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T-cell responses to MERS coronavirus infection in people with occupational exposure to dromedary camels in Nigeria: an observational cohort study



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Summary

Background Middle East respiratory syndrome (MERS) remains of global public health concern. Dromedary camels are the source of zoonotic infection. Over 70% of MERS coronavirus (MERS-CoV)-infected dromedaries are found in Africa but no zoonotic disease has been reported in Africa. We aimed to understand whether individuals with exposure to dromedaries in Africa had been infected by MERS-CoV.

Methods Workers slaughtering dromedaries in an abattoir in Kano, Nigeria, were compared with abattoir workers without direct dromedary contact, non-abattoir workers from Kano, and controls from Guangzhou, China. Exposure to dromedaries was ascertained using a questionnaire. Serum and peripheral blood mononuclear cells (PBMCs) were tested for MERS-CoV specific neutralising antibody and T-cell responses.

Findings None of the participants from Nigeria or Guangdong were MERS-CoV seropositive. 18 (30%) of 61 abattoir workers with exposure to dromedaries, but none of 20 abattoir workers without exposure ($p=0.0042$), ten non-abattoir workers or 24 controls from Guangzhou ($p=0.0002$) had evidence of MERS-CoV-specific CD4+ or CD8+ T cells in PBMC. T-cell responses to other endemic human coronaviruses (229E, OC43, HKU-1, and NL-63) were observed in all groups with no association with dromedary exposure. Drinking both unpasteurised camel milk and camel urine was significantly and negatively associated with T-cell positivity (odds ratio 0.07, 95% CI 0.01–0.54).

Interpretation Zoonotic infection of dromedary-exposed individuals is taking place in Nigeria and suggests that the extent of MERS-CoV infections in Africa is underestimated. MERS-CoV could therefore adapt to human transmission in Africa rather than the Arabian Peninsula, where attention is currently focused.

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Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is one of eight emerging pathogens identified in the WHO research and development blueprint requiring urgent action for development of effective vaccines and antiviral drugs.¹ The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a pandemic virus emphasises the threat posed by zoonotic coronaviruses. MERS-CoV causes a zoonotic disease, Middle East respiratory syndrome (MERS), with outbreaks in health-care facilities associated with transmission between humans. As of November, 2019, 2494 laboratory-confirmed cases of MERS, including 858 associated deaths (case-fatality ratio of 34.4%), were reported globally; the majority of these (2102 cases, including 780 deaths) occurred in Saudi Arabia.² Travel-associated outbreaks led to 186 cases and 39 deaths in South Korea.² Dromedary camels are the source of zoonotic MERS-CoV disease.³ The majority (>70%) of dromedaries are found in Africa. They have comparable seroprevalence and virus

shedding to those in the Arabian Peninsula,⁴ but no zoonotic disease has been reported in Africa.

Humans with prolonged close exposure to dromedaries in the Arabian Peninsula have serological evidence of MERS-CoV infection, sometimes having seroprevalence as high as 50%,^{5,6} but serological evidence is rare in Africa, even in dromedary-exposed individuals.^{7,8} However, virologically confirmed infection, especially if it is asymptomatic or mild, might not lead to a serological response.⁹ Thus, alternative and more sensitive methods for detection of past human MERS-CoV infection are needed.

Specific T-cell responses have been shown to be long-lasting in SARS-CoV and MERS-CoV infected humans,^{10,11} and persist longer than antibodies in SARS. We therefore aimed to test peripheral blood mononuclear cells (PBMC) in workers from an abattoir in Kano, Nigeria, for MERS-CoV-specific T-cell responses to understand if the dromedary-exposed individuals in Africa have been infected by MERS-CoV.

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Research in context

Evidence before this study

Middle East respiratory syndrome coronavirus (MERS-CoV) is recognised as one of eight emerging pathogens of greatest threat to global public health, and dromedary camels are the source of human zoonotic infection. The emergence of SARS-CoV-2 highlights the pandemic potential of zoonotic coronaviruses. Although zoonotic disease has been restricted to the Arabian Peninsula, the largest number (>70%) of MERS-CoV infected camels are found in Africa. We searched PubMed for articles published between Nov 8, 2012, and Dec 15, 2019, in English with the search terms “MERS” AND “coronavirus” AND “human” AND “Africa” and manually screened all retrieved articles. There was one MERS outbreak reported in Tunisia initiated by a traveller returning from the Arabian Peninsula but no reports of zoonotic disease in Africa. There were six sero-epidemiological studies of camel-exposed or other humans in Kenya, Egypt, Nigeria, and Morocco and only two (two of 1122 in Kenya and three of 476 tested in Morocco) found any evidence of MERS-CoV infection. Because there was evidence that serological assays for MERS-CoV had suboptimal sensitivity for past infection and because we had previous data showing that T-cell assays for MERS-CoV are specific and potentially more sensitive than antibody detection, we investigated T-cell responses in dromedary-exposed abattoir workers and controls in Nigeria.

Added value of this study

We found that 18 (30%) of 61 abattoir workers with exposure to dromedaries had MERS-CoV specific T-cell responses, but of 20 abattoir workers without exposure to dromedaries and ten non-abattoir workers from Kano, none had such T-cell responses. No individuals with MERS-CoV T-cell responses had detectable antibody. By contrast, T-cell responses to endemic human coronaviruses were detected comparably in abattoir workers with and without exposure to dromedaries and control groups. We document that dromedary-exposed individuals in Africa are frequently infected with MERS-CoV without evidence of severe disease.

Implications of all the available evidence

Our findings indicate that there is substantial zoonotic transmission of MERS-CoV to people with dromedary exposure in parts of Africa. The contribution of MERS-CoV to zoonotic respiratory disease remains to be established. Our findings have implications for global MERS-CoV control policy. There is a need to confirm our findings elsewhere in Africa and to include molecular testing for MERS-CoV in the investigation of patients with severe acute respiratory infections in dromedary-exposed populations in Africa.

