neutralization test-positive; n.d. = not done.



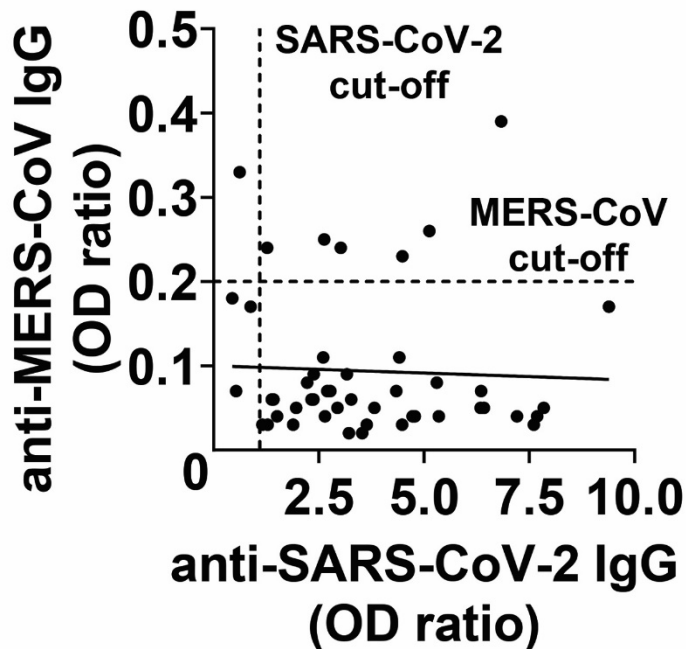
Appendix Figure 1. Origin of all MERS-CoV RNA-positive dromedaries. All dromedary samples (n = 2,711) in this study were sampled in an abattoir hub in Isiolo, where dromedaries from the sub-national regions of Marsabit, Isiolo, and Samburu are slaughtered. The number of total samples, MERS-CoV RNA-positive samples, and percentage positivity are shown for each region. Arrows indicate the direction of transportation from the regions to the Isiolo abattoir hub.



Appendix Figure 2. Phylogeny of newly identified MERS-CoV clade C2.2 strains in Kenya 2022–2023. Phylogenetic tree based on ORF1ab of MERS-CoV clade A (prototypic EMC/2012, blue), clade B (previous and contemporary MERS-CoV on the Arabian Peninsula, green) and clade C (Africa-specific MERS-CoV strains, orange (C1.1), purple (C1.2), black (C2.1), red (C2.2), magenta (C3)) reported previously (13,14). Newly identified MERS-CoV partial genomes were sequenced by NGS as described in the Material & Methods section. Sequence assembly was done by mapping the reads to the reference MERS-CoV strain (EMC/2012) using Geneious version 9.1.8 and phylogenetic analyses using Multiple Alignment using Fast Fourier Transform (MAFFT) for sequence alignment and a Tamura-Nei, neighbor-joining model for phylogenetic inference. The image was labeled using BioRender.com.



Appendix Figure 3. Overview of anti-MERS-CoV IgG (sera) and MERS-CoV upE PCR positivity (swabs) in randomized camel samples. (A) Anti-MERS-CoV S1 IgG ELISA of randomized dromedary sera (n = 369) according to the sampling month compared with MERS-CoV RNA positivity. ELISA cutoff was set to OD ratio = 0.3 according to previous studies (4). (B) Comparison of anti-MERS-CoV IgG levels (OD ratio) in MERS-CoV RNA negative- and positive-tested camels by MERS-CoV upE RT-qPCR (2). MERS-CoV RNA positivity is associated with low anti-MERS-CoV IgG levels. Statistical tests were done using GraphPad Prism (Mann Whitney test, p = 0.0002).



Appendix Figure 4. Antibody cross-reactivity profiling and correlation analysis of SARS-CoV-2 and MERS-CoV ELISA OD ratios in abattoir workers. The scatter plot illustrates the relationship between the SARS-CoV-2 S1 IgG ELISA OD ratio (x-axis) and the MERS-CoV S1 IgG ELISA OD ratio (y-axis) for serum samples from abattoir workers (n = 48). Dashed lines represent the cutoff values for SARS-CoV-2 ELISA (OD ratio = 1.1, vertical line) and MERS-CoV ELISA (OD ratio = 0.2, horizontal line), respectively. Data points are plotted to visually assess the correlation between the two ELISAs. The Spearman correlation coefficient ($r = -0.1449$, $p = 0.3257$) suggests a negligible linear relationship, indicating that the antibody responses to SARS-CoV-2 and MERS-CoV are largely independent ruling out a general cross-reactivity.

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