

Emerging Microbes & Infections



ISSN: (Print) (Online) Journal homepage: www.informahealthcare.com/journals/temi20

Genetic diversity and molecular epidemiology of Middle East Respiratory Syndrome Coronavirus in dromedaries in Ethiopia, 2017–2020

Ziqi Zhou, Abraham Ali, Elias Walelign, Getnet F. Demissie, Ihab El Masry, Takele Abayneh, Belayneh Getachew, Pavithra Krishnan, Daisy Y.M. Ng, Emma Gardner, Yilma Makonnen, Eve Miguel, Véronique Chevalier, Daniel K. Chu, Ray T. Y. So, Sophie Von Dobschuetz, Gezahegne Mamo, Leo L. M. Poon & Malik Peiris

To cite this article: Ziqi Zhou, Abraham Ali, Elias Walelign, Getnet F. Demissie, Ihab El Masry, Takele Abayneh, Belayneh Getachew, Pavithra Krishnan, Daisy Y.M. Ng, Emma Gardner, Yilma Makonnen, Eve Miguel, Véronique Chevalier, Daniel K. Chu, Ray T. Y. So, Sophie Von Dobschuetz, Gezahegne Mamo, Leo L. M. Poon & Malik Peiris (2023) Genetic diversity and molecular epidemiology of Middle East Respiratory Syndrome Coronavirus in dromedaries in Ethiopia, 2017–2020, Emerging Microbes & Infections, 12:1, e2164218, DOI: 10.1080/22221751.2022.2164218

To link to this article: https://doi.org/10.1080/22221751.2022.2164218

© 2023 The Author(s). Published by Ir UK Limited, trading as Taylor & Franci Group, on behalf of Shanghai Shangy Cultural Communication Co., Ltd	S View supplementary material =
Published online: 27 Jan 2023.	Submit your article to this journal 🗷
Article views: 3161	View related articles ☑
Uiew Crossmark data ☑	Citing articles: 1 View citing articles







Genetic diversity and molecular epidemiology of Middle East Respiratory Syndrome Coronavirus in dromedaries in Ethiopia, 2017–2020

Ziqi Zhou^a*, Abraham Ali^{b,c}*, Elias Walelign^d, Getnet F. Demissie^e, Ihab El Masry^f, Takele Abayneh^g, Belayneh Getachew⁹, Pavithra Krishnan^a, Daisy Y.M. Ng^a, Emma Gardner ¹, Yilma Makonnen^h, Eve Miguel ^{1,j}, Véronique Chevalier^{k,l,m}, Daniel K. Chu^{a,n}, Ray T. Y. So^a, Sophie Von Dobschuetz^f, Gezahegne Mamo ^{© c}, Leo L. M. Poon^a and Malik Peiris [©]

^aSchool of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, People's Republic of China; ^bBacterial, Parasitic and Zoonotic Diseases Research Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia; ^cDepartment of Veterinary Microbiology, Immunology and Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia; ^dFood and Agriculture Organization, Emergency Centre for Transboundary Animal Diseases, Addis Ababa, Ethiopia; ^eCollege of Veterinary Medicine, Department of Veterinary Epidemiology, Microbiology and Public Health, Haramaya University, Haramaya, Ethiopia; [†]Food and Agriculture Organization of the United Nations, Rome, Italy; ⁹National Veterinary Institute, Debre Zeit, Ethiopia; ^hFood and Agriculture Organization, Subregional Office for Eastern Africa, Addis Ababa, Ethiopia; ¹Animal, Santé, Territoires, Risques et Ecosystèmes, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Institut National de la Recherche Agronomique, Université de Montpellier, Montpellier, France; ¹Maladies Infectieuses et Vecteurs: Ecologie Genetique, Evolution et Controle, L'Institut de Recherche pour le Developpment, CNRS, Montpellier, France; kInternational Center of Research in Agriculture for Development (CIRAD), UMR ASTRE, Montpellier, France; CIRAD, UMR ASTRE, Antananarivo, Madagascar; mEpidemiology and Clinical Research Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar; ⁿUK Health Security Agency, London, UK

ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) is enzootic in dromedary camels and causes zoonotic infection and disease in humans. Although over 80% of the global population of infected dromedary camels are found in Africa, zoonotic disease had only been reported in the Arabia Peninsula and travel-associated disease has been reported elsewhere. In this study, genetic diversity and molecular epidemiology of MERS-CoV in dromedary camels in Ethiopia were investigated during 2017-2020. Of 1766 nasal swab samples collected, 61 (3.5%) were detected positive for MERS-CoV RNA. Of 484 turbinate swab samples collected, 10 (2.1%) were detected positive for MERS-CoV RNA. Twenty-five whole genome sequences were obtained from these MERS-CoV positive samples. Phylogenetically, these Ethiopian camel-originated MERS-CoV belonged to clade C2, clustering with other East African camel strains. Virus sequences from camel herds clustered geographically while in an abattoir, two distinct phylogenetic clusters of MERS-CoVs were observed in two sequential sampling collections, which indicates the greater genetic diversity of MERS-CoV in abattoirs. In contrast to clade A and B viruses from the Arabian Peninsula, clade C camel-originated MERS-CoV from Ethiopia had various nucleotide insertions and deletions in non-structural gene nsp3, accessory genes ORF3 and ORF5 and structural gene N. This study demonstrates the genetic instability of MERS-CoV in dromedaries in East Africa, which indicates that the virus is still actively adapting to its camel host. The impact of the observed nucleotide insertions and deletions on virus evolution, viral fitness, and zoonotic potential deserves further study.

ARTICLE HISTORY Received 28 September 2022; Revised 21 December 2022; Accepted 28 December 2022

KEYWORDS MERS-CoV; Ethiopia; genetic instability; molecular epidemiology; evolution

Introduction

The Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in a patient with the severe respiratory syndrome in Saudi Arabia in 2012 [1]. As of June 2022, 2591 laboratory-confirmed cases were reported to the World Health Organization with a fatality rate of 34.5% [2]. It remains a cause for global public health concern.

Dromedary camels are the source of zoonotic MERS-CoV infection [1,3]. According to the Food and Agriculture Organization (FAO), the number of

dromedary camels worldwide is estimated to be 39 million in 2020, of which over 80% are in Africa [4]. In East Africa, where there is the highest density of dromedary camels in the world, Ethiopia, Sudan, Somalia and Kenya have 1.6, 4.9, 7.3 and 4.7 million camels, respectively, accounting for almost 50% of the global population [4]. MERS-CoV is highly prevalent in dromedary camels, in both Africa and the Arabian Peninsula [5]. The earliest evidence of MERS-CoV infection in dromedaries dates back to 1983, based on the retrospective detection of MERS-CoV

^{*}These authors contributed equally and are co-first authors.



antibodies in camel sera collected in Sudan and Somalia [6]. In more recent studies in Egypt, Kenya, Ethiopia, and Somalia, over 90% of camels were seropositive to MERS-CoV and nasal viral RNA positive rate by RT-PCR ranged from 0.23% to 15.4% [5,7-9].

Despite the high prevalence of MERS-CoV in camels, zoonotic human disease has only been reported in the Arabia Peninsula although travelassociated outbreaks have been reported elsewhere [10]. Zoonotic infection has also sometimes caused transmission among humans with outbreaks of more than 100 individuals, which have repeatedly occurred in healthcare settings in the Arabian Peninsula and once in the Republic of Korea [11,12]. The apparent absence of zoonotic MERS disease from Africa remains an enigma. Previous studies showed that MERS-CoV circulating in Arabian Peninsula and Africa exhibit region-dependent genetic diversity and differ in their phenotypic traits [13,14]. Genetically distinct clade C MERS-CoV from Africa (Ethiopia, Kenya, Burkina Faso, and Nigeria) had lower replication competence in ex vivo cultures of the human bronchus and lungs in comparison with clade A and B strains from Saudi Arabia, which may possibly indicate the reduced human pathogenic potential of African MERS-CoV viruses [14]. It has been shown that deletion in the ORF4b gene and amino acid differences in the spike protein observed in clade C viruses may contribute to this phenotype difference [13,14]. While MERS-CoV antibodies have been detected in humans with exposure to camels in the Arabian Peninsula, with some studies showing up to 50% of camel workers (e.g. slaughterhouse workers and herders) to be seropositive, such evidence was less often reported from Africa, and if so, only at very low prevalence [15-19]. However, virologically confirmed infection is not always associated with antibody responses [20] and sero-epidemiology may markedly underestimate human infection in Africa. The recent detection of MERS-CoV specific T cell responses in around 30% of camel workers in an abattoir in Kano, Nigeria, despite an absence of detectable antibody to MERS-CoV, suggests that infection of camel-exposed populations may not be uncommon [21]. In a cohort of 262 camel handlers in Kenya followed longitudinally for two years, where camels were detected to be MERS-CoV positive, three camel handlers were detected MERS-CoV RNA positive [22,23]. Given the high prevalence and widespread presence of MERS-CoV infection in dromedary camels in Africa, there is a need for continued surveillance and studies at the camel-human interface in Africa in order to better define virus genetic diversity, evolution and phenotypic characterization of MERS-CoV circulating in Africa. This would be beneficial for understanding zoonotic risks, introducing riskreduction measures and developing countermeasures

against MERS-CoV in camel populations and humans. This study reports the genetic characteristics and molecular epidemiology of MERS-CoV in camels in Ethiopia.

