

# Genetic detection and characterization of HE and S genes of recent betacoronaviruses in rabbits from Egypt

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## SUMMARY

The current study is the first reported molecular characterization of rabbit coronavirus strains in Egypt. The cecal contents taken from young New Zealand rabbits (5-6 weeks old) with enteritis were tested for the presence of coronaviruses using RT-PCR with primers specific for the polymerase gene. The three positive samples showed the presence of the hemagglutinin-esterase (HE) gene indicative of Betacoronavirus subgroup A. Betacoronavirus strains designated as rabbit coronavirus (RCoV) Egypt/Qal/1, RCoV Egypt/Qal/2 and RCoV Egypt/Qal/3 were partially sequenced for their HE and spike (S) genes. In all RCoV Egypt/Qal strains, the nucleotide homology was 98.3-100% and 99.8-100% for HE and S genes respectively while the deduced amino acid homology was 98.7-100% and 99.5-100% for HE and S proteins respectively with characteristic cleavage site QGRSRR motif in their S proteins. High similarity was observed with RCoV HKU14 strains and bovine coronavirus strains (BCoV) than other Betacoronaviruses. Phylogenetic data of nucleotide and amino acid sequences of the HE and S genes revealed that all RCoV Egypt/Qal strains were more homologous to each other and were distinct from the other known Betacoronavirus published on GenBank. The findings suggest the existence of a novel variant of RCoV genetically related to RCoV HKU14 strains.

**Keywords: Egyptian RCoV; New Zealand rabbits; RT-PCR; HE and S gene analysis**

## RESUME

**Détection génétique et caractérisation des gènes HE et S de bêta-coronavirus récents chez des lapins en Egypte**

Cette étude est la première caractérisation moléculaire des souches de coronavirus chez le lapin en Egypte. La présence de coronavirus chez des jeunes lapins néo-zélandais (âgés de 5 à 6 semaines) souffrant d'entérite a été évaluée par RT-PCR avec des amorces spécifiques du gène de la polymérase. Les trois échantillons positifs ont montré la présence du gène de l'hémagglutinine-estérase (HE) indicatif du sous-groupe A de bêta-coronavirus. Les souches de bêta-coronavirus désignées comme coronavirus de lapin (RCoV) Egypte/Qal /1, RCoV Egypte/Qal/2 et RCoV Egypte / Qal /3 ont été partiellement séquencées pour leurs gènes HE et spike (S). Dans toutes les souches RCoV Egypt / Qal, l'homologie des nucléotides était respectivement de 98,3-100% et de 99,8 -100% pour les gènes HE et S tandis que l'homologie déduite des acides aminés était de 98,7-100% et de 99,5-100% pour les protéines HE et S respectivement avec un motif de clivage caractéristique QGRSRR dans leurs protéines S. Une forte similitude a été observée avec les souches RCoV HKU14 et les souches de coronavirus bovin (BCoV) comparativement aux autres bêta-coronavirus. Les données phylogénétiques des séquences nucléotidiques et d'acides aminés des gènes HE et S ont révélé que toutes les souches RCoV Egypte / Qal étaient plus homologues les unes par rapport aux autres et étaient distinctes des autres bêta-coronavirus connus publiés sur GenBank. Les résultats suggèrent l'existence d'une nouvelle variante de RCoV génétiquement liée à RCoV souche HKU14.

**Mots-clés : RCoV Egypte; Lapins de Nouvelle-Zélande; RT-PCR; Gènes HE et S, Coronavirus**

## Introduction

Coronaviruses (CoVs) are enveloped, single-stranded positive-sense RNA viruses, that are responsible for respiratory and enteric disease in a variety of avian and mammalian species, including humans, cattle, pigs, chickens, turkeys, dogs, cats, mice, rats, rabbits, and bats [29]. Currently, the family *Coronaviridae* is divided into three genera *Alphacoronavirus*, *Betacoronavirus*, and *Gammacoronavirus*, respectively [8].

All coronaviruses have four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins [5, 18]. Members of *Betacoronavirus* subgroup A contain an

additional structural haemagglutinin-esterase (HE) protein [18]. The S protein is post-translationally cleaved into two subunits, S1 and S2. The S1 mediates attachment to host cell receptors and fusion of the virion membrane to the host cell membrane. S1 sequences are variable in different CoV strains or isolates and mutations in S1 sequences have been associated with altered antigenicity and pathogenicity of the virus [3]. The S1 hypervariable region is useful to study the variability and evolution among CoV species [4, 16]. The HE glycoprotein has haemagglutinating and esterase activities as well as mediates initial adsorption of the virus to cell membranes [28]. This protein is frequently mutated or completely deleted during serial virus passaging in culture [32].