Methods

Study design and participants

In this observational cohort study, workers at an abattoir in Kano, Nigeria, consenting to participate in the cohort study in March 13–26, 2018, were recruited. Non-abattoir workers were also recruited randomly from the city of Kano during the same period, and blood donors aged 18–65 years sampled in May 10–Aug 31, 2018, at Guangzhou Blood Center, Guangzhou, China, were randomly included as healthy controls from a region with no dromedary camel exposure.

Convalescent blood samples collected from 14 people with symptomatic or asymptomatic virologically confirmed MERS-CoV infections detected at the King Abdulaziz Medical City, Riyadh, and King Faisal Specialist Hospital, Jeddah, Saudi Arabia, collected as part of a previously reported study¹¹ were included as positive controls. The clinical, serological and T-cell responses (using only interferon [IFN]- γ as a readout of positive cells) of this patient cohort have been previously reported.¹¹ PBMCs were collected at 6 months (patients 1–6, 8–9, 11–14 as reported in the previous publication) or 24 months (patients 18–19) after infection.

Written informed consent was obtained from all study participants in Nigeria and the study was approved by the Health Research Ethics Committee of the Ministry of Health, Nigeria (MOH/Off/797/T.I/630). We obtained

Institutional Review Board approval from the Health Commission of Guangdong Province to use the anonymised blood donor samples for this study. Written informed consent was obtained from all recovered patients with MERS to participate in this study and approval obtained from the Institutional Review Boards of the National Guard Hospital, Riyadh, and King Faisal Specialist Hospital, Jeddah.¹²

Procedures

8 mL of blood were collected from each study participant from the abattoir and from donors from Guangzhou. PBMCs were isolated from blood using Leucosep tubes (Greiner, Kremsmünster, Austria) and Ficoll-Paque PLUS (GE Healthcare, Chicago, IL) according to the manufacturer's instructions. PBMCs were stored in liquid nitrogen and plasma at -80°C or lower before and during shipping before analysis.

Plasma was heat inactivated for 30 min at 56°C before the serology testing. Anti-MERS-CoV antibody titres were determined using plaque reduction neutralisation tests.^{9,13}

A set of 20-mer peptides overlapping by ten amino acids encompassing the four MERS-CoV (HCoV-EMC/2012) structural proteins (peptides S1, S2, N, and ME encompassing the N-terminal and C-terminal portions of the spike [S] glycoprotein, the nucleocapsid [N] protein, and the transmembrane [M] and envelope [E] proteins) and five accessory proteins (ORF3, ORF4a,

ORF4b, ORF5 and ORF8b) were synthesised by Sino Biological (Shanghai, China), and used for stimulation of PBMCs. T-cell responses were measured using intracellular cytokine staining assays for interferon- γ (IFN- γ) and tumour necrosis factor (TNF). Structural proteins peptide libraries of HKU1-CoV, OC43-CoV, NL63-CoV, and 229E-CoV were also synthesised by Sino Biological to

detect viral-specific T-cell responses. To enhance specificity, only cells with dual expression of both IFN- γ and TNF after peptide stimulation were considered as positive.

Flow cytometry was used to determine the phenotype and function of T cells. The following anti-human monoclonal antibodies were used: BV510-CD3 (HIT3a; BD, San Jose, CA), PerCP-Cy5.5-CD4 (RPA-T4; BioLegend,

