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Citation	Eurosurveillance, 2015, v. 20 n. 49, p. 11-17
Issued Date	2015
URL	http://hdl.handle.net/10722/223373
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Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels in Nigeria, 2015

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Citation style for this article:
Chu D, Oladipo J, Perera R, Kuranga S, Chan S, Poon L, Peiris M. Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels in Nigeria, 2015. Euro Surveill. 2015;20(49):pii=30086. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30086

Article submitted on 10 November 2015 / accepted on 10 December 2015 / published on 10 December 2015

Evidence of current and past Middle East respiratory syndrome coronavirus (MERS-CoV) infection in dromedary camels slaughtered at an abattoir in Kano, Nigeria in January 2015, was sought by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and serology. MERS-CoV RNA was detected in 14 (11%) of 132 nasal swabs and antibody in 126 (96%) of 131 serum samples. Phylogenetic analyses demonstrate that the viruses in Nigeria are genetically distinct from those reported in the Arabian peninsula.

Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel lineage C betacoronavirus that can cause an acute viral respiratory disease in humans. As of 13 November 2015, there were 1,618 laboratory-confirmed cases of MERS reported to the World Health Organization (WHO), associated with 579 deaths [1,2]. Human disease is zoonotic in origin, although clusters of human-to-human transmission have been reported, especially within healthcare or family settings [1]. Dromedary camels in the Middle East have a high seroprevalence for MERS-CoV and MERS-CoV RNA has been consistently detected in these animals, especially in settings such as camel abattoirs, where camels from multiple origins are assembled. Camels are thus identified as a potential source of zoonotic MERS [3-6]. More than 60% of the global population of dromedary camels is distributed in African countries. Some of these countries are important camel exporters to the Arabian Peninsula [7]. MERS-CoV antibodies were also found with high prevalence in dromedary camels in these African countries with positive rates higher than 80% for the animals in Egypt, Ethiopia, Nigeria and Sudan, and of 30 to 54% in Tunisia [8]. However, zoonotic human disease has so far been reported only from countries in the Middle-East. The reason for the absence of zoonotic disease in Africa is unclear.

The genetic diversity of the virus in Africa is poorly defined. The only reports of genetic sequences from Africa have been those from viruses in Egypt and these were genetically distinct from those currently causing human disease in the Middle East [6]. However, phenotypic characterisation of these viruses revealed they have capacity to infect tissues of the human respiratory tract [9]. There is a need to better understand the ecology of MERS-CoV within Africa and the viral genetic diversity within this region. We report detection rates of MERS-CoV in dromedary camels in the abattoir in Kano, Nigeria, where around 55 camels are slaughtered per day, making it the largest camel abattoir in that country. Viral sequences derived from nasal swabs of these animals are also phylogenetically analysed.