Material and methods

Sample collection

1766 nasal swabs were collected from January 2017 to January 2020, and 484 turbinate swabs were collected from September 2019 to September 2020, from dromedary camels from camel herds and slaughterhouses in different regions of Ethiopia. Slaughterhouses sampled were located in Akaki and Babile. The Akaki slaughterhouse sourced camels from diverse regions in Ethiopia, predominantly the Borna area (Oromia region), Menze (Amahar region bordering Afar), from Afar (Afar region) and from the Methara area (Oromia Region). The number of camels slaughtered depends on demand and was around 3-10 camels per day. Camels brought to the abattoir stayed for days, ranging from a few days to a few weeks. The camels were kept in a single pen hence camels from different areas were mixed together. The slaughterhouse in Babile sourced camels from the Gursum, Babile and Somale regions nearby and camels were held for 3 or more days prior to slaughter. Camels were housed in pens where animals from different sources were held together and approximately 3-5 camels were slaughtered daily, depending on demand. In general, camels in slaughterhouse were apparently healthy and didn't show any clinical symptoms. Ante and post-mortem inspections of the camels slaughtered at the abattoir was carried out by veterinary inspectors assigned by the Addis Ababa Abattoirs enterprise. Camels with clinical signs of disease would not be accepted for slaughter at the abattoir.

After collection, swabs were put into virus transport medium and placed in cool boxes with ice packs for transport. On arrival at the local laboratories, samples were stored at -80 °C. When shipped to Hong Kong University (HKU) laboratory, samples were frozen in dry ice and then stored at -80 °C upon arrival, until testing.

MERS-CoV detection

Total nucleic acid was extracted from the samples using the automated MagPure 96 (Roche, Basel, Switzerland) or NucliSENS easyMAG 24 system (bioMérieux, Craponne, France). In the MagPure 96 system, viral RNA was extracted using the Viral RNA Small volume kit (Roche, Basel, Switzerland) by adding 200µl of the virus transport medium and eluting in 50µl H2O. In the NucliSENS easyMAG 24 system, viral RNA was extracted using 200µl of the virus transport medium and eluted in 60µl of elution buffer. The MERS-CoV RT-qPCR diagnostic tests were performed according to the WHO guideline [24]. A screening assay targeting the upE gene was first performed. Positive samples were confirmed by a second RT-qPCR assay targeting the ORF1a gene [25].

MERS-CoV whole genome sequencing

MERS-CoV whole genome sequencing was performed as previously described [26]. MERS-CoV cDNAs of different regions of the viral genome were synthesized using multiple gene-specific primers with the Super-Script IV First-Strand Synthesis System, according to the manufacture's recommendations (Thermofisher, Massachusetts, US). Nested PCRs with primers to produce overlapping DNA fragments were performed to amplify the whole genome using the TaKaRa LA Taq kit (Takara, Shiga, Japan). After purification using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), PCR products were sent to the HKU Centre for PanorOmic Sciences (CPOS) for NGS sequencing. The library was prepared by The Nextera XT DNA Library Preparation Kit (Illumina, California, US), followed by sequencing on the Illumina NovaSeq 6000 platform. Genome consensus sequences were generated through mapping to a reference MERS-CoV genome, the human strain EMC/ 2012 (Genbank: JX869059.2) using the BWA software, with a minimum coverage of 100 reads. Indels in nsp3, ORF3, ORF5 and N genes detected in the NGS sequencing were further confirmed by Sanger sequencing.

Phylogenetic analysis

Representative sequences (n = 75) in clades A, B and C of MERS-CoV (Table S1) and a sequence of MERS-CoV-related bat coronavirus (Genbank: KC869678.4) were downloaded from Genbank (All clade C whole genome sequences available in Genbank were included). These representative sequences and 25 whole genome MERS-CoV sequences from Ethiopian camels were first aligned using MAFFT version 6 software and phylogenetic analysis was done using the IQ-TREE (v.2.1.3) employing the GTR + F + R4 nucleotide substitution model (best-fit model searched by IQ-TREE) with the MERS-CoV-related bat coronavirus sequence as outgroup.

Results

Phylogenetic analysis of MERS-CoV in Ethiopia

Of 1766 nasal swab samples collected from January 2017 to January 2020, 61 (3.45%) were MERS-CoV RNA positive. Of 484 turbinate swab samples collected

from September 2019 to September 2020, 10 (2.07%) were detected positive. Twenty-five whole genome sequences of MERS-CoVs were obtained from these samples (sample information listed in Table S2). The location and type of collection site of the sequences obtained are indicated in the Figure 1. Phylogenetic analysis was done using the IQ-TREE with a MERS-CoV-related coronavirus KC869678.4) as an outgroup [27]. In the phylogenetic tree, our result supports the previous classification that all MERS-CoV from Africa are clade C viruses. Within clade C1, clade C1.1 viruses were West African strains from Burkina Faso, Nigeria and Morocco, and C1.2 are East African strains from Sudan, Djibouti, and Egypt. In this study, clade C2 was further divided into two novel subclades designated as C2.1 and C2.2. The newly sequenced Ethiopian camel-originated MERS-CoV fall within clades C2.1 and C2.2, along with other East African camel-originated strains from Sudan, Djibouti, Kenya, and Egypt (Figure 2(A)). A highly distinct Egyptian strain is designated as clade C3 based on a previous study [28].

Molecular epidemiology

Interestingly, of virus sequences collected from herds in 2017, those Gewane (CAC4787, CAC4791, CAC4802), Chifra (CAC4749, CAC4752, CAC4753) and Amibara (CAC4366, HKU4412, HKU4448, HKU4458, CAC4459) clustered within each region and were phylogenetically separate from virus sequences from the Babile slaughterhouse (CAC4849, CAC4855, CAC4868). Virus sequences from camels sampled from herds owned by different owners in the same district clustered together. However, in Amibara, viruses sampled in 2017 and 2019 from this study, and viruses sampled in 2017 from other study (Genbank: MK564475 MK564474) clustered separately [29]. Nonetheless, these sequences from Amibara did not cluster together with sequences from other districts.

Among the sequences collected from a local slaughterhouse in Akaki from September to November 2019, viruses CAC9648 and CAC9650 clustered together, falling into subclade C2.2. However, viruses (CAC9670, CAC9690, CAC9691) obtained from this same slaughterhouse in October (n = 1) or November (n = 2) 2019 clustered separately in clade C2.1 and were markedly different (over 250 nucleotide differences) compared to CAC9648 and CAC9650. Nor were these viruses within each subclade identical to each other. This finding was explained by the fact that slaughterhouses receive camels from geographically diverse areas, thus explaining genetically diverse viruses being detected. In another local slaughterhouse in Babile, viruses (CAC4849, CAC4855, CAC4868) from camels with different origins collected in Oct

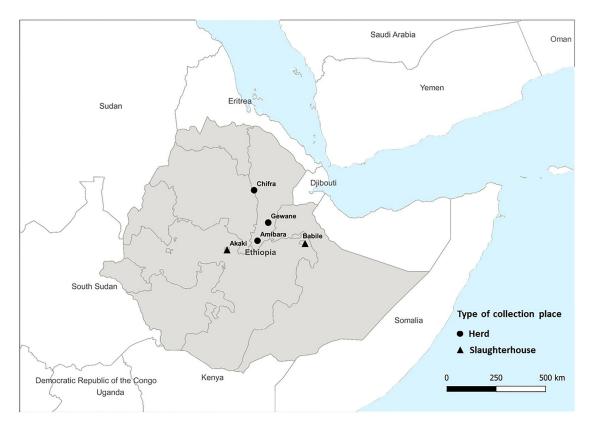


Figure 1. The location and type of collection site of the samples with whole genome sequences. Samples with whole genome sequences were collected in Amibara (n = 11), Gewane (n = 3), and Chifra (n = 3) from Afar region, Babile (n = 3) from Oromia region, and Akaki (n = 5) in the capital city, Addis Ababa.