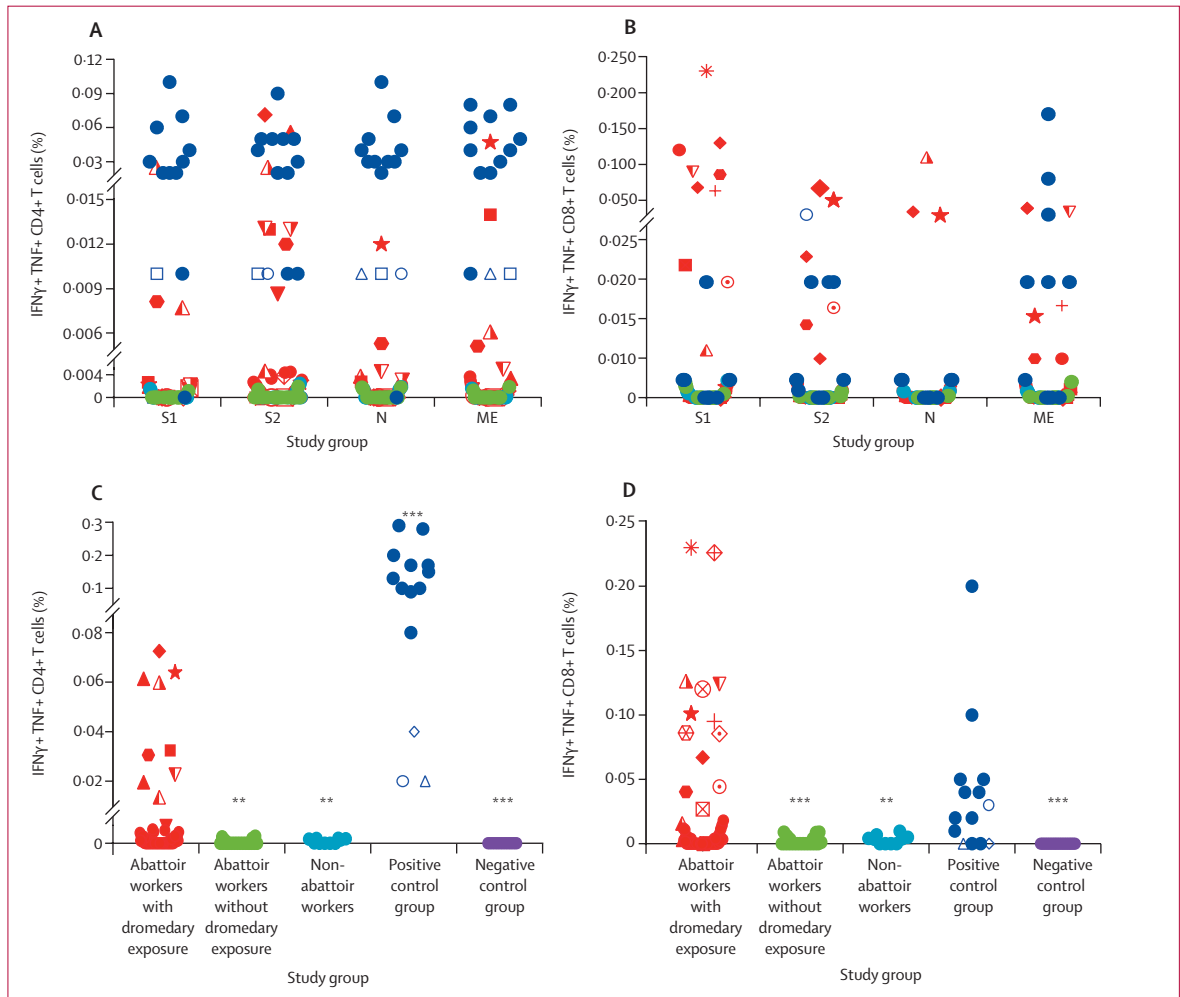
	Abattoir workers with exposure to dromedaries (N=61)	Abattoir workers without exposure to dromedaries (N=20)	Non-abattoir workers (N=10)	p value*
Mean age, years (SD)	27.7 (8.9)	29.5 (8.3)	25.9 (5.3)	0.57
Sex	1.00
Male	61 (100%)	20 (100%)	10 (100%)	..
Female	0	0	0	..
Marital status	0.059
Single	35 (57%)	9 (45%)	9 (90%)	..
Married	26 (43%)	11 (55%)	1 (10%)	..
Occupation				
Mean number of years working in the abattoir (range)	11.4 (0-25-30)	13.7 (0-25-35)	..	0.24
Nature of work in the abattoir	0.52
Cleaning	5 (8%)	0
Slaughtering	6 (10%)	1 (5%)
Flaying	22 (36%)	8 (40%)
Blood collection	8 (13%)	1 (5%)
Related to carcass	15 (25%)	9 (45%)
Related to viscera	4 (7%)	1 (5%)
Mean number of working days in abattoir per week (range)	6.5 (2-7)	6.5 (4-7)	..	0.80
Use of personal protection equipment during duty				
No protection	13 (21%)	5 (25%)	..	0.76
Boots	48 (79%)	14 (70%)	..	0.54
Gloves	1 (2%)	0	..	1.00
Coveralls	0	0	..	1.00
Dust masks	0	0	..	1.00
Goggles	0	0	..	1.00
Hand washing in abattoir	0.019
Rarely	13 (21%)	10 (50%)
Beginning and end of day	42 (69%)	7 (35%)
Before and after animal task	6 (10%)	3 (15%)
Personal				
Contact with camel carcasses, body fluids, secretions, or excrement in past 6 months	54 (89%)	14 (70%)	1 (10%)	<0.0001
Contact with camel bedding in past 6 months	18 (30%)	4 (20%)	0	0.11
Feeding camels around your home in past 6 months	7 (11%)	0	0	0.25
Cleaning camel pens around your home in past 6 months	3 (5%)	0	0	0.70
Cleaning farm equipment around your home in past 6 months	2 (3%)	0	0	1.00
Slaughtering camels at your home in past 6 months	17 (28%)	0	0	0.0035
Assisting with camel birth around your home in past 6 months	2 (3%)	0	0	1.00
Milking camels at your home in past 6 months	38 (62%)	11 (55%)	0	0.0006
Keeping livestock around home in past 6 months	35 (57%)	10 (50%)	5 (50%)	0.76
Camel	0	0	0	1.00
Sheep	19 (31%)	6 (30%)	3 (30%)	1.00
Cattle	13 (21%)	3 (15%)	0	0.33
Goat	23 (38%)	4 (20%)	3 (30%)	0.34

(Table 1 continues on next page)

	Abattoir workers with exposure to dromedaries (N=61)	Abattoir workers without exposure to dromedaries (N=20)	Non-abattoir workers (N=10)	p value*
(Continued from previous page)				
Regularly drink unpasteurised camel milk	46 (75%)	15 (75%)	0	<0.0001
Drink or use camel urine for medicinal purposes	33 (54%)	14 (70%)	0	0.0005
Travel outside Kano in the past 6 months	24 (41%)	9 (45%)	7 (70%)	0.21
Participated mass gathering	37 (62%)	9 (45%)	7 (70%)	0.37
Hospitalised with respiratory illness in past year	39 (64%)	16 (80%)	9 (90%)	0.16
Other				
Mean number of other people living in the household (SD)				
<18 years old	5.8 (4.9)	6.1 (8.5)	4.1 (3.2)	0.66
≥18 years old	5.4 (5.9)	6.3 (8.3)	9.7 (9.9)	0.47
Others household members frequently visit to camel farm or abattoir	35 (57%)	8 (40%)	0	0.0013

Data are n (%), unless otherwise specified. *Testing group difference, using Kruskal-Wallis test for age, number of years working in the abattoir, number of working days in abattoir per week, and number of people living in the household; Fisher's exact test for other variables.

Table 1: Demographic and exposure characteristics of workers from Kano, Nigeria



(Figure 1 continues on next page)