Two human coronaviruses, Severe acute respiratory syndrome-related coronavirus (SARS-CoV) and Middle-East respiratory syndrome coronavirus (MERS-CoV) are likely to reside in an animal reservoir, and have recently initiated an epidemic in humans through zoonotic transmission [14, 31]. This zoonotic potential implies the need for surveillance of coronaviruses associated with domestic animals in close contact with the human population.

Coronavirus-like particles have been demonstrated in laboratory rabbits from Scandinavia with a syndrome called rabbit cardiomyopathy and in laboratory rabbits in Canada with contagious diarrheal disease [19, 24]. Similar particles were reported in the Netherlands [10] and in the USA [11].

A rabbit coronavirus HKU14 (RCoV HKU14) belonging to the *Betacoronavirus* clade A was isolated from the feces of domestic rabbits in Guangzhou, China [20]. This clade also contains Bovine coronavirus (BCoV), Bovine-like coronavirus (BCoV-like) originating from captive wild ruminants [2] and camelids [6, 31], Human coronavirus OC43 (HCoV-OC43), Equine coronavirus (ECoV) and Porcine haemagglutinating encephalomyelitis virus (PHEV) [8]. Also, canine respiratory coronavirus (CRCoV) has shown a high genetic similarity to *Betacoronavirus* clade A [12].

To date, no coronavirus infection has been reported in rabbits in Egypt. In this study, we detect three Egyptian *Betacoronavirus* strains designated as rabbit coronavirus (RCoV) from cecal contents of rabbits, and characterized the strains using partial HE and S genes sequencing and their phylogenetic relatedness to other known *Betacoronavirus* clade A viruses.

## Material and methods

### SAMPLE COLLECTION

In May 2015, three hundreded sixty young New Zealand rabbits (5-6 weeks old) from three private rabbit farms (120 rabbits/farm) in Qalubia province, Egypt were reported to have clinical signs of enteritis. Five fecal samples per farm were aseptically collected from the caecum of diseased rabbits submitted to our virology laboratory and pooled as a single specimen for each farm, then tested for RCoV as an attempt to identify a novel virus due a lack of diagnosis of other pathogens.

### RNA extraction

Fecal samples were homogenized in phosphate buffered saline solution (PBS, pH 7.4) followed by centrifugation at 3000g for 10 min. Total RNA was extracted from fecal supernatant with the QIAamp RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's

instructions. Template RNAs were eluted in 50 µl of RNase-free water and stored at -70 °C until their use.

### Detection of RCoV by RT-PCR

Detection of RCoV RNA was carried out using SuperScript™ One-Step RT-PCR for Long Templates (Life Technologies, Invitrogen, Milan, Italy) and primers specific for the RNA dependant RNA polymerase (RdRp) gene [20]. The following thermal protocol was used: reverse transcription at 50 °C for 30 min, inactivation of Superscript II RT at 94 °C for 2 min, 40 cycles of 94 °C for 1 min, 50 °C for 1min, 72 °C for 1 min, with a final extension at 72 °C for 10 min. The expected size of the PCR product was 320 bp.

Primers for the HE gene were designed from nucleotide sequences of the HE gene of BCoV (GenBank Accession No. U00735) and RCoV strain HKU14 (GenBank Accession No. NC017083). The sequence and location of the primers were HE-F 5'-AGCTCTTTGTA-AATCTGGTAG-3' and HE-R 5'-GTGAAA-AACAAGGTGAATC-3'. RT-PCR was performed by using SuperScript™ One-Step RT-PCR kit. The following temperature profile was used for the RT-PCR, reverse transcription at 50 °C for 30 min, inactivation of Superscript II RT at 94 °C for 2 min, followed by 35 cycles of 95 °C for 1 min, 50 °C for 30 s, and 68 °C for 1 min, followed by a final extension at 68 °C for 10 min. The expected size of the PCR product was 572 bp.

The PCR products were detected by electrophoresis through a 1.5% agarose gel and visualization under UV light after ethidium bromide staining.

### Sequencing of RCoV HE and S1 gene

The PCR-amplified products of the HE-gene of RCoV Egypt/Qal strains were extracted with a QIAquick PCR purification kit (Qiagen) and the RT-PCR primers were used in sequencing. Sequencing of the purified PCR products was performed by the manufacturer in BigDye® Terminator v. 3.1. The cycle sequencing was performed using an ABI 3130 Genetic Analyzer (Applied Biosystems).