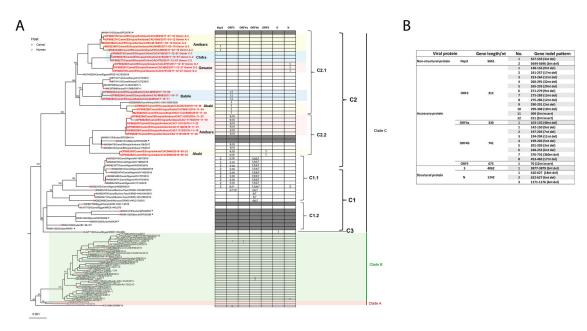


Figure 2. (A) Phylogenetic analysis of MERS-CoV whole genome sequences obtained in this study using IQ-TREE. The tree is rooted against MERS-CoV-related bat coronavirus Coronavirus Neoromicia/PML-PHE1/RSA/2011(GenBank: KC869678.4), which was removed from the tree due to the long branch length. Selective bootstrap values are shown. The MERS-CoV clades designations are denoted based on the previous studies [28,47]. Whole genome sequences labelled in red are from this study and those in black are sequences downloaded from Genbank. S gene partial sequences from the previous study with the length of 1046 bp are labelled with asterisk [28]. Indel patterns of nsp3, ORF3, ORF4a, ORF4b, ORF5, S, and N genes observed in the sequences are shown in the table within the tree (S gene partial sequences and sequence MK967708/Egypt/Camel/AHRI—FAO—1/2018 are excluded). Two clade B Saudi Arabian viruses that show deletions in ORF4b are included for comparison. The sampling places (Amibara, Chifra, Gewane, Babile, Akaki) of the sequences are labelled in the tree. (B) Details of the indel patterns of the sequences in the phylogenetic trees.

2017 clustered together, which indicate the possible virus cross-transmission occurred within the slaughterhouse (Table S2).

Genetic characteristics of MERS-CoV in Ethiopia

Of the total 35 whole genome sequences currently available from East Africa (Egypt = 3; Kenya = 5; Ethiopia = 27) from this and previous studies, 23 (66.7%) virus sequences harboured indels (partial sequences and sequence MK967708/Egypt/Camel/ AHRI-FAO-1/2018 without peer review are excluded). Of the 25 whole genome sequences obtained in Ethiopia in the present study, 19 (76%) sequences harboured indels. These indels were observed in nsp3, ORF3, ORF5 and N gene (Table S1). Unlike East African MERS-CoV, all the whole genome sequences (n = 13) from West Africa available in Genbank (Nigeria = 9; Burkina Faso = 3; Morocco = 1) harboured indels, mainly in ORF3 and ORF4b [30].

It was previously reported that clade C1.1 viruses from Burkina Faso and Nigeria all harboured large ORF4b gene deletions, which contributed to a reduction of replication competence in human respiratory cells [13]. Viruses from our study in Ethiopia did not have ORF4b gene deletions. However, a substitution at the position of G733 T in ORF4b gene in (CAC9648, CAC9650, viruses CAC11175, CAC11179, CAC11181, CAC11188, CAC11191 and CAC11202) led to a premature stop codon, deleting 2 amino acids at the C-terminus of the ORF4b protein (Figures 2 and 3).

Multiple ORF3 indel patterns were observed in our sequences, similar to other clade C1 viruses. The patterns are geographic subclade specific. For example, viruses from Babile (CAC4855, CAC4868 and CAC4849) contained 2nt insertion at the C-terminus of ORF3, causing the frameshift and predicted to extend ORF3 protein with 11 more amino acids. Among 2 subclade viruses from Akaki, CAC9648 and CAC9650 had Pattern 4 and 10 deletions (42nt in total), while the other three (CAC9670, CAC9690 and CAC9691), have Pattern 3 deletion (12nt) in ORF3 gene. In clade C1.1 viruses, Pattern 5 and 10 deletions were found in most of the Nigerian viruses resulting in 15 amino acid deletions. Notably, KX108943/D998/15 from UAE and a clade B virus KU233362/Jordan_10/2015 from Jordan contained a 9nt deletion in ORF3 gene, resulting in 3 amino acid deletions (Figure 3).

Indels of nsp3, ORF5 and N protein were also observed in the sequences obtained from this study but were less frequent than ORF3 changes. In nsp3 gene, clade C2.1 virus HKU4448 and HKU4458 collected from Amibara in 2017 contained a 3nt deletion from position 557-559 resulting in a 1 amino acid

deletion. Viruses from Nigeria in the same subclade of Clade C 1.1 also contained 3nt deletions in nsp3 gene resulting in a 1 amino acid deletion but in a different position. In contrast, a substitution at the position G456A in clade C2.2 virus CAC9650 collected from Akaki slaughterhouse in 2019 led to a premature stop codon and the nsp3 protein was predicted to be 151aa in length, much shorter than that of the reference strain JX869059/EMC/2012 (1887aa in length). Interestingly, viruses CAC9648 and CAC9650 had 4 amino acid insertions in ORF5 protein. In N gene, there were 18nt deletions in sequences collected from Gewane in 2017, predicted to be a 9 amino acid deletion in the protein encoded. N protein deletions were also found in a clade C1.1 virus (NS004) from Nigeria and a clade B virus (KC667074/England-Qatar/2012) (Figure 3).

Nucleotide insertions and deletions were rare in viruses from the Arabian Peninsula. Large ORF4a deletions (48nt) were found in two clade B sequences from a hospital outbreak in Jordan in 2015, but these were different to deletions observed so far from clade C in Africa [31]. While the gene deletions observed in West (Nigeria, Burkina Faso) and North (Morocco) Africa appear to be related (e.g. ORF4b deletion of nucleotides 248-253) with deletions increasing progressively, those in East Africa appear diverse and independent. There was only one example of a deletion (N gene nucleotides 610-627) common between West (MG923472/camel/Nigeria/NS004/ 2015) and East Africa (OP866280/Camel/Ethiopia/ Gewane/CAC4787/2017-12-27).

Discussion

Ethiopia has an estimated 1.6 million dromedary camels and is an important source of camels for trade to Egypt and the Arabian Peninsula through Djibouti and Somalia [32]. With evidence of human infection found in camel-exposed populations in Africa, it is critical to conduct MERS-CoV surveillance studies to understand the risk of zoonotic infection and monitor ongoing virus evolution. In the present study, we investigated MERS-CoV genetic diversity and molecular epidemiology of MERS-CoV in camels

Phylogenetic analysis of whole genome sequences obtained from this study showed that MERS-CoVs from Ethiopia belong to clade C2.1 and C2.2 within the African clade C, clustering with other East African strains. Within Ethiopia, MERS-CoV sequences of 2017 strains clustered geographically by location of sample collection, e.g. those from Amibara, Chifra, and Gewane (Figure 2). This is plausible because camel herders from the same clans in the same districts may graze camels in the same area where there were abundant feed and water sources, thus