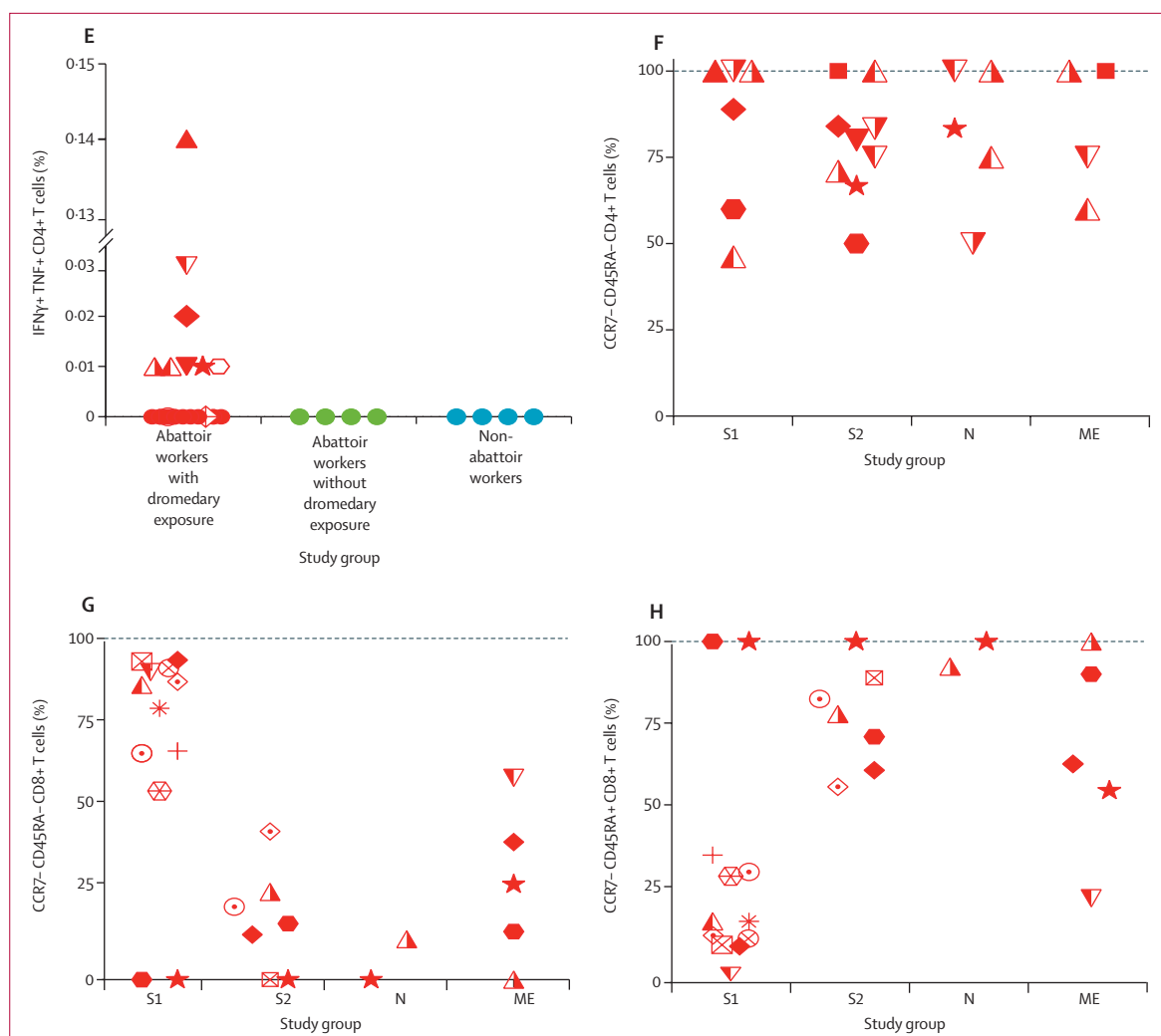


Figure 1: MERS-CoV-specific CD4+ and CD8+ T-cell responses in camel workers and controls

(A) Frequencies of MERS-CoV-specific CD4+ T cells. (B) Frequencies of MERS-CoV-specific CD8+ T cells. (C) Summary of aggregate CD4+ T-cell responses to all structural peptide pools in different study groups. (D) Summary of aggregate CD8+ T-cell responses to all structural peptide pools in different study groups. (E) CD4+ T-cell responses to MERS-CoV accessory protein-specific peptide pools. (F) Phenotypes of virus-specific CD4+ T cells. (G,H) Phenotypes of virus-specific CD8+ T cells. Abattoir workers with exposure to dromedaries are represented by red symbols, those without exposure to dromedaries by green symbols, non-abattoir workers by light blue symbols, MERS-positive controls by dark blue symbols (open shapes represent asymptomatic patients), and negative controls from Guangzhou by purple symbols. Symbol shape identifies the same individual. IFN=interferon. MERS-CoV=Middle East respiratory syndrome coronavirus. TNF=tumour necrosis factor.

= $p < 0.01$. *= $p < 0.001$.

San Diego, CA), APC Fire750-CD8 (SK1; BioLegend), APC-IFN- γ (B27; BD), PE-TNF (MAB11; Invitrogen, Carlsbad, CA), BB515-CD45RA (HI100; BD), and PE-Cy7-CCR7 (G043H7; BioLegend). Fc receptor-blocking solution was obtained from BioLegend. For surface staining, about 1×10^5 cells were blocked with Fc receptor blocking solution, stained with the indicated antibodies at 4°C , and labelled with live–dead staining dye (Thermo Fisher, Waltham, MA). For in-vitro intracellular cytokine staining, 2×10^5 to 6×10^5 cells per well were cultured in 96-well round-bottom plates at 37°C for 12 h in the presence of $10 \mu\text{M}$ peptides (Sino Biological) and brefeldin A (BD Biosciences). Cells were then labelled for cell surface markers, fixed and permeabilised with Cytofix/Cytoperm

solution (BD Biosciences), and stained with anti-cytokine antibodies. All flow cytometry data were acquired on a BD FACSVerser flow cytometer and analysed using FlowJo software. PBMCs were considered MERS-CoV positive if they expressed both IFN- γ and TNF in response to peptide stimulation as described previously.¹⁴

Statistical analysis

In a previous study of dromedary abattoir workers in Saudi Arabia, ten of 30 workers sampled had detectable T-cell responses to MERS-CoV.¹⁴ On the basis of this finding, and the assumption that abattoir workers without dromedary exposure and the other control groups would have no detectable T-cell responses,