For partial sequencing of the S gene hyper-variable and cleavage site, the primers S-S1, 5'-GATAAGTTTGCTATACCCCAATGG-3' (nt 24966-24988, sense primer); S-AS1, 5'-ACTGTCATTTACTGCATTAACAG-3' (nt 26137-26159, antisense primer) were designed based on the sequence of the RCoV HKU14 strain (GenBank accession no. NC\_017083). RT-PCR was performed by using one step RT-PCR Kit (Qiagen), followed by purification of the DNA fragments (1194 bp) using a QIAquick PCR purification kit (Qiagen). The sequencing reaction was performed by using a BigDye Terminator v. 3.1 cycle sequencing kit (Applied Bio-systems) according to the manufacturer's instructions. The sequencing

primers were S-S2, 5'- AATCCTTGACTTGCCAACCAC -3' (nt 25510–25531, sense primer) and S-AS2, 5'- TTGTAACAAGAATCCACAGACC -3' (nt 25549–25571, antisense primer) in addition to S-S1 and S-AS1. Sequencing was performed by using an ABI 3130 Genetic Analyzer (Applied Biosystems).

### GenBank accession numbers

The Partial HE gene sequence of RCoV Egypt/Qal strains was assigned accession number KX806587, KX806589, KX806588. The Partial S gene sequence of RCoV Egypt/Qal strains was assigned accession number KX806584, KX806585, KX806586.

### Phylogenetic analysis

Multiple nucleotide and amino acid sequence alignments were carried out with Clustal W [27], and phylogenetic tree was constructed with MEGA 6 software [26], using the Neighbor-joining tree method with 1000 bootstrap replicates to assign confidence levels to branches. The HE and S sequences of RCoV Egypt/Qal strains were aligned and compared with other *Betacoronaviruses*. The sequences were retrieved from GenBank (National Centre of Biotechnology Information) (Table I) and BLAST search was carried out.

## Results

### RT-PCR USING PRIMERS FOR RCOV RDRP AND HE GENE

Using the specific primers targeted a 320-bp fragment of the RdRp gene of RCoV, all samples were found to be positive by RT-PCR (Fig.1). An additional structural protein gene, the HE was detected in the three fecal samples by RT-PCR with the primer set for the HE gene emphasized by sequence analysis (Fig.2) indicates *Betacoronavirus* strains.

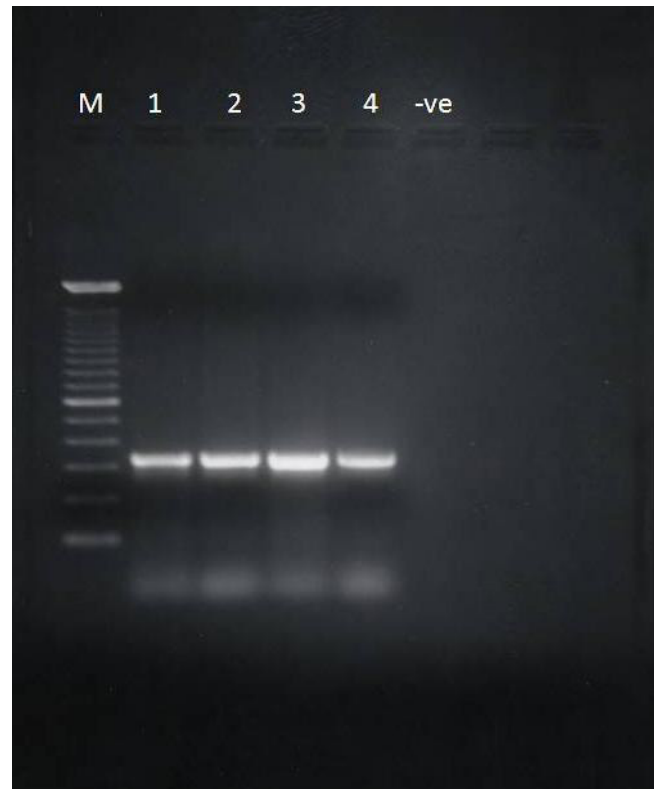


FIGURE 1: The amplified products (320 bp) of the coronavirus polymerase gene by RT-PCR from fecal samples of rabbits. (M) 100bp DNA ladder (Invitrogen); (1) RCoV Egypt/Qal/1; (2) RCoV Egypt/Qal/2 (3) RCoV Egypt/Qal/3 (4) BCoV Mebus reference strains as positive control and (-ve) the negative control

### SEQUENCE ANALYSIS OF PARTIAL HE GENES

The homology analysis of the HE gene nucleotide and deduced amino acid demonstrated that the nucleotide similarity ranged from 98.3% to 100% while the deduced amino acid similarity ranged from 98.7% to 100% between RCoV Egypt/Qal strains. Our strains shared nucleotide identities 91.2% to 94.3% with RCoV HKU14 strains and