10004000	ein	Virus	Clade	Gen	Indel pattern		ct of Indels on protein mutation		Schematic diagrams
				No.	Detail	Detail	Туре	Protein length/aa	
		JX869059/EMC/2012	A		1		1	1887	1 1000 2000 8000 4000 5661H
		OP866286/Camel/Ethiopia/Akaki/CAC9650/2019	C2.2		/	Premature stop codon at 152 because of the G456A substitution in the gene	Premature termination	151	351AA
		MG923467/Carnel/Ethiopia/Amibara/HKU4448/2017-03-15	G.1	1	557-559 (3nt del)	Laa del at position 186	Deletion	1886	
		MG923468/Camel/Ethiopia/Amibara/HKU4458/2017-03-15 MG923472/Camel/Nigeria/NS004/2015							
		MG923474/Camel/Nigeria/NV1465/2016 MG923475/Camel/Nigeria/NV1657/2016							
ion-structural protein	Nsp3	MG923476/Camel/Nigeria/NV1989/2016							
,		MG923477/Camel/Nigeria/NV2040/2016 MG923478 /Camel/Nigeria/NV1673/2016	C1.1	2	5644-5646 (3nt del)	1aa del at position 1882	Deletion	1886	H H H H H H H H H H H H H H H H H H H
		MG923479/Camel/Nigeria/NV1712/2016							
		MG923480/Camel/Nigeris/NV1787/2016 MG923481/Camel/Nigeris/NV2020/2016							
		JX869059/EMC/2012	A		1		/	103	200 240 260 280 312mt
		KX108943/D998/15	Unclassified	6	271-279 (9nt del)	3aa del at position 91-93	Deletion	100	W 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
			Uncassined	*			Desetion		100AA
		XU233362/Jordan_10/2015		1	148-156 (9nt del)	3aa del at position 50-52	Deletion	100	100AA
		MG923473/Camel/Burkina Faso/CIRAD-HKU697/2015	CL1	2 7	241-257 (17nt del) 275-289 (15nt del)	Frameshift at position 81	Frameshift and premature termination	84	De De De
		mostar y centry out the resolution in most y total		10	299-308 (10nt del)	THE POST OF THE PO	Transport and premature termination		
		MG923472/Camel/Nigeria/NS004/2015	C1.1	8	275-286 (12nt del) 309 (3nt insert)	4aa del at position 92-95; stop codon at position 103	Deletion and premature termination	98	
		MG923474/Camel/Nigeria/NV1405/2016 MG923475/Camel/Nigeria/NV1657/2016							
		MG923476/Camel/Nigeria/NV1989/2016							
		MG923477/Camel/Nigeria/NV2040/2016 MG923478 /Camel/Nigeria/NV1673/2016	C1.1	10	265-293 (29nt del) 299-308 (10nt del)	Frameshift at position 89; stop codon at position 89	Frameshift and premature termination	88	No.
		MG923479/Camel/Nigeria/NV1712/2016							38 I I I I I
	UHF3	MG923480/Camel/Nigeris/NV1787/2016 MG923481/Camel/Nigeris/NV2020/2016							
		OP866282/Camel/Ethiopia/Babile/CAC4849/2017-10-11 OP866283/Camel/Ethiopia/Babile/CAC4855/2017-10-09	C2.1	12	311 (2nt insert)	Frameshift at position 104 and 11 amino acids were inserted at the C-terminus	Frameshift and insertion	114	<u> </u>
		OP866284/Camel/Ethiopia/Babile/CAC4858/2017-10-11 OP866287/Camel/Ethiopia/Babile/CAC4858/2017-10-12			(end andres)	inserted at the Oterminus		***	
		MZ268404/Camel/Ethiopia/Akaki/HKU-CAC9690/2019-11-24	C2.1	3	253-264 (12nt del)	4aa del at position 85-88	Deletion	99	38 1 1 1 1 1
		OP866288/Camet/Ethiopia/Akaki/CAC9691/2019-11-24 MZ268405/Camet/Xenya/HRU-CAC10200/2020	46.1	3	213-504 (1516 000)	THE WEST OF THE PROPERTY OF T	Second Se	39	9944
		OP866289/Camel/Ethiogia/Amibara/CAC11175/2019-11-19							10 1 1 1 1
		OP866290/Carnel/Ethiogia/Amibara/CAC11179/2019-11-19 OP866291/Carnel/Ethiogia/Amibara/CAC11181/2019-11-19							
		OP866292/Carnel/Ethiopia/Amibara/CAC11188/2019-11-19 OP866293/Carnel/Ethiopia/Amibara/CAC11191/2019-11-19	C2.2	9	290-291 (2nt del) 299-308 (10nt del)	Frameshift at position 97	Frameshift and premature termination	97	Fran
		OP866294/Camel/Ethiopia/Amibara/CAC11202/2019-11-19		10	239-300 (2010 50)			37	131 1 1 1 1
		MKS64474/Camel/Ethiopia/Amibara/118/2017 MKS64475/Camel/Ethiopia/Amibara/126/2017							
		OP866285/Camel/Ethiopia/Akaki/CAC9648/2019-09-23 OP866285/Camel/Ethiopia/Akaki/CAC9650/2019-09-23	C2.2	4	260-291 (32nt del) 299-308 (10nt del)	Frameshift at position 86	Frameshift and premature termination	87	No. 104
		JX869059/EMC/2012	A	10	/		1	109	1 60 120 180 160 3304
	ORF4a	KU233362/Jordan_10/2015							- LOSAA
Accessory		KU233363/Jordan_1/2015		1	103-150 (48nt del)	16aa del at position at 35-50	Deletion	93	53.44
protein		JX869059/EMC/2012	A		/		/	246	1 150 300 450 600 74tet
		KF600612/Ryadh_1_2012	161		453-469 (17nt del)	Frameshift at position 152	Frameshift and premature termination	157	
		x/600620/8isha_1/2012		2	197-203 (7nt del)				10 mm 10 m
		MG923473/Camel/Burkina Faso/CRAD-HKU697/2015	C1.1	2 6 7	197-203 (7nt del) 248-253 (6nt del) 376-735 (860nt del)	Frameshift at position 66	Frameshift and premature termination	92	300 300
		MG923473/Camel/Burbine Faso/CIRAO-HIXX697/2015 MG923470/Camel/Burbine Faso/CIRAO-HIXX697/2015		2 6 7 6	197-203 (7nt del) 248-253 (6nt del) 376-735 (860nt del) 248-253 (6nt del)	Frameshift at position 66			20 MA
		MG923473/Camel/Burkins faso/CRAO-HXX897/2015 MG923473/Camel/Burkins faso/CRAO-HXX847/2015 MG923473/Camel/Burkins faso/CRAO-HXX847/2015	Cl.1	7 6 7 4	197-203 (7nt del) 248-253 (6nt del) 376-735 (360nt del) 248-253 (6nt del) 376-735 (360nt del) 199-203 (5nt del)	Frameshift at position 66 Premature stop codon at 15 because of the C43T substitution in the gene	Frameshift and premature termination Premature termination	92	9 9 9
		MG923473/Camel/Burbine Faso/CIRAO-HIXX697/2015 MG923470/Camel/Burbine Faso/CIRAO-HIXX697/2015	C1.1	7	197-203 (7nt del) 248-253 (6nt del) 376-735 (360nt del) 248-253 (6nt del) 376-735 (360nt del)	Frameshift at position 66	Frameshift and premature termination	92	9 9 9
		M0292413/Cmw/8buline fram/CRAC-H0X097/2015 M0923473/Cmw/8buline fram/CRAC-H0X047/2015 M0923473/Cmw/8buline fram/CRAC-H0X447/2015 M0923473/Cmw/8buline fram/CRAC-H0X447/2015 M0923480/Cmw/8buline fram/CRAC-H0X47/2015 M0923480/Cmw/8Anorout/SRAC-H0X2131/2015	CL1 CL1	7 6 7 4	197-203 (7nt del) 248-253 (6nt del) 376-735 (360nt del) 248-253 (6nt del) 376-735 (360nt del) 199-203 (5nt del) 248-253 (6nt del) 376-735 (360nt del) 48-259 (6nt del)	Frameshiff at position 66 Premature floor codon at 35 because of the C43T substitution in the gene Frameshiff at position 67	Frameshift and premature termination Premature termination Frameshift and premature termination	92 14 80	
		MG923473/Camel/Burkins faso/CRAO-HXX897/2015 MG923473/Camel/Burkins faso/CRAO-HXX847/2015 MG923473/Camel/Burkins faso/CRAO-HXX847/2015	Cl.1	7 6 7 4 6 7	197-203 (7nt del) 248-253 (fint del) 376-735 (Biden del) 142-150 (9nt del) 142-150 (9nt del) 194-204 (11nt del) 248-253 (fint del)	Frameshift at position 66 Premature stop codon at 15 because of the C43T substitution in the gene	Frameshift and premature termination Premature termination	92	1555
		M032247/Came/Burkin Fasc(PARA-HRA097/205 M032247/Came/Burkin Fasc(PARA-HRA097/205 M032247/Came/Burkin Fasc(PARA-HRA097/205 M032248/Came/Burkin Monrous/CARA-HRA017/205 M032248/Came/Monrous/CARA-HRA017/205 M032248/Came/Monrous/CARA-HRA017/205 M032248/Came/Monrous/Pasc(PARA-HRA017/205)	CL1 CL1	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 248-253 (fert del) 249-253 (fert del) 248-253 (fert del) 248-253 (fert del) 248-253 (fert del) 142-150 (5ht del) 142-150 (5ht del) 378-755 (5fert del) 378-755 (5fert del) 378-755 (5fert del)	Frameshift at position 66 Preventure stop codes at 35 because of the C43T substitution in the game Frameshift at position 67 Jaa del at position 65,00 Frameshift at position 65	Frameshift and premature termination Premature termination Frameshift and premature termination	92 14 80	
	ORF4b	MG23473C-amil@units FanCERA-HD0877255 MG23473C-amil@units FanCERA-HD0877255 MG23476C-amil@units FanCERA-HD0847255 MG23476C-amil@units FanCERA-HD0847255 MG23446C-amil@units FanCERA-HD08727253 MG23446C-amil@units FanCERA-HD08727253 MG23446C-amil@unitsFanCA-HD08727253 MG23446C-amil@unitsFanCA-HD08727253 MG23476C-amil@unitsFanCA-HD08727253 MG23476C-amil@unitsFanCA-Amil@unitsFanCA-HD08727253 MG23476C-amil@unitsFanCA-Amil@unitsF	CL1 CL1	7 6 7 4 6 7 1 3	197-203 (7mt del) 248-253 (fent del) 248-253 (fent del) 248-253 (fent del) 248-255 (fent del)	Frameshift at position 66 Frameshire drop codon at 15 because of the CGIT Frameshift at position 67 Sau del at position 65 Sau del at position 65 Frameshift at position 65 Jau del at position 65 Jau del at position 65 Jau del at position 65	Frameshift and premature termination Premature termination Frameshift and premature termination	92 14 80	
	ORF4b	M0232476_came/fluvrise facu(CRIAD-HRUBRI72ES M0232476_came/fluvrise facu(CRIAD-HRUBRI72ES M0232476_came/fluvrise facu(CRIAD-HRUBRI72ES) M0232476_Came/fluvrise facu(CRIAD-HRUBRI72ES) M0232476_Came/flugeris/M024676_CRIAD-HRUBRI72ES M0232476_Came/flugeris/M024676_CRIAD-HRUBRI72ES M0232476_Came/flugeris/M024676_CRIAD-HRUBRI72ES M0232476_Came/flugeris/M024676_CRIAD-HRUBRI72ES M02324776_Came/flugeris/M024676_CRIAD-HRUBRI72ES M02324776_Came/flugeris/M02467ES M0232476_CAME/flugeris/M02467ES M0232476_CAME/flugeris/M02467E	CL1 CL1	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Framewith as position 66 Framewith as top codes at 33 because of the COTT substruction in the gene Framewith as position 65 Sae del al position 65-00.	Frameshift and premature termination Premature termination Frameshift and premature termination	92 14 80	