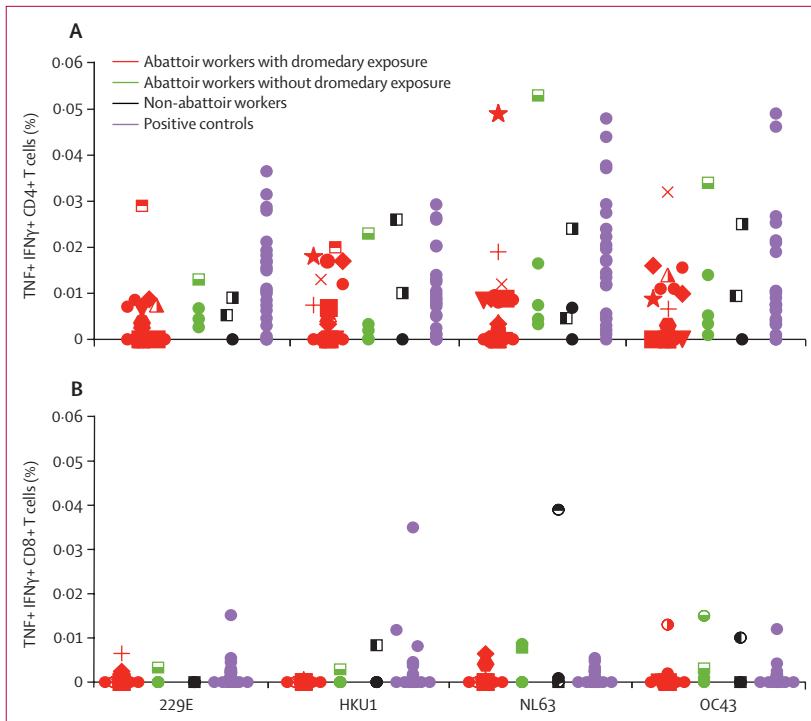


Figure 2: T-cell responses against endemic human coronaviruses in the study cohort
Aggregate CD4+ (A) and CD8+ (B) T-cell responses to the structural peptide pools from human coronaviruses (229E, HKU1, NL63, and OC43). Abattoir workers with exposure to dromedaries are represented by red symbols, those without exposure to dromedaries by green symbols, non-abattoir workers by black symbols, and MERS-positive controls by purple symbols. Symbol shape identifies the same individual. IFN=interferon. TNF=tumour necrosis factor.

eight abattoir workers would be the minimal sample size required to detect a positive result with 95% probability, where the detection probability is given by: $1 - (1 - p)^n$ with p equivalent to 10/30 and n being the sample size. We aimed at sampling all abattoir workers who consented to participate, as long as we successfully sampled at least eight dromedary-exposed abattoir workers.

Association of T-cell responses with different exposure to dromedaries was done using Fisher's exact test. In univariate analysis, we estimated the crude odds ratio (OR) for each potential epidemiological exposure factor in relation to MERS-CoV T-cell positivity using a logistic regression model. Independent risk factors for T-cell positivity were identified using multivariable logistic regression. We included a-priori variables, such as years of work in abattoir and whether other household members frequently visited camel farms, and other variables with a crude OR of more than 2 or less than 0.5 in the univariate analysis. Due to small sample size and cross-related practices of drinking camel milk and camel urine, we first fitted a logistic regression model which considered all four combinations of the two practices (eg, drinking camel milk only, drinking camel urine only, drinking both camel milk and urine or not drinking either), adjusted for potential confounding factors (Model 1). Then we further assessed the effect of

drinking camel milk and camel urine separately in two models (Models 2 and 3). Missing data were handled using multiple imputation with 50 imputations by predictive mean matching using the *AregImpute* function in R. All statistical analyses were done using R version 3.5.1.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We recruited 81 volunteers working in an abattoir in Kano, Nigeria. Dromedaries, sheep, goats, and cattle were slaughtered in different areas of this abattoir (appendix p 4), and workers usually restrict themselves to work with one animal type. 61 (75%) workers had occupational exposure to dromedaries, whereas 20 (25%) were only involved in the slaughtering of sheep, goats, or cattle. Ten people residing in Kano not involved in abattoir-work and 24 volunteers from Guangzhou, China, with no exposure to dromedaries, were also recruited as additional controls. 14 patients with confirmed MERS from Saudi Arabia were included in this study as positive controls.¹¹

All participants were adults (aged ≥ 18 years). Boots were the main protective equipment used by abattoir workers with 48 [79%] of 61 and without 14 [70%] of 20 exposure to dromedaries, whereas other protection, such as gloves, coveralls, masks, or goggles, were rarely used. There was no significant difference in the demographic characteristics between the three groups recruited in Kano (table 1).

None of the sera collected neutralised the Nig1657 virus (previously isolated at the same abattoir) at the dilution of 1:10 to levels of greater than 50% of control, the lowest threshold for a positive result (data not shown).

PBMCs possessed good viabilities in all groups from which they were collected (appendix p 5) and responded to anti-human CD3 stimulation (appendix p 6). 18 (30%) of 61 samples from workers with exposure to dromedaries contained CD4+ or CD8+ T cells that responded to at least one peptide pool, particularly S1 and S2 pools (figure 1A, B; appendix p 7). No MERS-CoV specific CD4+ or CD8+ T-cell responses were detected in the three groups without exposure to dromedaries (figure 1C, D). The proportion of individuals with both CD4+ and CD8+ T-cell responses was significantly larger among dromedary-exposed abattoir workers than in workers without exposure (CD4+ $p=0.0014$; CD8+ $p=0.0009$), non-abattoir workers (CD4+ $p=0.0038$; CD8+ $p=0.0018$), or the Guangzhou control group (CD4+ $p=0.0005$; CD8+ $p=0.0003$). The magnitude of the CD4+ T-cell responses in abattoir workers with exposure to dromedaries was similar to

individuals in the Saudi Arabian positive control group with a subclinical condition ($p=0.094$), whereas the CD8+ T-cell responses were comparable to the symptomatic group ($p=0.49$). For stimulation with peptide pools derived from MERS-CoV accessory proteins (ORF3, ORF4a, ORF4b, ORF5 and ORF8b), PBMCs were available from 11 workers with exposure to dromedaries who had T-cell responses to MERS-CoV structural proteins, from 11 who had negative T-cell responses to MERS-CoV structural proteins, and from four each from abattoir workers without exposure to dromedaries and non-abattoir workers. Eight of the 11 dromedary-exposed workers who had T-cell responses to structural proteins also had T-cell responses to accessory proteins (figure 1E). None of the abattoir workers with dromedary exposure who did not have T-cell responses previously, nor those without dromedary exposure and non-abattoir workers had T-cell responses to accessory proteins (figure 1E). All the T-cell responses detected to accessory proteins were CD4+ T-cell responses and no CD8+ T-cell responses were detected (data not shown). Taken together, of 61 workers with exposure to dromedaries in our cohort, six had both CD4+ and CD8+ T-cell responses against MERS-CoV structural proteins, four had only CD4+, and eight had only CD8+ T-cell responses.