Strain	Host	Accession no.
RCov HKU14 strain	Rabbit	NC_017083
RCov HKU14 strain HKU14-1	Rabbit	JN874559
RCov HKU14 strain HKU14-3	Rabbit	JN874560
RCov HKU14 strain HKU14-8	Rabbit	JN874561
RCov HKU14 strain HKU14-10	Rabbit	JN874562
Bovine coronavirus strain Mebus	Bovine	U00735
Bovine coronavirus strain Quebec	Bovine	AF220295
SD cov WD388/1994	Wild ruminant	FJ425190
HcoV-OC43-8942_09	Human	KF572869
Human coronavirus HKU1	Human	NC_006577
MERS coronavirus isolate Jeddah_1_2013	Human	KJ556336
Dromedary camel coronavirus HKU23 strain HKU23-362F	Camel	KF906250
PHEV	Swine	AF481863
CR cov strain K37	Canine	JX860640
Equine coronavirus strain NC99	Equine	EF446615
Murine coronavirus MHV-1	Murine	FJ647223

TABLE I: *Betacoronaviruses* strains included in the phylogenetic analysis of the HE and S gene in this study

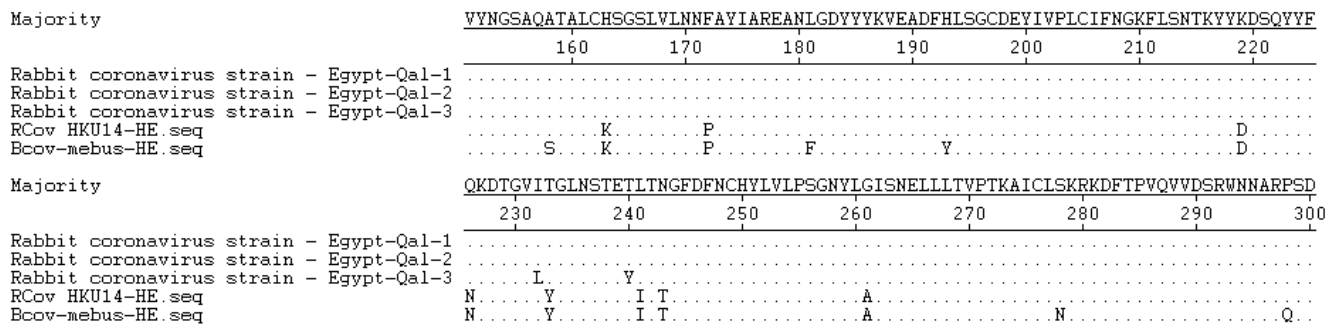


FIGURE 2: Comparative HE amino acid sequence alignment of RCoV Egypt/Qal strains sequenced in this study, RCoV HKU14 and BCoV Mebus reference strains. Identical amino acids from the majority sequence are indicated with dots

87.7% to 88.8% with BCoV strains and CR CoV strain K37. Percent identities of the deduced amino acid of partial HE protein revealed that RCoV Egypt/Qal strains had 92.3% to 94.5% similarities with RCoV HKU14 strains and 90.3% to 91.8% similarities with BCoV strains and CR CoV strain K37 (Table II).

RCoV Egypt/Qal/3 had unique amino acids substitution at 232 (I to L) and 240 (T to Y) in comparison to other two RCoV Egypt/Qal strains, RCoV HKU14 strain and BCoV mebus strain. Our strains possess 13 amino acids changes with BCoV mebus strain, 8 from which were shared by RCoV HKU14 strain. The substitution at 219 (D to K) was unique and altered the charge to be positive (Fig 2).

Phylogenetic data of amino acid sequences of the HE gene revealed that all Egyptian RCoV strains were more

homologous to each other and were distinct from the other known *Betacoronavirus* (Fig 3).

SEQUENCE ANALYSIS OF S1 GENES

A pairwise comparison of S1 gene analysis between RCoV Egypt/Qal strains revealed nucleotide similarity ranging from 99.8% to 100% and amino acid similarity ranging from 99.5% to 100%. Our strains shared nucleotide identities 91.9% to 92.5% with RCoV HKU14 strains, 85.4% to 85.8% with BCoV like of wild ruminants, 84.9% to 85.3% with CR CoV strain K37 and 84.6% to 85.1% with BCoV strains. Percent identities of the deduced amino acid of partial S1 protein revealed that RCoV Egypt/Qal strains had 89.2% to 89.4% similarities with RCoV HKU14 strains, 83.6% to 84.4% similarities with BCoV like of wild ruminants and 83.1% to 83.9% similarities with BCoV strains (Table II).