	ORF4b	M0232473Came/Burkin Fasa(CRA0-HR08077255 M0232473Came/Burkin Fasa(CRA0-HR08077255 M0232473Came/Burkin Fasa(CRA0-HR08077255 M0232473Came/Burkin Fasa(CRA0-HR08072555 M0232485Came/Boorco/CRA0-HR071317253 M023245CACame/Boorco/CRA0-HR071317253 M0232474Came/Boorco/CRA0-HR071317253 M023247453 M023247453 M02324745	G71 G72 G73	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 248-253 (fert del) 248-253 (fert del) 248-253 (fert del) 248-253 (fert del) 376-755 (560nt del) 248-253 (fert del) 248-253 (fert del) 248-253 (fert del) 142-150 (9ht del) 142-255 (fert del) 376-755 (560nt del) 142-250 (fert del) 248-253 (fert del)	Frameshift as positions 66 Frameshift as specified as 13 Securise of the CGIT substitution in the game Frameshift as positions 67 has del as positions 65-50; Frameshift as positions 65-50; Jaca del as positions 65-50; Jaca del as positions 65-50; Jaca del as positions 67-50; Jaca del as positions 67-50; Jaca del as positions 67-50;	Frameshilt and premature termination Premature termination Frameshilt and premature termination Deletion; Frameshilt and premature termination	92 14 80 75	752
	ORF4b	M0232473Cammil Burker I RanCPEAD-H020977255 M0232473Cammil Burker I RanCPEAD-H020977255 M0232473Cammil Burker I RanCPEAD-H020477255 M0232473Cammil Burker I RanCPEAD-H02047255 M0232476Cammil Burker I RanCPEAD-H02047255 M0232476Cammil Burker I RanCPEAD-H02047255 M0232476Cammil Burker I RanCPEAD-H02047255 M0232476Cammil Burker I RanCPEAD-H0204755 M0232476Cammil Burker I RanCPEAD-H02047255 M0232476Cammil Burker I RanCPEAD-H0204725 M0232476Cammil Burker I RanCPEAD-H020	G71 G72 G73	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Framewith as position 66 Framewith as top codes at 33 because of the COTT substruction in the gene Framewith as position 65 Sae del al position 65-00.	Frameshilt and premature termination Premature termination Frameshilt and premature termination Deletion; Frameshilt and premature termination	92 14 80 75	752
	ORF4b	M02247/Came/Burkin Fast(CRAD-HRABI7/205 M02247/Came/Burkin Fast(CRAD-HRABI7/205 M02247/Came/Burkin Fast(CRAD-HRABI7/205 M02248/Came/Bornous/CRAD-HRAI7/205 M02248/Came/Bornous/CRAD-HRAI7/205 M02248/Came/Bornous/CRAD-HRAI7/205 M02247/Came/Bor	G71 G72 G73	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Framewith as position 66 Framewith as top codes at 33 because of the COTT substruction in the gene Framewith as position 65 Sae del al position 65-00.	Frameshilt and premature termination Premature termination Frameshilt and premature termination Deletion; Frameshilt and premature termination	92 14 80 75	752
	ORFAL	M0232473Came/Burkin Fasa(CRAA-HADRI7255 M0232473Came/Burkin Fasa(CRAA-HADRI7255 M0232473Came/Burkin Fasa(CRAA-HADRI7255 M0232486Came/Boorca/CRAA-HADRI7255 M0232486Came/Boorca/CRAA-HADRI7255 M0232486Came/Boorca/CRAA-HADRI7255 M0232345Came/Boorca/CRAA-HADRI7255 M0232345Came/Boorca/M02325 M023235Came/Boorca/M02325 M023235Came	G71 G72 G73	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Framewith as position 66 Framewith as top codes at 33 because of the COTT substruction in the gene Framewith as position 65 Sae del al position 65-00.	Frameshilt and premature termination Premature termination Frameshilt and premature termination Deletion; Frameshilt and premature termination	92 14 80 75	752
	ORF4b	MG232473Came/Burkin Fasa(CRAD-HRAB/7205 MG232473Came/Burkin Fasa(CRAD-HRAB/7205 MG232473Came/Burkin Fasa(CRAD-HRAB/7205 MG232473Came/Burkin Fasa(CRAD-HRAB/7205 MG232473Came/Burkin Fasa(CRAD-HRAB/7205 MG232473Came/Burkin Fasa(CRAD-HRAB/7205) MG232473Came/Burkin Fasa(GRAD-HRAB/7205) M	G.1 G.1 G.1	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Frameshin as positions 66 Premotives stop codes at 13 because of the COT substitutions in the gare. Frameshiff as position 67 Saw did at position 65 50; Frameshiff as position 65 50; Frameshiff as position 65 50; Jan did at position 65 50; Jan did at position 67 Jan did at position 69	Frameshift and premature termination Premature termination Frameshift and premature termination Deletions, Frameshift and premature termination Deletions and truncation	92 14 80 75	752
	ORF46	M0232473Came/Burkin Fasic/Stub - H004977255 M0232473Came/Burkin Fasic/Stub - H004977255 M0232473Came/Burkin Fasic/Stub - H004977255 M0232486Came/Burkin Fasic/Stub - H0047257255 M0232486Came/Moreout/CMD- H0072572553 M0232486Came/Moreout/CMD- H0072572553 M0232474Came/Moreout/Stub - H0072572553 M0232474Came/Moreout/More	G71 G72 G73	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Framewith as position 66 Framewith as top codes at 33 because of the COTT substruction in the gene Framewith as position 65 Sae del al position 65-00.	Frameshift and premature termination Premature termination Frameshift and premature termination Deletions, Frameshift and premature termination Deletions and truncation	92 14 80 75	752
	ORF6b	M0232473Cammil Burkins FasaCPEA0-H004977255 M0232473Cammil Burkins FasaCPEA0-H004977255 M0232473Cammil Burkins FasaCPEA0-H004877255 M0232473Cammil Burkins FasaCPEA0-H00487255 M0232473Cammil Burkins FasaCPEA0-H0072137255 M0232473Cammil Burkins FasaCPEA00-H0072137255 M0232473Cammil Burkins FasaCPEA000-H0072137255 M0232	G.1 G.1 G.1	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Frameshirs a position 66 Permitten days coders at 15 liceuses of the COT administration has gained. Permitten this particular in the gained are considered in the gained and administration of the considered and administration of the	Frameshift and premature termination Premature termination Frameshift and premature termination Deletions, Frameshift and premature termination Deletions and truncation	92 14 80 75	752
	ORF4b	MOSTATINE CHARGE PLANS PRANCE PLANS PRANCE PLANS	G.1 G.1 G.1	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Frameshirs a position 66 Permitten days coders at 15 liceuses of the COT administration has gained. Permitten this particular in the gained are considered in the gained and administration of the considered and administration of the	Frameshift and premature termination Premature termination Frameshift and premature termination Deletions, Frameshift and premature termination Deletions and truncation	92 14 80 75	752
	ORF4b	M0232473Came/Burkin Fasa(CRAD-HRABI72ES) M0232473Came/Burkin Fasa(CRAD-HRABI72	G1 G	7 6 7 4 6 7 1 3 6 7	197-200 (Pm 4ed) 240-230 (Pm 4ed) 376-275 (Sibbri ed) 376-275 (Sibri ed)	Frameshim as positions 66 Permettion risp roder at 15 lineauser of the COT solution for the gater Frameshim is the gater Associated to the gater of the COT solution of the COT solution 65 db; Frameshim is providen 65 Jana 64 at a position 65 db; Jana 64 at a position 65 db; Jana 64 at a position 67, Jan 64 at position 62, Jana 64 at a position 12, Jana 64 at a	Frameshift and premature termination Premature termination Frameshift and premature termination Duletion, Frameshift and premature termination Duletion and truncation Premature termination	92 14 80 75	752
	ORF4b	MOSTATINE CHARGE PLANS PRANCE PLANS PRANCE PLANS	G.1 G.1 G.1	7 6 7 4 6 7 1 3 6 7	197-203 (7ht del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2481-253 (fert del) 2491-253 (fert del) 199-203 (5rt del) 2491-253 (fert del)	Frameshirs a position 66 Permitten days coders at 15 liceuses of the COT administration has gained. Permitten this particular in the gained are considered in the gained and administration of the considered and administration of the	Frameshift and premature termination Premature termination Frameshift and premature termination Deletions, Frameshift and premature termination Deletions and truncation	92 14 80 75	752
		M0232473Came/Burkin Fasa(CRAD-HRABI72ES) M0232473Came/Burkin Fasa(CRAD-HRABI72	G1 G	7 6 7 4 6 7 1 3 6 7	197-200 (Pm 4ed) 240-230 (Pm 4ed) 376-275 (Sibbri ed) 376-275 (Sibri ed)	Frameshim as positions 66 Premature day roder at 15 because of the COT sold indicates to the gate. Premature day roder at 15 because of the COT sold indicates to the gate. San did as position 65 50; Frameshim as position 65 50; Frameshim as position 65 50; Jans did as po	Frameshift and premature termination Premature termination Frameshift and premature termination Duletion, Frameshift and premature termination Duletion and truncation Premature termination	92 14 80 75	