The MERS-CoV-specific CD4+ and CD8+ T cells were multifunctional with dual expression of two cytokines (IFN- γ and TNF). The majority of MERS-CoV-specific CD4+ T cells from dromedary-exposed workers were phenotypically effector memory (CD45RA⁺ CCR7⁻) cells (figure 1F), whereas CD8+ T cells consisted of effector memory (CD45RA⁺ CCR7⁻) and effector (CD45RA⁺ CCR7⁺) cells (figure 1G, H), comparable to the TEMRA subset (effector memory T cells expressing CD45RA) described in MERS survivors.¹¹ Thus, these multifunctional cells are expected to rapidly and efficiently respond to subsequent MERS-CoV reinfection.

61 (53%) of the 115 participants had PBMCs available for additional testing for four endemic human coronaviruses (229E, HKU1, NL63, and OC43), including 18 dromedary-exposed workers positive and ten negative for a MERS-CoV T-cell response and 33 from the negative control groups who were all MERS-CoV T-cell negative. 47 (77%) of 61 were T-cell positive to one or more of the human coronaviruses, with CD4+ T-cell responses being detected in all four groups (figure 2A), whereas CD8+ T-cell responses were found less often (figure 2B). In this group of 61 people, MERS-CoV T-cell responsiveness was not significantly associated with T-cell responses to any of the other coronaviruses (Fisher's exact test; 229E $p=0.57$, HKU1 $p=0.58$, NL63 $p=0.37$, and OC43 $p=0.40$). Of the 47 with T-cell response to any of the other coronaviruses, ten (21%) had T-cell responses to MERS-CoV. By contrast, seven (50%) of 14 with no detectable T-cell response to any other coronavirus had T-cell responses to MERS-CoV, the negative association being statistically significant (Fisher's exact test $p=0.047$).

Seven (41%) of 17 with T-cell responses to MERS-CoV, had no T-cell responses to 229E, OC43, HKU1, or NL63. In conclusion, T-cell responses to these four endemic

	n (%)	Crude odds ratio (95% CI)	p value
Age, years*	..	0.99 (0.93–1.06)	0.87
Years working in abattoir†	..	1.01 (0.95–1.07)	0.77
Personal protection in abattoir			
Yes	48 (29%)	0.93 (0.24–3.51)	0.91
No	13 (31%)	1 (ref)	
Hand washing in abattoir			
Rarely	13 (15%)	0.41 (0.08–2.10)	0.28
Beginning and end of day	42 (31%)	1 (ref)	
Before and after animal task	6 (50%)	2.23 (0.40–12.6)	0.36
Contact with camel carcasses, body fluids, secretions, or excrement in past 6 months around your home			
Yes	54 (28%)	0.51 (0.10–2.57)	0.42
No	7 (43%)	1 (ref)	
Contact with camel bedding around your home in past 6 months			
Yes	18 (33%)	1.29 (0.39–4.22)	0.67
No	43 (28%)	1 (ref)	
Feeding camels around your home in past 6 months			
Yes	7 (14%)	0.36 (0.04–3.25)	0.37
No	54 (31%)	1 (ref)	
Cleaning camel pens around your home in past 6 months			
Yes	3 (33%)	1.21 (0.10–14.2)	0.88
No	58 (29%)	1 (ref)	
Cleaning farm equipment around your home in past 6 months			
Yes	2 (50%)	2.47 (0.15–41.8)	0.53
No	59 (29%)	1 (ref)	
Slaughtering camels at your home in past 6 months			
Yes	17 (35%)	1.45 (0.44–4.81)	0.54
No	44 (27%)	1 (ref)	
Assisting with camel birth around your home in past 6 months			
Yes	2 (50%)	2.47 (0.15–41.8)	0.53
No	59 (29%)	1 (ref)	
Milking camels at your home in past 6 months			
Yes	38 (32%)	1.31 (0.41–4.15)	0.65
No	23 (26%)	1 (ref)	
Keeping livestock around home in the past 6 months			
Yes	35 (23%)	0.47 (0.16–1.45)	0.19
No	26 (38%)	1 (ref)	
Other household members frequently visited camel farms			
Yes	35 (34%)	2.09 (0.63–6.95)	0.23
No	25 (20%)	1 (ref)	
Do you regularly drink unpasteurised camel milk			
Yes	46 (22%)	0.24 (0.07–0.83)	0.025
No	15 (53%)	1 (ref)	
Do you drink or use camel urine for medicinal purposes			
Yes	33 (18%)	0.30 (0.09–0.94)	0.040
No	28 (43%)	1 (ref)	

(Table 2 continues on next page)

	n (%)	Crude odds ratio (95% CI)	p value
(Continued from previous page)			
Travel outside Kano in the past 6 months			
Yes	24 (17%)	0.30 (0.08–1.07)	0.063
No	35 (40%)	1 (ref)	
Participated mass gathering			
Yes	37 (30%)	0.97 (0.31–3.01)	0.95
No	23 (30%)	1 (ref)	
Hospitalised with respiratory illness in past year			
Yes	39 (31%)	1.19 (0.37–3.78)	0.77
No	22 (27%)	1 (ref)	

One abattoir worker with exposure to dromedaries had missing data for years working in abattoir, one for other household members frequently visited camel farms, two for travel outside Kano in the past 6 months, and one for participated in mass gathering. *Mean for age was 27.7 years (SD 8.9). †Mean for years working in abattoir was 11.4 years (SD 9.8).