Strain	RCov Egypt/Qal/1				RCov Egypt/Qal/2				RCov Egypt/Qal/3			
	HE		S1		HE		S1		HE		S1	
	%nt	%aa	%nt	%aa	%nt	%aa	%nt	%aa	%nt	%aa	%nt	%aa
<b>RCov Egypt/Qal/1</b>	100	100	100	100	100	100	100	100	98.3	98.7	99.8	99.5
<b>RCov Egypt/Qal/2</b>	100	100	100	100	100	100	100	100	98.3	98.7	99.8	99.5
<b>RCov Egypt/Qal/3</b>	98.3	98.7	99.8	99.5	98.3	98.7	99.8	99.5	100	100	100	100
<b>RCov HKU14 strain</b>	94.3	94.5	92.5	89.7	94.3	94.5	92.5	89.7	92.5	93.5	92.3	89.2
<b>RCov HKU14-1</b>	94.3	94.5	92.5	89.7	94.3	94.5	92.5	89.7	92.5	93.5	92.3	89.2
<b>RCov HKU14-3</b>	94.1	94.9	91.7	88.7	94.1	94.9	91.7	88.7	92.3	93.5	91.5	88.2
<b>RCov HKU14-8</b>	93.7	94.3	92.3	89.7	93.6	94.3	92.3	89.7	91.8	92.9	92.1	89.2
<b>RCov HKU14-10</b>	93.0	93.7	92.0	89.2	93.0	93.7	92.1	89.2	91.2	92.3	91.9	89.7
<b>BCov strain Mebus</b>	88.8	91.8	85.1	83.9	88.7	91.8	84.8	83.6	87.7	90.3	84.6	83.1
<b>BCov strain Quebec</b>	88.8	91.8	85.1	83.7	88.7	91.8	84.8	83.3	87.7	90.3	84.6	82.8
<b>SD cov WD388/1994</b>	*	*	85.8	84.4	*	*	85.6	84.1	*	*	85.4	83.6
<b>HcoV-OC43-8942_09</b>	*	*	84.3	78.9	*	*	83.9	78.4	*	*	83.8	78.2
<b>HcoV HKU1</b>	57.0	44.8	62.3	53.5	57.0	44.8	62.1	53.3	57.3	46.6	62.0	53.3
<b>MERS Jeddah 2013</b>	*	*	42.2	19.7	*	*	42.1	19.9	*	*	42.0	19.9
<b>Ccov HKU23</b>	87.6	91.1	83.3	82.2	87.6	91.1	83.2	82.3	86.8	89.7	83.1	82.1
<b>PHEV</b>	84.8	89.9	69.2	64.2	84.8	89.9	69.0	64.2	83.7	88.4	68.9	64.0
<b>CR Cov strain K37</b>	88.8	91.8	85.3	83.2	88.7	91.8	85.0	82.8	87.7	90.3	84.9	82.6
<b>EqCov strain NC99</b>	63.1	63.3	70.9	66.5	63.1	63.3	70.8	66.6	63.0	62.6	70.8	66.8
<b>Murine hepatitis virus</b>	58.0	37.7	58.5	54.6	58.0	37.7	85.2	54.2	57.4	38.4	58.3	54.4

\* Not analyzed

TABLE II: Nucleotide and amino acid identities of RCoV Egypt/Qal strains with selected *betacoronaviruses* sequences from GenBank based on partial HE and S gene analysis.