	ORF4b	M023247/Came/Burkin Fasa(*Pabo* HR08/17/205 M023247/Came/Burkin Fasa(*Pabo* HR08/17/205 M023247/Came/Burkin Fasa(*Pabo* HR08/17/205 M023247/Came/Burkin Fasa(*Pabo* HR08/17/205) M023247/Came/Burkin Fasa	G.1 G.1 G.1 G.2 G.2 B. B. A.	7 6 7 4 6 6 7 1 1 3 6 7 7	197-200 (Prie del) 240-230 (Prie del) 370-792 (Morie del)	Frameshim as positions 66 Permettion risps roders at 15 lineases of the COT abolish fides in the galaxy Frameshir as positions 67 Sau 66 et a position 65-50; Frameshir as positions 65 Sau 66 et a position 65-50; Frameshir as positions 65. Sau 66 et a position 65-70; Sau 66 et a position 67. Zau 66 et a position 67. Zau 66 et a position 67. Zau 66 et a position 67. Frameshir et 66. Frameshir et 67. Frameshir e	Frameshift and premature termination Premature termination Frameshift and premature termination Ouletion, Frameshift and premature termination Defection and frameshift and premature termination Premature termination	92 14 80 75 120 244	
	ORFS	M0232473;Camirli Burkin Fata (CRAD-HADRI77255 M0232473;Camirli Burkin Fata (CRAD-HADRI77255 M0232473;Camirli Burkin Fata (CRAD-HADRI77255 M02324745;Camirli Burkin Fata (CRAD-HADRI77255 M0232475;Camirli Burkin M020420;M0231251 M0232475;Camirli Burkin M020420;M0232475;Camirli Burkin M020420;M0232475;Camirli Burkin M020420;M023475;Camirli Burkin M020420;M023475;Camirli Burkin M020420;M023475;Camirli Burkin M020420;M023475;M0204200420;M020420;M020420;M020420;M020420;M020420;M020420;M020420;M02	G.3 G.1 G.1 G.3 G.3 G.3 G.3 G.4 G.3	7 6 7 4 6 7 1 3 6 7	197-200 (Pris del) 240-230 (Iris del) 376-735 (Sibiri del)	Frameshirs a position 66 Permitter day coders at 15 leavant of the COT admitted in the garde Frameshir at position 45 to 15 miles and 15 leavant of the COT admitted in the garden See of at position 45 to 15 miles and 15 mil	Frameshift and premature termination Premature termination Frameshift and premature termination Orderion, Frameshift and premature termination Orderion and transaction Premature termination Premature termination	92 14 80 75 120 244 98 224 228	
	ORFS	M023247/Came/Burkin Fasa(*Pabo* HR08/17/205 M023247/Came/Burkin Fasa(*Pabo* HR08/17/205 M023247/Came/Burkin Fasa(*Pabo* HR08/17/205 M023247/Came/Burkin Fasa(*Pabo* HR08/17/205) M023247/Came/Burkin Fasa	G.1 G.1 G.1 G.2 G.2 B. B. A.	7 6 7 4 6 6 7 1 1 3 6 7 7	197-200 (Prie del) 240-230 (Prie del) 370-792 (Morie del)	Frameshirs a position 66 Permitter day coders at 15 leavant of the COT admitted in the garde Frameshir at position 45 to 15 miles and 15 leavant of the COT admitted in the garden See of at position 45 to 15 miles and 15 mil	Frameshift and premature termination Premature termination Frameshift and premature termination Ouletion, Frameshift and premature termination Defection and frameshift and premature termination Premature termination	92 14 80 75 120 244	
	ORFS	M0232473;Camirli Burkin Fata (CRAD-HADRI77255 M0232473;Camirli Burkin Fata (CRAD-HADRI77255 M0232473;Camirli Burkin Fata (CRAD-HADRI77255 M02324745;Camirli Burkin Fata (CRAD-HADRI77255 M0232475;Camirli Burkin M020420;M0231251 M0232475;Camirli Burkin M020420;M0232475;Camirli Burkin M020420;M0232475;Camirli Burkin M020420;M023475;Camirli Burkin M020420;M023475;Camirli Burkin M020420;M023475;Camirli Burkin M020420;M023475;M0204200420;M020420;M020420;M020420;M020420;M020420;M020420;M020420;M02	G.3 G.1 G.1 G.3 G.3 G.3 G.3 G.4 G.3	7 6 7 4 6 6 7 1 1 3 6 7 7	197-200 (Pris del) 240-230 (Iris del) 376-735 (Sibiri del)	Frameshirs a position 66 Permitter day coders at 15 leavant of the COT admitted in the garde Frameshir at position 45 to 15 miles and 15 leavant of the COT admitted in the garden See of at position 45 to 15 miles and 15 mil	Frameshift and premature termination Premature termination Frameshift and premature termination Orderion, Frameshift and premature termination Orderion and transaction Premature termination Premature termination	92 14 80 75 120 244 98 224 228	
	ORFS S	M0232473Camel@unites FascCRAD-H02097255 M0232473Camel@unites FascCRAD-H02097255 M0232473Camel@unites FascCRAD-H02047255 M0232474Camel@unites FascCRAD-H020472555 M0232474Camel@unites FascCRAD-H020472555 M0232474Camel@unites FascCRAD-H0204725555 M0232474Camel@unites FascCRAD-H0204725555 M0232474Camel@unites FascCRAD-H02047255555 M0232474Camel@unites FascCRAD-H0204725555555555555555555555555555555555	G1 G	7 6 7 4 6 7 7 1 1 3 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	187-200 (Pris del) 248-230 (En del) 378-778 (Silvin del)	Frameshirs a position 66 Permitter stop coders at 15 because of the COT solitothoris. In the gare Frameshirs at position 67 Frameshirs at position 65 Insertable of position 650, Frameshirs at position 65 Sale of a position 650, Frameshirs at position 650, Frameshirs at position 650, Frameshirs 650, Fr	Frameshift and premature termination Premature termination Frameshift and premature termination Deletion, Frameshift and premature termination Deletion and transaction Premature termination Premature termination J Deletion	92 14 80 75 120 244 98 224 228 1353	
Structural	ORFS S	M032247Ccame/fluvius fau:CRAD-HRADI725S M032247Ccame/fluvius fau:CRADI725S M032247Ccame/flu	G.3 G.1 G.1 G.2 G.3	7 6 7 4 6 7 7 1 1 3 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	197-200 (Pris del) 240-230 (Pris del) 376-775 (Silvin del)	Frameshirs a position 66 Permitter stop coders at 15 because of the COT solitothoris. In the gare Frameshirs at position 67 Frameshirs at position 65 Insertable of position 650, Frameshirs at position 65 Sale of a position 650, Frameshirs at position 650, Frameshirs at position 650, Frameshirs 650, Fr	Frameshift and premature termination Premature termination Frameshift and premature termination Deletion, Frameshift and premature termination Outside and transaction Premature termination Premature termination I	92 14 80 75 120 244 98 224 228	
Structural	ORFS 5	M0232477Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-HR08172557255 M023247Came/Burstin Fass(CRAD-HR0817257255) M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-H	G1 G	7 6 7 4 6 7 7 1 1 3 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	187-200 (Pris del) 248-230 (En del) 378-778 (Silvin del)	Frameshirs a position 66 Permitter stop coders at 15 because of the COT solitothoris. In the gare Frameshirs at position 67 Frameshirs at position 65 Insertable of position 650, Frameshirs at position 65 Sale of a position 650, Frameshirs at position 650, Frameshirs at position 650, Frameshirs 650, Fr	Frameshift and premature termination Premature termination Frameshift and premature termination Deletion, Frameshift and premature termination Deletion and transaction Premature termination Premature termination J Deletion	92 14 80 75 120 244 98 224 228 1353	
	ORFS 5	\$M323477\Careft Pure Village Intel (Village)	G1 G	7 6 7 4 4 6 7 1 3 6 7 7 1 5 6 7 7	187-200 (Pri	Frameshirs a position 66 Permitter stop coders at 15 because of the COT solutions in the gares. Permitter stop coders at 15 because of the COT solutions in the gares. Permitter stop position 65 db. (Frameshir at position 65 db.) Frameshir at position 65 db. Frameshi	Frameshift and premature termination Premature termination Frameshift and premature termination Deletion, Frameshift and premature termination Deletion, Frameshift and premature termination Premature termination Premature termination I Insertion J Deletion J Deletion	92 14 80 75 120 244 98 224 228 1353 1352 413 407	
	ORFS 5	M0232477Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08077255 M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-HR08172557255 M023247Came/Burstin Fass(CRAD-HR0817257255) M023247Came/Burstin Fass(CRAD-HR08172555) M023247Came/Burstin Fass(CRAD-H	G1 G	7 6 7 4 4 6 7 7 1 3 6 7 7 7	197-200 (Pris del) 308-230 (Pris del) 378-778 (Silvin del)	Frameshirs approached in Statement of the COST advisionables in the game in the COST advisionables in the game in COST advisionables in the game in COST advisionables in the game in COST advisionable of Statementh of public of State of a position 65 db. Frameshir of public of State of a position 65 db. Take of all of public of State of a position 65 db. Take of all of public of State of a position 65 db. Take of a public of State of a position 164 db. Take of a public of the State of a position 164 db. Take of a public of the State of the COST between of the C	Frameshift and premature termination Premature termination Frameshift and premature termination Deletion, Frameshift and premature termination Deletion and transaction Premature termination Premature termination J Insertion	92 14 80 75 120 244 98 224 228 1333 1352 413	