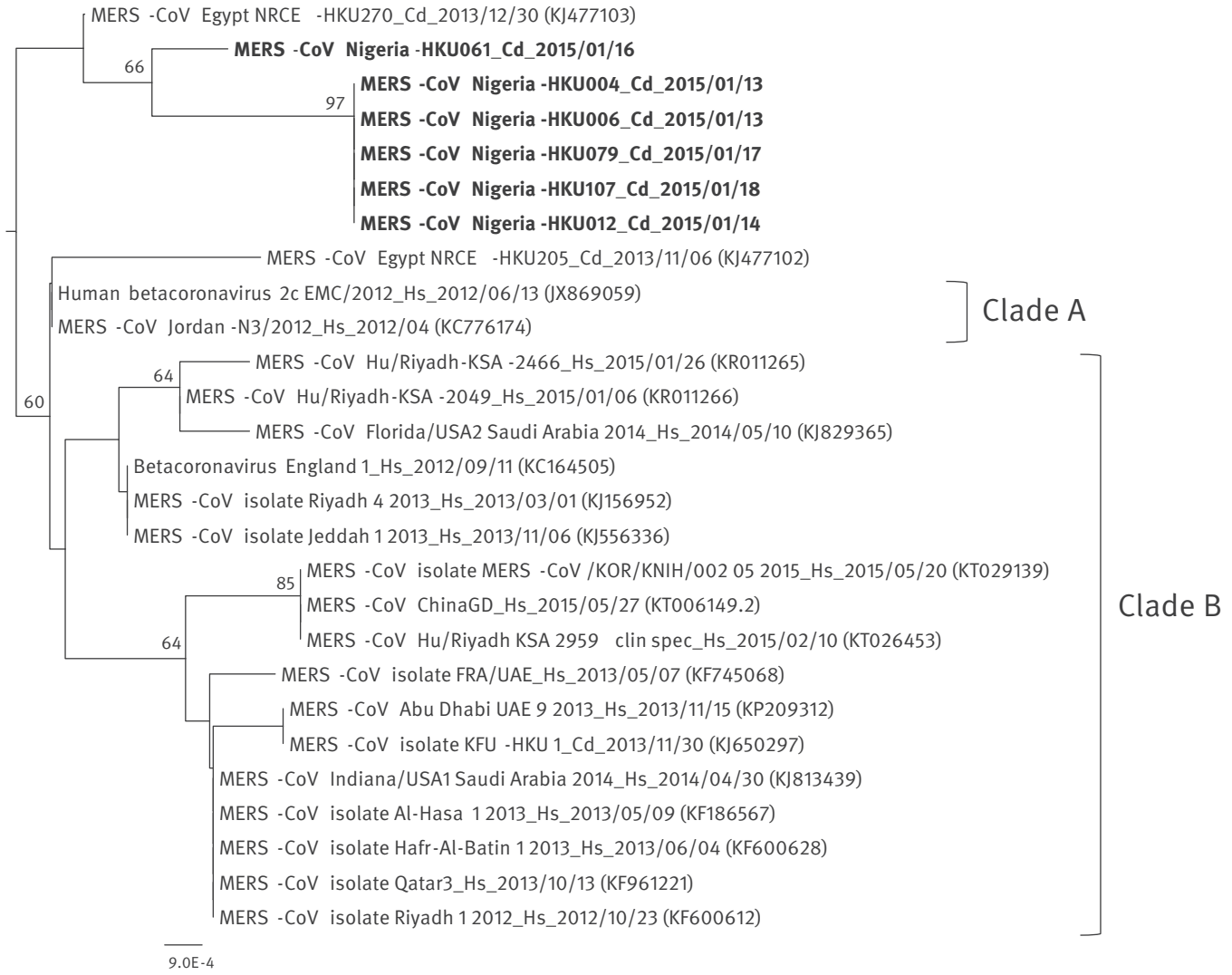
Methods

The study was carried out on seven consecutive days in January 2015. Nasal swabs and blood were collected from camels shortly after slaughter at the abattoir in Kano, Nigeria. Some of the animals slaughtered at this abattoir originate from northern Nigeria, as well as neighbouring African countries including Chad, Libya, Mali, Niger and Sudan. Nasal swabs were collected in RNALater (Ambion) or in virus transport medium and stored at -80°C. Blood samples were collected and serum separated. The age of the sampled camels was assessed by dental examination. Samples were shipped to the laboratory at The University of Hong Kong on dry ice for laboratory investigation.

Total nucleic acid was extracted from swab samples using EasyMag (Biomerieux), and tested for the presence of MERS-CoV RNA by upstream of the Envelope gene (UpE) reverse transcription-quantitative polymerase chain reaction (RT-qPCR) hydrolysis probe assay. All positive specimens were confirmed by a second RT-qPCR assay targeting the open reading frame (ORF)1a region of the genome [6]. The S2 region of

Figure 1

Phylogenetic analysis of the Middle East respiratory syndrome coronaviruses (MERS-CoVs) recovered from Nigeria



Phylogenetic tree of S2 (600 bp) gene sequences obtained by neighbour-joining method with bootstrap values >60 indicated. The tree is mid-point rooted. Sequences generated from this study are in bold with GenBank accession numbers: KU201953–58. Accession numbers of other sequences retrieved from GenBank are shown in brackets.

the positive samples were amplified and sequenced for phylogenetic characterisation [10]. The full spike gene and 5kb of the ORF1b region of one virus were sequenced for more detailed analysis.

Sera were screened at a dilution of 1:20 for the detection of MERS-CoV antibody using a validated MERS-spike pseudoparticle neutralisation test (ppNT) as previously described [5].

Results

Overall, 132 of the 385 animals slaughtered during this period (34%; daily range: 24%–43%) were sampled shortly after slaughter. Nasal swabs were collected from all 132 camels, while serum samples were obtained from 131.