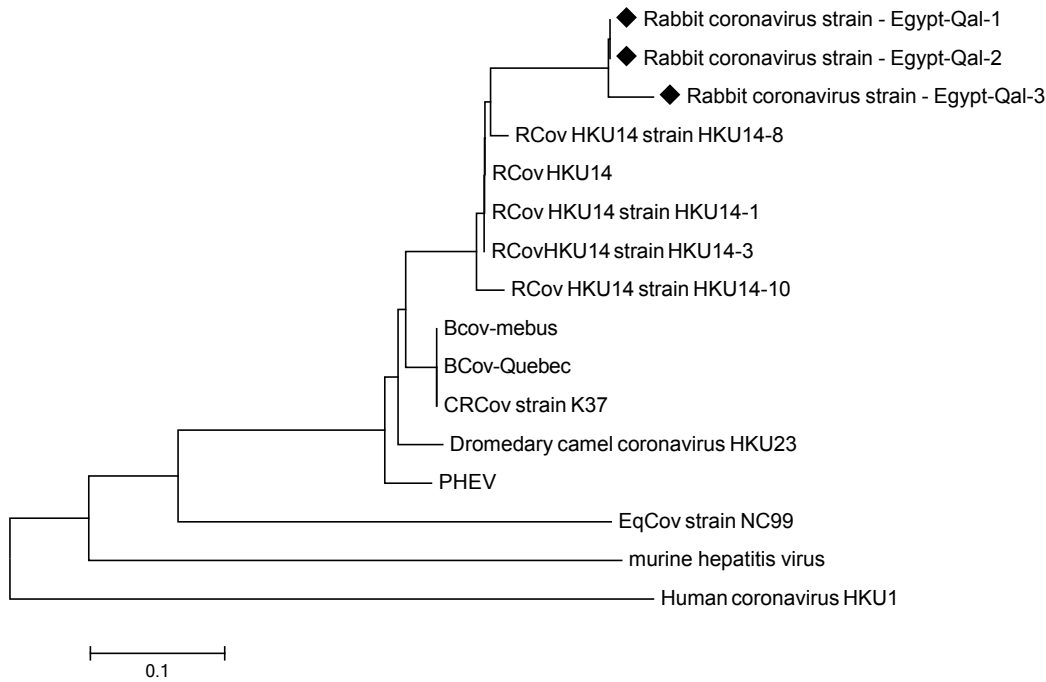


FIGURE 3: Phylogenetic tree for partial HE nucleotide sequence of RCoV Egypt/Qal strains compared with other *Betacoronaviruses* reference sequence obtained from GenBank database. The scale bar represents the number of substitutions per nucleotide. RCoV Egypt/Qal strains from the present study are indicated by black rectangular

Majority	RKVDLQIGNLGYLQSFNTKIDTFSATSCOLYANLPAYNVSVSRIDPSTWNHFRGFTEENFAFKPQAPAGVFTAKDVVVAQHCF							
	410	420	430	440	450	460	470	480
Rabbit coronavirus strain -Egypt-Qal-1.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-2.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-3.	.	.	.	.	.	.	.	.
RCoV HKU14-S.seq	.	Y	.	Y	A	N	R	QSV
BCov-Mebus-S.seq	.	YR	T	Y	A	FN	R	QV
Majority	KAPTTFPCPKLDGSGVCGSGSLLEAGFRHSGIGTFCPAGYNVLTCTYSLECNCOCTPDPIVSKASGPYKCFQTKYIVGVGE							
	490	500	510	520	530	540	550	560
Rabbit coronavirus strain -Egypt-Qal-1.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-2.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-3.	.	E	.	.	.	.	.	.
RCoV HKU14-S.seq	.	L	GWD	K	T	T	N	VQ
BCov-Mebus-S.seq	SN	L	N	PGID	YKN	T	HNAAQ	L
Majority	HCSVLAIAKSDTCGGNPECTQCPQAFGLGWSVDSCLOGDRCNIFANLLHGVNSGTTCTSDLQKANTDIIIVAVCVNVDLYGIS							
	570	580	590	600	610	620	630	640
Rabbit coronavirus strain -Egypt-Qal-1.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-2.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-3.	.	.	.	.	.	.	.	.
RCoV HKU14-S.seq	G	Y	.	.	.	I	.	G
BCov-Mebus-S.seq	G	Y	.	.	.	FI	D	S
Majority	LQGI FVDVNGTTVNSWQNLIVDSNGNAYGFRDYLTRNRFMIRSCYTGRRVSAAFHANSSEPALVFRNIKCNVLFNNTFGKQ							
	650	660	670	680	690	700	710	720
Rabbit coronavirus strain -Egypt-Qal-1.	.	.	.	.	.	.	.	E
Rabbit coronavirus strain -Egypt-Qal-2.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-3.	.	.	.	.	.	.	.	.
RCoV HKU14-S.seq	G	E	A	Y	.	L	.	S
BCov-Mebus-S.seq	G	E	A	Y	.	L	.	S
Majority	LQPINYFDSYLGCVNVDNASTATTAQTCDLTVGSGYCVDYSTQGRSRRAITTGVRFTNFEPFTVNSVND							
	730	740	750	760	770	780	790	
Rabbit coronavirus strain -Egypt-Qal-1.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-2.	.	.	.	.	.	.	.	.
Rabbit coronavirus strain -Egypt-Qal-3.	.	.	.	.	.	.	.	.
RCoV HKU14-S.seq	.	.	S	V	.	L	.	A
BCov-Mebus-S.seq	.	.	SSV	.	.	KR	.	T

FIGURE 4: Comparative S1 amino acid sequence alignment of RCoV Egypt/Qal strains sequenced in this study, RCoV HKU14 and BCoV Mebus reference strains. Identical amino acids from the majority sequence are indicated with dots

For the sequenced S1 subunit, RCoV Egypt/Qal/3 had unique amino acids substitution at 492 (D to E) and 720 (Q to E) in comparison to other two RCoV Egypt/Qal strains. The analyzed S1 segment showed 7 potential N-linked glycosylation sites in all Egyptian RCoV strains, with three NXS (V437, S696, D788) and 4 NXT (G649, R676, N714, S739) sites, while additional N-linked glycosylation NXS (P444) was observed in RCoV HKU14 strain and BCoV

mebus strain. Our strains possess 29 amino acids changes at the site previously identified as being hyper variable among S1 protein of BCoV mebus and RCoV HKU14 strains. The substitution at 458 (S to F) and 510 (T to S) increased the hydrophobicity of the protein compared with RCoV HKU14 strain. The conserved amino acid at 531 (N) among the Egyptian RCoV, RCoV HKU14 and BCoV mebus strains emphasized the enteric virus tropism (Fig 4).