Figure 3. Schematic of the indels and their impact on encoded proteins. A clade A prototype virus, JX869059/EMC/2012 served as reference.

facilitating transmission of the viruses within and among herds through direct or indirect contact. Even though there were three different phylogenetic clusters of virus sequences from Amibara, these sequences formed separate subclades, genetically different to those from other districts. It is likely that our sampling was not representative of the whole area of the Amibara district and there may be more diverse MERS-CoV circulating in the area. Across the whole virus genome sequenced (around 30 kb), viruses within the same district had 0-25 nucleotide differences while the nucleotide differences among different districts was greater, ranging from 62 to 275.

However, virus sequences obtained from the camel slaughterhouse in Akaki showed greater genetic diversity (from 4 to 262 nucleotide differences across the approx. 30 kb genome sequenced) reflecting the fact that camels of different sources were held and slaughtered in slaughterhouses. Dromedary camels experimentally infected with MERS-CoV started to shed infectious virus from 1-2 days after inoculation (10⁷TCID50/ml, high dose of inoculation), and kept shedding virus until day 7 [33]. Thus, the conditions in slaughterhouses with camels from different sources being kept from several days to weeks before being slaughtered, provide optimal conditions for virus cross-transmission and for amplification of virus infection and explains the greater virus genetic heterogeneity observed in the samples collected there.

Nineteen (76%) of the 25 full genome sequences obtained in the present study had genetic insertions, deletions or frame-shift mutations. Previous studies had reported nucleotide deletions or insertions in ORF3, ORF5 and ORF4b accessory genes in African camel MERS-CoV [13,23,28]. Thus, Ethiopian cameloriginated MERS-CoV appears to demonstrate even greater genetic instability with the detection of various nucleotide indels in nsp3, ORF3, ORF5 and N genes, but not in ORF4b gene as was observed in West Africa. In the sequences obtained from the present study we confirmed that the observed indels were not minor subpopulations within the intra-host genetic diversity by variant-calling analysis and Sanger sequencing. Furthermore, we have virus isolates from four of these specimens and the virus isolates had the same genetic deletions observed in the original specimen (unpublished data) indicating the viability of these viruses. In contrast, such frequent and diverse nucleotide indels have rarely been seen in clade B strains which are enzootic in camels in the Arabian Peninsula.

Given the role of nsp3, ORF3, ORF5 and N protein in the virus replication cycle, it is important to explore the impact of indels observed in those genes of the Ethiopian camel-originated MERS-CoV on the virus phenotype, to further understand its zoonotic risk. Nsp3 protein of MERS-CoV is important for virus replication through inducing the formation of double-membrane vesicles (DMV) [34]. It encodes several domains, including papain-like protease (PLpro) which can proteolytically cleave the junction from nsp1 to nsp4 and act as an interferon antagonist [35,36]. However, the single amino acid deletion found in Ethiopian and Nigerian camel-originated MERS-CoVs is not in the PLpro domain and the implication of this deletion is unknown to date. The diversity of indel patterns in ORF3 and ORF5 gene in Ethiopian camel-originated MERS-CoV is of interest. An in vitro study demonstrated that ORF3 protein of MERS-CoV is able to promote cell apoptosis, similar with the ORF3a protein of SARS-CoV and SARS-CoV-2 [37–39]. The deletions of accessory gene ORF3-5 (ORF3, -4a, -4b, and -5) have been shown to attenuate the virus replication competence and increase the INF- β and IFN- λ response and ORF5 likely modulates NF-kB-mediated inflammation [40]. Still, the implication of these indel patterns of ORF3 and ORF5 genes on viral phenotype remains unknown, unless functional studies are carried out.

The deletions observed in N protein in Ethiopian camel-originated MERS-CoV are also interesting. N protein of coronaviruses packages the viral RNA to form a nucleocapsid and involves in other functions including virus replication and evasion of the innate immune response [41-43]. MERS-CoV N protein can also act as an IFN antagonist, inhibiting type I and type III interferon induction by targeting RIG-I signalling [44]. In the host cell life cycle, MERS-CoV N protein interacts with human translation elongation factor 1A(EF1A) to inhibit cytokinesis and cell proliferation [45]. These findings imply that N protein may play a role in pathogenesis. Therefore, it is of interest to explore the functional significance of the deletions found in MERS-CoV. The implication of genetic instability of MERS-CoV in camels in East Africa warrants more investigation and functional characterization is needed.

Unlike West African strains (Nigeria and Burkina Faso) with large ORF4b deletions, no ORF4b gene deletions were observed in our sequences from Ethiopia. Phenotypic characterization showed that all African clade C viruses studied, whether with (West African strains from Nigeria and Burkina Faso) or without large ORF4b deletions (some East African strains from Ethiopia and Kenya) had reduced replication competence in a human airway Calu-3 cell line, in ex vivo cultures of human lung and bronchus, and in the lungs of an *in vivo* mouse model, when compared to clade A and B viruses from Saudi Arabia [13,14]. Since the Ethiopian and Kenyan viruses we studied phenotypically had no ORF4b deletions, the difference in observed phenotype was not primarily determined by the ORF4b deletions. Similar studies are needed to phenotypically characterize the newly observed genetic indels in some of the Ethiopian viruses detected in this study.

Overall, the frequency and diversity of genome indels observed in African clade C viruses reported in this study indicates the virus genetic instability and raises the possibility that MERS-CoV is a virus that is still adapting to its ecologic niche in this region. There is little surveillance or virus genetic data from regions of Central Asia, East of the Arabian Peninsula and such information is important to assess whether this genetic instability is also observed outside the African Continent.

This study has contributed to the existing knowledge on the transmission dynamics and the genetic characteristics of MERS-CoV in dromedary camels in Ethiopia and Eastern Africa. The frequency and diversity of nucleotide insertions and deletions observed in African MERS-CoV suggesting genetic instability of MERS-CoV in camels raise the concern about the possibility of the emergence of novel viruses with unexpected transmissibility and pathogenicity for humans. A deletion in ORF8 gene was associated with the SARS-CoV-1 acquiring efficient transmission in humans[46]. The high camel density and high prevalence of MERS-CoV infection in camels in East Africa, evidence of human infection in Africa and the observed genetic instability of MERS-CoV in East Africa strongly suggest that further surveillance of MERS-CoV in camels and humans in Africa is critically needed in order to better understand the zoonotic risk and monitor the virus evolution.

Acknowledgements

The views and opinions expressed in this paper are those of the authors and are not necessarily the views of USAID and FAO.



Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was supported by research grants from the US National Institutes of Health (contract no. U01-Grant AI151810) (MP, AA); the Health and Medical Research Fund Research on Control of Infectious Diseases (Phase IV) - Control of emerging, epidemic and endemic infectious diseases (Project no.: CID-HKU2). Field studies in Ethiopia were supported through the Food and Agriculture Organization of the United Nations (FAO) project OSRO/GLO/505/ USA, funded by the United States Agency for International Development. The views and opinions expressed in this paper are those of the authors and are not necessarily the views of USAID and FAO. Centre for PanorOmic Sciences was not a funder. We simply acknowledge technical support from colleagues at the Centre for PanorOmic Sciences, which is a core facility of the University of Hong Kong.