MERS-CoV RNA was detected by both UpE and ORF-1a RT-qPCR assays in 14 (11%), nasal swabs with positive samples being found in five of the seven days sampled (Table). Threshold cycles of the positives by UpE assay ranged from 23.8 to 37.4, which represented $3.54x10^2$ to $2.60x10^6$ copies of virus RNA per 1 mL of original swab sample. Ages of the MERS-CoV positive animals ranged from three to 12 years-old.

On a daily basis, the seroprevalence ranged from 82% to 100%, giving an overall seroprevalence of 95% (Table). All 14 animals positive by RT-qPCR were also positive for MERS-CoV antibody by ppNT. The highest MERS-CoV RNA positive rate by RT-qPCR was detected on the days with lowest seroprevalence.

We sequenced a 600 bp region in S2 gene of six selected RT-qPCR positive samples, representing at

Figure 2

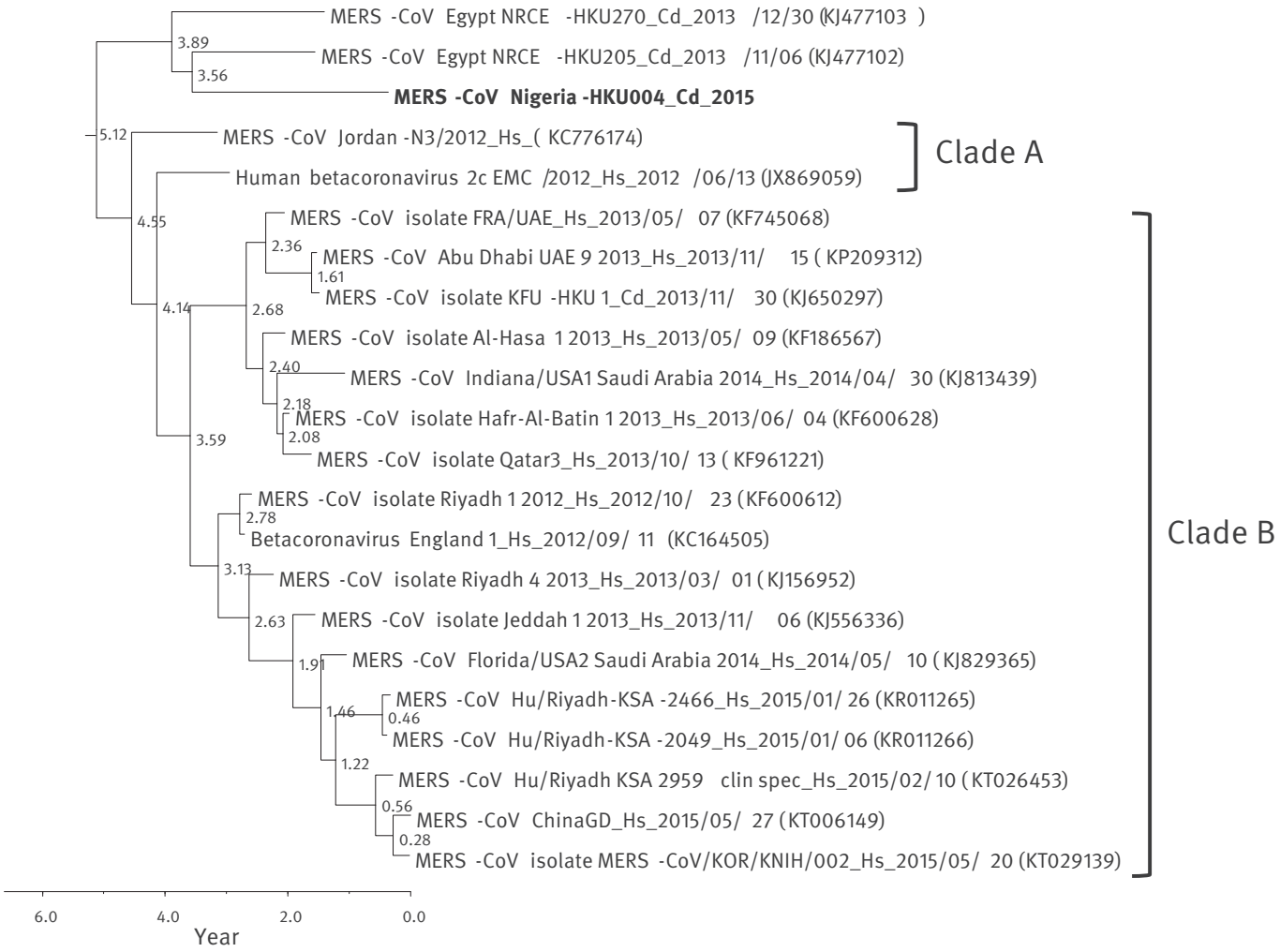
Pairwise similarities between nucleotide sequences of spike S2 region of Middle East respiratory syndrome coronaviruses, including several recovered from Nigeria and other representative viruses