Phylogenetic data of amino acid sequences of the S1 gene revealed that all Egyptian RCoV strains were more homologous to each other and were distinct from the other known *Betacoronavirus* (Fig 5).

ANALYSIS OF CLEAVAGE SITE OF S GENES

Comparative analysis of the consensus S1/S2 cleavage site among the RCoV Egypt/Qal strains and other

*Betacoronaviruses* published strains revealed characteristic cleavage site QGRSRR motif for all the Egyptian RCoV from position 763 to 768. The amino acid substitution was observed in the 764 position (L to G) with RCoV HKU14, in the 763 and 764 positions (KR to QG) with BCoV strains and CR CoV strain K37 that considered mostly related strains (Table III).

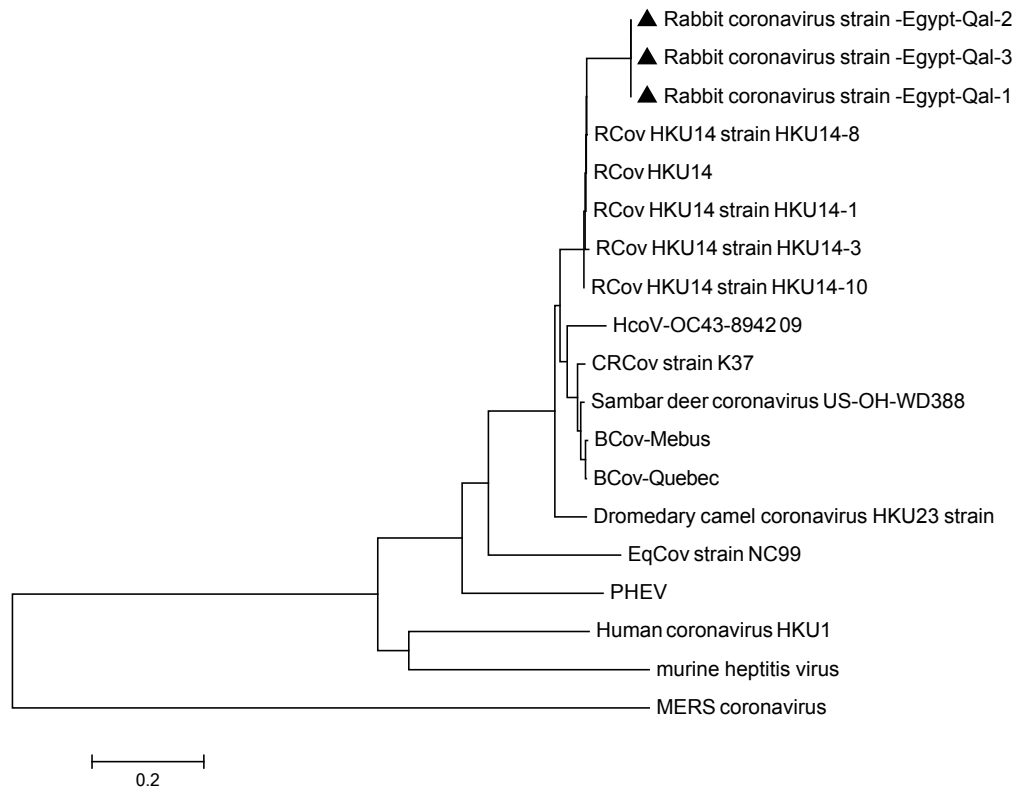


FIGURE 5: Phylogenetic tree for partial S1 nucleotide sequence of RCoV Egypt/Qal strains compared with other *Betacoronaviruses* reference sequence obtained from GenBank database. The scale bar represents the number of substitutions per nucleotide. RCoV Egypt/Qal strains from the present study are indicated by black triangles