ORCID

Emma Gardner http://orcid.org/0000-0002-5270-6916 Gezahegne Mamo http://orcid.org/0000-0001-7886-5475 Malik Peiris http://orcid.org/0000-0001-8217-5995

References

- [1] Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012 Nov 8;367(19):1814-1820.
- [2] WHO. MERS Situation Update June 2022; 2022.
- [3] Azhar EI, El-Kafrawy SA, Farraj SA, et al. Evidence for camel-to-human transmission of MERS coronavirus. N Engl J Med. 2014 Jun 26;370(26):2499-2505.
- [4] https://www.fao.org/statistics/eni [Internet]; 2020.
- [5] Sikkema RS, Farag E, Islam M, et al. Global status of Middle East respiratory syndrome coronavirus in dromedary camels: a systematic review. Epidemiol Infect. 2019 Jan;147:e84.
- [6] Muller MA, Corman VM, Jores J, et al. MERS coronavirus neutralizing antibodies in camels, Eastern Africa, 1983-1997. Emerg Infect Dis. 2014 Dec;20(12):2093-2095.
- [7] Miguel E, Chevalier V, Ayelet G, et al. Risk factors for MERS coronavirus infection in dromedary camels in Burkina Faso, Ethiopia, and Morocco, 2015. Euro Surveill. 2017 Mar 30;22(13).
- [8] Ali M, El-Shesheny R, Kandeil A, et al. Cross-sectional surveillance of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels and other mammals in Egypt, August 2015 to January 2016. Euro Surveill. 2017 Mar 16;22(11).
- [9] Hemida MG, Chu DK, Poon LL, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis. 2014 Jul;20(7):1231-1234.
- [10] Ki M. MERS outbreak in Korea: hospital-to-hospital transmission. Epidemiol Health. 2015;2015(37):e2015033.
- [11] Korea Centers for Disease C, Prevention. Middle East Respiratory Syndrome Coronavirus Outbreak in the Republic of Korea. (2015). Osong Public Health Res Perspect. 2015 Aug;6(4):269-278.

- [12] Balkhy HH, Alenazi TH, Alshamrani MM, et al. Notes from the field: nosocomial outbreak of Middle East Respiratory Syndrome in a large tertiary care hospital-Riyadh, Saudi Arabia, 2015. MMWR Morb Mortal Wkly Rep. 2016 Feb 19;65(6):163-164.
- [13] Chu DKW, Hui KPY, Perera R, et al. MERS coronaviruses from camels in Africa exhibit region-dependent genetic diversity. Proc Natl Acad Sci U S A. 2018 Mar 20;115(12):3144-3149.
- [14] Zhou Z, Hui KPY, So RTY, et al. Phenotypic and genetic characterization of MERS coronaviruses from Africa to understand their zoonotic potential. Proc Natl Acad Sci USA. 2021 Jun 22;118(25):e2103984118.
- [15] Liljander A, Meyer B, Jores J, et al. MERS-CoV antibodies in humans, Africa, 2013-2014. Emerg Infect Dis. 2016 Jun;22(6):1086–1089.
- [16] Farag E, Sikkema RS, Mohamedani AA, et al. MERS-CoV in camels but not camel handlers, Sudan, 2015 and 2017. Emerg Infect Dis. 2019 Dec;25(12):2333-2335.
- [17] So RT, Perera RA, Oladipo JO, et al. Lack of serological evidence of Middle East respiratory syndrome coronavirus infection in virus exposed camel abattoir workers in Nigeria, 2016. Eurosurveillance 2018; 23(32):1800175.
- [18] Alshukairi AN, Zheng J, Zhao J, et al. High prevalence of MERS-CoV infection in camel workers in Saudi Arabia. mBio. 2018 Oct 30;9(5):e01985-18.
- [19] Muller MA, Meyer B, Corman VM, et al. Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study. Lancet Infect Dis. 2015 May;15 (5):559-564.
- [20] Choe PG, Perera R, Park WB, et al. MERS-CoV antibody responses 1 year after symptom onset, South Korea, 2015. Emerg Infect Dis. 2017 Jul;23(7):1079-1084.
- [21] Mok CKP, Zhu A, Zhao J, et al. T-cell responses to MERS coronavirus infection in people with occupational exposure to dromedary camels in Nigeria: an observational cohort study. Lancet Infect Dis. 2021 Mar;21(3):385-395.
- [22] Munyua PM, Ngere I, Hunsperger E, et al. Low-level Middle East Respiratory Syndrome Coronavirus among Camel Handlers, Kenya, 2019. Emerg Infect Dis. 2021;27(4):1201-1205.
- [23] Ngere I, Hunsperger EA, Tong S, et al. Outbreak of Middle East Respiratory Syndrome Coronavirus in camels and probable spillover infection to humans in Kenya. Viruses. 2022 Aug 9;14(8):1743.
- [24] WHO. Laboratory testing for Middle East Respiratory Syndrome Coronavirus.
- [25] Corman VM, Eckerle I, Bleicker T, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012 Sep 27;17(39):20285.
- [26] Hemida MG, Chu DKW, Chor YY, et al. Phylogenetic analysis of MERS-CoV in a Camel Abattoir, Saudi Arabia, 2016-2018. Emerg Infect Dis. 2020 Dec;26 (12):3089-3091.
- [27] Ithete NL, Stoffberg S, Corman VM, et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg Infect Dis. 2013 Oct;19(10):1697-1699.
- [28] El-Kafrawy SA, Corman VM, Tolah AM, et al. Enzootic patterns of Middle East respiratory syndrome coronavirus in imported African and local Arabian dromedary camels: a prospective genomic study. Lancet Planet Health. 2019 Dec;3(12):e521-e528.



- [29] Shirato K, Melaku SK, Kawachi K, et al. Middle East Respiratory Syndrome Coronavirus in dromedaries in Ethiopia is antigenically different from the Middle East isolate EMC. Front Microbiol. 2019;10:1326.
- [30] MERS coronavirus database [Internet]. (2022). Available from: https://www.ncbi.nlm.nih.gov/genomes/Virus Variation/Database/nph-select.cgi?cmd = database&taxid = 1335626.
- [31] Lamers MM, Raj VS, Shafei M, et al. Deletion variants of Middle East respiratory syndrome Coronavirus from Humans, Jordan, 2015. Emerg Infect Dis. 2016 Apr;22(4):716-719.
- [32] Faye B. Camel meat and meat products 2013.
- [33] Adney DR, van Doremalen N, Brown VR, et al. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerg Infect Dis. 2014 Dec;20(12):1999-2005.
- [34] Oudshoorn D, Rijs K, Limpens R, et al. Expression and cleavage of Middle East Respiratory Syndrome Coronavirus nsp3-4 polyprotein induce the formation of double-membrane vesicles that mimic those associated with Coronaviral RNA replication. mBio. 2017 Nov 21;8(6):e01658-17.
- [35] Yang X, Chen X, Bian G, et al. Proteolytic processing, deubiquitinase and interferon antagonist activities of Middle East respiratory syndrome coronavirus papain-like protease. J Gen Virol. 2014 Mar;95(Pt 3):614-626.
- [36] Lei J, Kusov Y, Hilgenfeld R. Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. Antiviral Res. 2018 Jan;149:58-74.
- [37] Freundt EC, Yu L, Goldsmith CS, et al. The open reading frame 3a protein of severe acute respiratory syndrome-associated coronavirus promotes membrane rearrangement and cell death. J Virol. 2010 Jan;84 (2):1097-1109.

- [38] Ren Y, Shu T, Wu D, et al. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. Cell Mol Immunol. 2020 Aug;17(8):881-883.
- [39] Zhou Y, Zheng R, Liu S, et al. Host E3 ligase HUWE1 attenuates the proapoptotic activity of the MERS-CoV accessory protein ORF3 by promoting its ubiquitindependent degradation. J Biol Chem. 2022 Feb;298 (2):101584.
- [40] Menachery VD, Mitchell HD, Cockrell AS, et al. MERS-CoV accessory ORFs play key role for infection and pathogenesis. mBio. 2017 Aug 22;8(4):e00665-17.
- [41] McBride R, van Zyl M, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein. Viruses. 2014 Aug 7;6(8):2991-3018.
- [42] Chang CK, Hou MH, Chang CF, et al. The SARS coronavirus nucleocapsid protein-forms and functions. Antiviral Res. 2014 Mar;103:39-50.
- [43] Szelazek B, Kabala W, Kus K, et al. Structural characterization of human coronavirus NL63 N protein. J Virol. 2017 Jun 1;91(11):e02503-16.
- [44] Chang CY, Liu HM, Chang MF, et al. Middle East Respiratory Syndrome Coronavirus nucleocapsid protein suppresses type I and type III interferon induction by targeting RIG-I signaling. J Virol. 2020 Jun 16;94(13):e00099-20.
- [45] Zhu L, Gao T, Fu Y, et al. The MERS-CoV N protein regulates host cytokinesis and protein translation via interaction with EF1A. Front Microbiol. 2021;12:551602.
- [46] Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-288.
- [47] Sabir JS, Lam TT, Ahmed MM, et al. Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. Science. 2016 Jan 1;351 (6268):81-84.