Table 2: Risk and exposure factors associated with T-cell positivity in dromedary abattoir workers

	Model 1		Model 2		Model 3	
	aOR (95% CI)	p value	aOR (95% CI)	p value	aOR (95% CI)	p value
Years working in abattoir	1.00 (0.92–1.09)	0.99	1.01 (0.92–1.09)	0.89	0.99 (0.91–1.07)	0.78
Hand washing in abattoir						
Rarely	0.16 (0.01–1.65)	0.12	0.21 (0.02–2.24)	0.19	0.26 (0.03–2.08)	0.21
Beginning and end of day	1 (ref)	..	1 (ref)	..	1 (ref)	..
Before and after animal task	4.95 (0.36–68.14)	0.23	3.71 (0.34–40.43)	0.28	4.44 (0.37–53.08)	0.24
Feeding camels around your home in past 6 months						
Yes	0.35 (0.01–9.56)	0.53	0.35 (0.02–8.21)	0.52	0.27 (0.01–6.23)	0.42
No	1 (ref)	..	1 (ref)	..	1 (ref)	..
Assisting with camel birth around your home in past 6 months						
Yes	5.30 (0.12–226.07)	0.39	2.70 (0.07–97.51)	0.59	5.72 (0.15–217.69)	0.35
No	1 (ref)	..	1 (ref)	..	1 (ref)	..
Keeping livestock around home in the past 6 months						
Yes	0.39 (0.09–1.72)	0.21	0.46 (0.11–1.89)	0.28	0.40 (0.09–1.67)	0.21
No	1 (ref)	..	1 (ref)	..	1 (ref)	..
Other household members frequently visited camel farms						
Yes	3.98 (0.79–20.11)	0.091	3.61 (0.79–16.53)	0.097	3.61 (0.74–17.77)	0.11
No	1 (ref)	..	1 (ref)	..	1 (ref)	..
Travel outside Kano in the past 6 months						
Yes	0.77 (0.14–4.23)	0.76	0.66 (0.13–3.32)	0.60	0.57 (0.12–2.81)	0.48
No	1 (ref)	..	1 (ref)	..	1 (ref)	..
Regularly drink unpasteurised camel milk						
Yes	0.14 (0.02–0.81)	0.026
No	1 (ref)
Drink camel urine for medicinal purposes						
Yes	0.19 (0.04–0.84)	0.030
No	1 (ref)	..

(Table 3 continues on next page)

human coronaviruses did not differ between the exposure groups and this was in marked contrast with the observations with MERS-CoV, which was observed exclusively in the dromedary-exposed group.

Drinking unpasteurised camel milk (OR 0.24, 95% CI 0.07–0.83) and drinking camel urine (0.30, 0.09–0.94) were significantly and negatively associated with T-cell positivity (table 2). In the multivariate analysis, drinking both camel milk and urine was significantly negatively associated with T-cell responses (0.07, 95% CI 0.01–0.54; Model 1; table 3). Similar findings were obtained from a model without adjustment for potential confounders (data not shown). We further assessed the effect of each practice separately (Models 2 and 3; table 3) and found that drinking unpasteurised camel milk (0.14, 0.02–0.81) and camel urine (0.19, 0.04–0.84) remained a significant factor for T-cell negativity. The two practices of drinking camel milk and camel urine were closely cross-related; 48 (79%) of 61 dromedary-exposed workers drank camel milk or urine, 15 drank milk without drinking urine, and two drank urine without drinking milk. Our results indicated that drinking camel milk or camel urine was associated with a protective effect against MERS-CoV infection, but we could not separate their independent effects in the analysis.

Discussion

Dromedaries in Africa have comparable seroprevalence of MERS-CoV and virus shedding to those in the Arabian Peninsula, but zoonotic disease has not been reported.^{4,12,15} Even serological evidence of MERS-CoV infection in dromedary-exposed populations is uncommon. We previously found no serological evidence of MERS-CoV infection in 261 dromedary-exposed abattoir workers in an abattoir in Kano, Nigeria, although virus RNA was repeatedly detected in the camels slaughtered during the winter months, with a peak of 11% of animals shedding virus in some weeks.¹⁶ The negative serological results in workers from the same abattoir in this study were thus consistent with those of other studies of dromedary-exposed populations in Kenya and Egypt, which also did not find MERS-CoV-specific antibodies.^{7,16,17} One study in Kenya¹⁸ found two seropositive individuals among 1010 people tested, and our study⁸ in Morocco detected three seropositive individuals among 476 people living in dromedary herding areas. Because some patients with confirmed MERS disease might not manifest neutralising antibody responses and because such antibody responses can wane over time, serological studies could underestimate the extent of MERS-CoV infections in Africa.⁹ Furthermore, antibody responses might not be positive in those with mild or asymptomatic infection,^{9,19–21} and T-cell responses are known to be more sensitive and long-lasting following SARS-CoV infections.²²

We have therefore previously analysed T-cell responses to MERS-CoV.¹¹ In these studies, both MERS survivors (symptomatic and asymptomatic) and camel workers

(asymptomatic) identified in Saudi Arabia were shown to have MERS-CoV specific T cells in their blood, and some of those with T-cell responses did not have neutralising antibodies. Comparable findings were observed in the Korean outbreak; some patients with mild MERS did not produce neutralising antibodies but had MERS-CoV-specific T cells in their peripheral blood.²¹ We have shown that MERS-CoV-specific T cells were present in 18 (30%) of 61 dromedary-exposed workers but not in controls without exposure to dromedaries, and we conclude that MERS-CoV infections in people with occupational contact with dromedaries is underestimated in Nigeria, and probably elsewhere in Africa. T-cell responses in these workers recognised the highly variable S1 region and unique accessory proteins found in MERS-CoV, arguing for the MERS-CoV specificity of the T-cell responses. By contrast, T-cell responses to human coronaviruses NL63, HKU1, 229E, and OC43 were found equally distributed in the dromedary-exposed worker group and the control groups (abattoir workers without dromedary exposure, non-abattoir workers, and Guangzhou negative control). Cross-reactive T-cell responses to other human endemic coronaviruses were not likely to be an explanation for the MERS-CoV T-cell responses in the dromedary-exposed workers, the association being a negative one.