Strain	Amino acid sequence of cleavage site
RCoV Egypt/Qal/1	ST <b>QGRSRR</b> *AIT
RCoV Egypt/Qal/2	ST <b>QGRSRR</b> *AIT
RCoV Egypt/Qal/3	ST <b>QGRSRR</b> *AIT
RCoV HKU14 strain	ST <b>QLRSRR</b> *AIT
RCoV HKU14-1	ST <b>QLRSRR</b> *AIT
RCoV HKU14-3	ST <b>QLRSRR</b> *AIT
RCoV HKU14-8	ST <b>QLRSRR</b> *AIT
RCoV HKU14-10	ST <b>QLRSRR</b> *AIT
BCov strain Mebus	ST <b>KRRSRR</b> *AIT
BCov strain Quebec	ST <b>KRRSRR</b> *AIT
SD cov WD388/1994	ST <b>KRRSRR</b> *SIT
HCoV-OC43-8942_09	SK <b>NRRSRR</b> *AIT
HCoV HKU1	SS <b>SRKRR</b> *SIS
Ccov HKU23	SID <b>RRSRR</b> *AIT
PHEV	VT <b>ALRSRR</b> *SFT
CR Cov strain K37	ST <b>KRRSRR</b> *SIT
EqCov strain NC99	ST <b>ARRQRR</b> *SPS
Murine hepatitis virus	ST <b>SHRARR</b> *SIS

Residues in bold represents proteolytic cleavage signal sequence of the RCoV Egypt/Qal and other *Betacoronaviruses* strains. The asterisk between the amino acids indicates the cleavage site between S1 and S2.

TABLE III: Predicted amino acid sequences for the spike gene in the cleavage site proximities of RCoV Egypt/Qal strains compared with other *Betacoronaviruses*.

## Discussion

A number of studies investigated the presence of coronavirus-like particles in fecal material collected from young rabbits with clinical signs of enteritis [11, 19, 23]. Proper identification of coronavirus in rabbit feces requires a sensitive assay, probably because it is often present in low titer and difficult to culture in cell lines [10, 19].

As little is known about the genetic characters of rabbit coronavirus except for RCoV HKU14, isolated from the feces of domestic rabbits in Guangzhou, China [20]. The main objective of this study was to conduct a molecular characterization of the RCoV strains that circulate in Egypt by partially amplifying the HE and S genes.

The RCoV Egypt/Qal/1, RCoV Egypt/Qal/2 and RCoV Egypt/Qal/3 strains were detected in the cecal contents of 5-6 weeks old New Zealand rabbits with enteritis using RT-PCR primers directed to the polymerase gene of coronaviruses [20, 25]. We were able to demonstrate the presence of a HE gene in our strains by PCR, therefore they belong to *Betacoronavirus* subgroup A [22, 28].

It has been suggested that the high degree of variation in host range and tissue tropism of *Betacoronavirus* subgroup A is largely attributable to variations in the HE and S glycoproteins [13, 17 and 22]. The S protein is cleaved into the S1 and S2 domains with the sequences of the S1 domains much more variable than the S2 domains [1, 9].

Sequence analysis of HE and S1 genes showed that all Egyptian RCoV strains, had high genetic similarity to each other and form a distinct group from the other known *Betacoronavirus*. The amino acids changes found in residues 232 and 240 of the HE protein and in residue 492 and 720 of the S1 protein could distinguish between RCoV Egypt/Qal/3 and other two RCoV Egypt/Qal strains.

In the present study, our strains appeared to be most genetically related to the RCoV HKU14 strain and to bovine coronavirus (BCoV) [20]. A comparison of the HE proteins, the Egyptian RCoV strains possess 13 amino acids changes with BCoV mebus strain, 8 from which were shared by RCoV HKU14 strain with unique change found in residue 219 (D to K). The analyzed S1 protein for all Egyptian RCoV strains revealed absence of N-linked glycosylation NXS (P444) that observed in RCoV HKU14 strain and BCoV mebus strain. Our strains possess 29 amino acids changes at the site previously identified as being hyper variable among S1 protein of BCoV mebus and RCoV HKU14 strains [7, 15] with unique changes found in residues 458 and 510 increased the hydrophobicity of the protein. The conserved amino acid at 531 (N) among the Egyptian RCoV emphasized the enteric virus tropism [20, 33].

A potential S1/S2 cleavage site located was identified in the S proteins of most strains of coronaviruses [30]. The

predicted proteolytic cleavage site (QGRSRR) was conserved in all Egyptian RCoV strains indicating that it is functionally essential for RCoV replication in the enteric epithelium [21]. Our data may provide crucial information about genetic characterization of Egyptian RCoV strains detected in young New Zealand rabbits with enteritis.

## Conclusion

This study is the first report of a molecular characterization of the RCoV strains in Egypt. The results showed that two types of RCoV circulate among young rabbits with enteritis. All Egyptian RCoV strains were more homologous to each other and were genetically related to RCoV HKU14 strains, suggesting a novel variant of RCoV strains.

## Conflict of interest statement

None of the authors of this paper has financial or personal relationships with other people or organization that could inappropriately influence or bias the content of the paper.

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