	Virus clade	Nigeria-HKU004	Nigeria-HKU006	Nigeria-HKU012	Nigeria-HKU061	Nigeria-HKU079	Nigeria-HKU107	NRCE-HKU205	NRCE-HKU270	HCoV_2c_EMC	Jordan-N3/2012	Abu_Dhabi_UAE_9	Florida/USA2	England_1	KFU-HKU_1	Hafr-Al-Batin_1	Riyadh_4	FRA/UAE	KOR/KNIH/002	Hu/Riyadh-2049	Hu/Riyadh-2466	ChinaGD
Nigeria-HKU004	Undefined	ID																				
Nigeria-HKU006	Undefined	1	ID																			
Nigeria-HKU012	Undefined	1	1	ID																		
Nigeria-HKU061	Undefined	0.993	0.993	0.993	ID																	
Nigeria-HKU079	Undefined	1	1	1	0.993	ID																
Nigeria-HKU107	Undefined	1	1	1	0.993	1	ID															
Egypt NRCE-HKU205	Undefined	0.986	0.986	0.986	0.989	0.986	0.986	ID														
Egypt NRCE-HKU270	Undefined	0.993	0.993	0.993	0.996	0.993	0.993	0.993	ID													
Human_CoV_2c_EMC	A	0.991	0.991	0.991	0.994	0.991	0.991	0.994	0.998	ID												
Jordan-N3/2012	A	0.991	0.991	0.991	0.994	0.991	0.991	0.994	0.998	1	ID											
Abu_Dhabi_UAE_9	B	0.986	0.986	0.986	0.989	0.986	0.986	0.989	0.993	0.994	0.994	ID										
Florida/USA2_Saudi_Arabia	B	0.986	0.986	0.986	0.989	0.986	0.986	0.989	0.993	0.994	0.994	0.989	ID									
England_1	B	0.989	0.989	0.989	0.993	0.989	0.989	0.993	0.996	0.998	0.998	0.993	0.996	ID								
KFU-HKU_1	B	0.986	0.986	0.986	0.989	0.986	0.986	0.989	0.993	0.994	0.994	1	0.989	0.993	ID							
Hafr-Al-Batin_1	B	0.988	0.988	0.988	0.991	0.988	0.988	0.991	0.994	0.996	0.996	0.998	0.991	0.994	0.998	ID						
Riyadh_4	B	0.989	0.989	0.989	0.993	0.989	0.989	0.993	0.996	0.998	0.998	0.993	0.996	1	0.993	0.994	ID					
FRA/UAE	B	0.989	0.989	0.989	0.989	0.989	0.989	0.989	0.993	0.994	0.994	0.996	0.989	0.993	0.996	0.998	0.993	ID				
KOR/KNIH/002	B	0.984	0.984	0.984	0.988	0.984	0.984	0.988	0.991	0.993	0.993	0.994	0.991	0.991	0.994	0.996	0.991	0.994	ID			
Hu/Riyadh-KSA-2049	B	0.988	0.988	0.988	0.991	0.988	0.988	0.991	0.994	0.996	0.996	0.991	0.998	0.998	0.991	0.993	0.998	0.991	0.993	ID		
Hu/Riyadh-KSA-2466	B	0.986	0.986	0.986	0.989	0.986	0.986	0.989	0.993	0.994	0.994	0.989	0.996	0.996	0.989	0.991	0.996	0.989	0.991	0.998	ID	
ChinaGD	B	0.984	0.984	0.984	0.988	0.984	0.984	0.988	0.991	0.993	0.993	0.994	0.991	0.991	0.994	0.996	0.991	0.994	1	0.993	0.991	ID

ID: identical virus.