The observation that dromedary-exposed individuals with MERS-CoV T-cell responses did not have antibody responses is consistent with previous studies on MERS and the underlying mechanisms needs further investigation. A question of relevance to public health is why no human zoonotic MERS has been documented in Africa even though zoonotic infection seems to be taking place as assessed by specific T-cell responses. The perception that MERS does not occur in Africa might reduce the use of MERS-CoV diagnostics in patients who have travelled to the Arabian Peninsula, precluding detection of zoonotic MERS in Africa. Our finding that zoonotic MERS-CoV infection is occurring in dromedary-exposed populations in Africa highlights that MERS-CoV needs to be considered in the differential diagnosis of patients with severe acute respiratory infections in these regions.

An alternative hypothesis is that MERS-CoV strains in Africa differ in pathogenic potential to those circulating in the Arabian Peninsula—ie, causing infection but less likely to cause severe disease. We have shown that MERS-CoVs identified from Africa (clade C), including those isolated in Nigeria (clade C1), are phylogenetically distinct from contemporary viruses causing disease in the Arabian Peninsula (clade B).^{12,23,24} Viruses from the African clade C1-lineage were found to replicate less efficiently in human respiratory epithelial cell lines, in ex-vivo cultures of the human lung and in experimentally infected human DPP4 transgenic mice, possibly suggesting impaired pathogenic potential.¹² The absence of antibodies in individuals with T-cell responses might

	Model 1		Model 2		Model 3	
	aOR (95% CI)	p value	aOR (95% CI)	p value	aOR (95% CI)	p value
(Continued from previous page)						
Regularly drank unpasteurised camel milk or camel urine*						
Both	0.07 (0.01-0.54)	0.011
Unpasteurised camel milk only	0.32 (0.05-2.19)	0.83
Camel urine only†	1.71 (0.02-184.19)	0.24
No	1 (ref)

aOR=adjusted odds ratio. *31 workers with dromedary exposure regularly drank unpasteurised camel milk and drank camel urine for medicinal purposes, and 15 regularly drank unpasteurised camel milk only. Two drank camel urine only, so the estimate had large uncertainty.

Table 3: Multivariable logistic regression on T-cell positivity with multiple imputations

also be indicative of less severe infections, because patients with mild or asymptomatic MERS-CoV infections often do not have detectable antibody in both the acute and convalescent stages of infection.^{9,21}

Irrespective of whether MERS-CoV in Africa is less pathogenic than the virus strains in the Arabian Peninsula, our findings argue for more intensive investigation of MERS-CoV in both humans and camels in Africa. If repeated unsuspected zoonotic transmission of MERS-CoV continues to take place in Africa as our findings indicate, given the much larger number of MERS-CoV-infected dromedaries in Africa, the possibility of the virus adapting and efficiently transmitting between humans is probably more likely here than in the Arabian Peninsula where MERS control efforts have been focused. The phylogenetic diversity of clade C viruses in Africa suggests that these are the precursors that gave rise to the potentially more pathogenic clade B viruses currently enzootic in the Arabian Peninsula.^{12,25} If so, similar pathogenic MERS-CoV might independently emerge in Africa. Overall, our findings suggest that the MERS control in the Arabian Peninsula needs to be extended to Africa.

Occupational contact with camels was found to be a key risk factor for MERS-CoV infection, as defined by the positive T-cell responses against MERS-CoV. A univariate analysis of exposure factors associated with MERS-CoV infection (ie, MERS-CoV T cell reactivity) in the dromedary-exposed worker group revealed that drinking unpasteurised camel milk and drinking camel urine for medicinal purposes were significantly and negatively associated with infection risk. Because the practices of drinking raw camel milk and urine were often associated and because of the small sample size, it was not possible to estimate their independent effects in a multivariate analysis in which both factors were concurrent variables. The finding that drinking unpasteurised camel milk was negatively correlated with infection risk is surprising and requires independent confirmation. Camel milk has been previously thought of as a potential risk factor for MERS-CoV infection because MERS-CoV virus has

sometimes been detected in camel milk. However, camel milk contains high titre antibodies to MERS-CoV, which is likely to neutralise any infectious virus particles, and viable MERS-CoV was not isolated from milk samples in which MERS-CoV RNA was detected.²⁶ Thus, MERS-CoV antibody present in camel milk could provide protection against MERS-CoV infection.

Our study had some limitations. Exposure and risk factors associated with T-cell positivity were self-reported and the details on frequency or intensity for different modes of contacts with dromedaries were not collected. A small sample size reduced the power of the multi-variable logistic regression analysis, although we were still able to identify a large protective effect of drinking unpasteurised camel milk or urine on T-cell positivity.

In conclusion, we have shown that detection of virus-specific T-cell responses was a more sensitive method for detecting past infection compared with the serological tests being used hitherto, findings that may be also relevant to assessment of population-based infection attack rates of SARS-CoV-2 using seroprevalence that are currently under way. Our findings suggest that the incidence of MERS infections taking place in Africa is underestimated. These findings have implications for policies on global MERS prevention and control and highlight the need for attention towards camel-herding regions in Africa as well as the Arabian Peninsula.

Contributors

CKPM, JincZ, and MP designed the study. CKPM, JOO, and SAK coordinated and carried out the field work. AZ, JingZ, and JincZ designed and performed the experiments. JW, ZC, ZZ, and RAPMP participated in the experiments. CKPM, AZ, JingZ, and MP analysed the data. EHYL and WL did the statistical analysis. YW collected PBMC from Guangzhong blood donors. ANA and SAB provided MERS patients samples from Saudi Arabia. WW and WT contributed new reagents. CKPM, AZ, JincZ, and MP drafted the manuscript. All authors critically reviewed and commented on the manuscript.

Declaration of interests

We declare no competing interests.

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