Figure 3

Time resolved phylogenetic tree of open reading frame (ORF)1b of Middle East respiratory syndrome coronaviruses (MERS-CoVs) recovered in Africa and the Middle East, 2012–2015



The time resolved phylogenetic tree was constructed using Bayesian evolutionary analysis by sampling trees (BEAST) with an uncorrelated lognormal relaxed clock based on open reading frame (ORF)1b (5,347 bp) gene sequences of representative MERS-CoVs. Median ages of the nodes with respective to MERS-CoV ChinaGD (the latest virus included) are shown. The tree is rooted to bat coronavirus Neoromicia. The sequence generated from this study (Nigeria-HKU004) is in bold and has GenBank accession number KU201959. GenBank accession numbers of other sequences retrieved for the phylogenetic analysis are shown in brackets.

least one positive sample from each day with PCR-positive samples. Phylogenetic analysis showed that these six viruses formed two distinct lineages, the virus from a camel sampled on 16 January being distinct from the others (Figure 1).

Both of the Nigerian virus groups were distinct from previously known virus lineages. The Nigerian camel viruses were most closely related to virus NRCE-HKU270 previously found in Egypt with nt sequence similarity of 99.3 to 99.6%. MERS-CoV in Nigeria were genetically distinct from viruses found in camels in the Middle East and viruses detected in humans in more recent years (nt sequence similarity ranging from 98.4 to 99.4%) (Figure 2).

The time-resolved phylogeny of the ORF1b region of one virus confirms these overall findings (Figure 3).

We derived the nt sequence of the full spike gene of one virus Nigeria HKU004 and the deduced amino acid sequence is aligned and compared to those of other human and animal MERS-CoV in Figure 4. This reveals that the receptor binding domain, which is crucial for virus host range and tropism, is fully conserved. There are only two amino acid residues not previously reported in other MERS-CoV spike proteins; these being S 656 T and L 1200 F.

Discussion

We report high MERS-CoV seroprevalence in camels gathered in an abattoir in Nigeria with relatively high detection rates of virus in nasal swabs. Phylogenetic analysis revealed that these viruses are genetically distinct from viruses reported from camels or humans in the Arabia peninsula and cluster with, but are clearly distinct from those reported from Egypt. However, it is

FIGURE 4

Alignment of the full spike protein amino acid sequence of a Middle East respiratory syndrome coronavirus (MERS-CoV) found in Nigeria with those of other MERS-CoVs

Viurus clade	Virus sequences compared	Spike region and spike protein amino acid position																																					
		S1	S1	S1	S1	S1	S1	S1	S1	COR	COR	COR	COR	COR	RBD	RBD	RBD	S1	S1	S1	S1	S1	S1	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
		23	26	94	125	137	158	194	301	411	431	434	457	460	506	509	530	656	666	677	683	696	744	756	773	810	833	856	886	888	914	918	1,020	1,158	1193	1,200	1,208	1,314	1,333
A	MERS-CoV ChinaGD	P	V	G	N	S	D	H	R	L	A	A	S	S	L	D	V	S	E	V	S	M	T	E	L	V	Q	S	A	S	Q	A	R	A	A	L	Q	V	C
A	MERS-CoV isolate MERS-CoV/KOR/KNIH/002	.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV Hu/Riyadh KSA 2959 clin spec	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV Abu Dhabi UAE 9 2013	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	P	.	.	I	.	.	.	.	.	.	.	.	S	.	.	.	.
A	MERS-CoV Florida/USA2 Saudi Arabia 2014	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F	.	.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV Indiana/USA1 Saudi Arabia 2014	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV isolate Al-Hasa 1 2013	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.	.
A	Human Betacoronavirus England 1	.	.	.	.	.	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.	.	.	.	.	.
A	MERS-CoV isolate KFU-HKU 1	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	F	I	.	.	.	.	.	.	.	.	S	.	.	.	.
A	MERS-CoV isolate Hafr-Al-Batin 1 2013	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV isolate Qatar3	.	.	.	.	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV isolate Riyadh 1 2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A	MERS-CoV isolate Riyadh 4 2013	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.	.	.	.	.	.
A	MERS-CoV isolate FRA/UAE	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.	.	.	S	.	.	.	.
A	MERS-CoV isolate Jeddah 1 2013	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H	.	.	.	.	.	.
A	MERS-CoV Hu/Riyadh-KSA-2049	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
A	MERS-CoV Hu/Riyadh-KSA-2466	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
B	Human betacoronavirus 2c EMC/2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Q	.	.	.	.	.	.
B	MERS-CoV Jordan-N3/2012	.	.	V	.	.	.	Y	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Q	S	.	.	.	.	.
UD	MERS-CoV Egypt NRCE-HKU205	L	A	.	.	.	.	Y	.	.	.	S	.	.	.	.	.	.	K	.	.	T	.	Q	.	.	.	.	S	I	.	S	Q	S	.	.	.	.	R
UD	MERS-CoV Egypt NRCE-HKU270	.	A	.	.	.	Y	Y	.	.	T	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	Y	.	.	.	.	Q	S	.	.	.	.	.
UD	MERS-CoV Nigeria HKU004	.	A	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	Y	.	.	H	.	Q	L	.	F	.	.	.

GenBank accession number for MERS-CoV Nigeria HKU004 was KU201953.
RBD: receptor binding domain; UD: undefined.

Table

Swabs and sera collected from dromedary camels and results of reverse transcription-quantitative polymerase chain reaction and serology for Middle East respiratory syndrome coronavirus, Nigeria, January 2015

Sampling date	Number of camels slaughtered	Swabs sampled	Swabs positive n (%)	Sera sampled	Sera positive n (%)
13 Jan 2015	45	11	4 (36)	11	9 (82)
14 Jan 2015	54	17	6 (35)	17	16 (94)
15 Jan 2015	50	18	0 (0)	18	18 (100)
16 Jan 2015	48	15	1 (7)	15	15 (100)
17 Jan 2015	70	30	2 (7)	30	28 (93)
18 Jan 2015	55	19	1 (5)	19	19 (100)
19 Jan 2015	63	22	0 (0)	21	20 (95)
Total	**385**	**132**	**14 (11)**	**131**	**125 (95)**

important to note that these Nigerian viruses remain genetically closely related to other MERS-CoV with an overall nt similarity of›0.98 for the S2 gene. Thus these are almost certainly pertaining to one viral species, and very likely (though not directly studied in the present work) also form one serotype.

Although the animals in this study were sampled in Kano, Nigeria, some animals originated from neighbouring African countries. The camels are kept within the abattoir for a number of days before slaughter allowing opportunity for virus cross-infection and amplification. Thus the viruses detected in this study may reflect viruses circulating in this wider region.

A limitation of this study is the short period within which the sampling was carried out. Thus it is unclear whether the high rate of virus detection reflects an all year-round pattern or if there are seasonal differences in virus activity. It has been suspected that virus activity in camels may increase during the calving season. In Nigeria, as in the Arabian peninsula, camel calving peaks during the January to March period. Thus it is possible that the rate of virus detection observed in this study reflects a seasonal peak. Indeed this was the rationale for targeting sampling within this time frame. Even if the observations of this study reflect a seasonal peak, the detection of MERS-CoV in camels slaughtered on five of seven days sampled, suggests intense exposure of humans to virus infected camels, whether seasonally or year round, and thus potentially a significant zoonotic threat.

It is not known if the MERS-CoV in Nigeria has similar zoonotic potential to viruses currently circulating in the Arabian peninsula. Other explanations for the lack of zoonotic MERS in Africa include differences in patterns of exposure to infected animals or animal products or differences in human susceptibility to MERS-CoV. Alternatively, MERS may indeed be occurring but unrecognised in Africa due to lack of awareness or diagnostic testing.

A second limitation of this study is that there were no virus isolates available for phenotypic characterisation and full genome sequence data were not available for genetic comparison across the whole genome with viruses known to infect humans. Comparison of the sequence of the spike gene with other MERS-CoV suggests that the virus isolated in Nigeria appears to have competence to bind the dipeptidyl peptidase (DPP)-4 receptor. Viruses isolated from Egypt, though genetically distinct, had comparable tropism and replication competence in ex vivo cultures of the human bronchus and lung to those viruses isolated from humans in Saudi Arabia [9]. Thus zoonotic potential of these viruses cannot be excluded. Further studies to determine the genetic diversity and biological characterisation of MERS-CoV across Africa are urgently needed. Studies looking into seroprevalence of humans exposed to settings associated with such high levels of exposure to MERS-CoV such as camel abattoirs across Africa are also important to assess the extent of zoonotic spillover, if any.

Acknowledgements

This study is supported by a research grant from the US National Institutes of Health (contract No. HHSN272201400006C).

Conflict of interest

None declared.

Authors' contributions

JOO and MP designed the study, JOO and SAK carried out the field work, DKWC, RAPMP, SMSC and LLMP supervised and carried out the laboratory analysis, DKWC and MP drafted the manuscript. All authors critically reviewed the manuscript.

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