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Isolation and subtyping of BoHV-1 associated with respiratory disease in cattle at Dakahlia, Egypt 2018

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ABSTRACT

The aim of the present study was to isolate, identify and genetically characterize bovine herpes virus Type 1 (BoHV-1) from suspected cattle showing clinical symptoms of respiratory disease in Dakahlia, Egypt, September 2018. BoHV-1 was isolated on specific pathogen free-embryonated chicken eggs (SPF-ECE) from suspected nasal swabs and lung tissue samples. Pock lesions on the chorio-allantoic membrane (CAM) of SPF-ECE ranged from 1 to 2 mm in diameter and scattered all over the membrane that were more pronounced after the third passage. PCR assay specific for BoHV-1 gC gene was also performed for molecular identification of the viral isolate and the gC PCR product was sequenced. Phylogenetic analysis of gC gene sequence identified and clustered our isolate with BoHV1. This highlights the isolation, rapid and sensitive detection, and identification of BoHV-1 virus from clinical cases and its subtyping by nucleotide sequencing and subsequent phylogenetic analysis which gives valuable information about the molecular epidemiology of BoHV-1 subtypes prevalent in the country

1. INTRODUCTION

Bovine herpesvirus 1 (BoHV-1) causes highly contagious disease which affects cattle and buffaloes. It causes initial respiratory infection and can predispose secondary bacterial infections which lead to severe pneumonia and death if not treated (Rivera-Rivas et al., 2009). It causes also reproductive disease, abortion, and neonatal infections. BoHV-1 is a member of the Herpesviridae family and Alpha herpes virinae subfamily (Fulton et al., 2016). BoHV-1 genome consists of a linear double-stranded DNA molecule encoding 73 proteins. The genome of the virus is subdivided into unique long (UL) and unique short (US) segments. Alpha herpes viruses, including bovine herpesvirus 1, encode several glycoproteins of the viral envelope that play crucial roles in viral pathogenicity and regulate virus attachment, entry, egress, and cell to cell spread (Haque et al., 2016). All isolated BoHV-1 strains belong to one single viral species and are classified into three subtypes; BoHV-1.1, BoHV-1.2 (1.2a and 1.2b) and BoHV-1.3. However, all subtypes are antigenically similar. BoHV-1.3, which is a neuropathogenic agent, has been re-classified as BoHV-5 (Muylkens et al., 2007). Most of the BoHV-1.1 isolates are of respiratory origin while BoHV-1.2 are isolated from genital infections. In addition, BoHV-1.1 and 1.2a are also associated with the occurrence of abortions (Kaley et al., 2017).

After primary infection, BoHV-1 replicates in the mucous membranes of the respiratory or genital tract. Latency and reactivation also occur within germinal centers of pharyngeal tonsils (Ackermann et al., 1990; Winkler et al., 2000; Muylkens et al., 2007).

BoHV-1 can be readily isolated in cell culture of primary or secondary bovine kidney, lungs, testis, turbinate bone, trachea, and established cell lines such as Madin–Darby bovine kidney (MDBK) cell line (Nandi et al., 2009). Embryonated chicken eggs are commonly used for isolation of animal viruses due to its several advantages and adaptation of virus in embryonated eggs. In the embryonated chicken eggs (ECE), BoHV-1 produces pock lesion when inoculated via chorio-allantoic membrane (CAM) route (Samrath et al., 2016).

Several laboratory methods are available for diagnosis of BoHV-1 including virus isolation, fluorescent antibody technique (FAT) and enzyme linked immunosorbent assay (ELISA). The polymerase chain reaction (PCR) is used to detect the presence of the viral DNA in nasal swabs, blood, tissue and semen, and it has advantages over above mentioned methods for its sensitivity and rapidity (Van Engelenburg et al., 1993; Moore et al., 2000; Belak and Hakhverdyan, 2006).

In the present study, nasal swabs and tissue samples derived from clinically suspected animals from Dakahlia governorate, Egypt were subjected for isolation of BoHV-1 on CAM of developing ECE then identification using PCR followed by sequencing and phylogenetic analysis of gC gene of BoHV-1. The nucleotide sequence of identified BoHV-1 strain was compared with corresponding nucleotide sequences available in GenBank database (http://www.ncbi.nlm.nih. gov/BLAST/) in order to reveal the similarity or differences with other reference BoHV-1.

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2. MATERIAL AND METHODS

2.1. Clinical specimens:
In 2018, suspected cases of BoHV-1 infection were observed among cattle in Dakahlia governorate, Egypt. Suspected animals showed severe respiratory symptoms with high fever. Nasal swabs (n= 37) and lung tissue samples (n= 6) were collected from the clinically affected and emergency slaughtered animals. Samples after preparation were used for virus isolation.

2.2. Sample preparation:
Nasal swabs were squeezed against wall of container then removed from soaked fluid. All samples were centrifuged in cooling centrifuge 4 °C at 2000 rpm for 20 minutes. The supernatant fluid was collected in sterile screw-capped vials and kept at -20 °C until used.
Lung tissue samples were ground with PBS in sterile mortar to make 10% Suspension then three cycles of freezing and thawing were made followed by centrifugation at 3000 rpm for 30 minutes and the supernatant fluid was collected then 5% of stock antibiotic solution was added and kept at -70 °C till use.
The supernatants from nasal swabs and lung tissue samples were filtered through 0.45 µm filters before being used for virus isolation and viral DNA extraction.

2.3. Virus isolation:
The supernatant of prepared samples was filtrated through 0.22 µm filter (Millipore, Milford, MA) and inoculated to chorio-allantoic membrane (CAM) of specific pathogen free-embryonated chicken eggs. The eggs were incubated at temperature 37 °C and humidity and candled daily. The inoculated eggs were harvested aseptically and examined for specific signs. The suspected isolates were propagated on SP-ECE for three times to be used for virus identification using electron microscope and PCR.

2.4. Virus identification:
Nucleic acid was extracted from the homogenized CAMs with positive specific signs using QIAamp DNA mini kit instructions according to the manufacturer's protocol (Qiagen Inc. Valencia CA). The nucleic acid was detected for BoHV-1 by PCR (Esteves *et al*., 2008). Oligonucleotide primers have specific sequence to amplify a specific product of gC gene of BoHV-1

PF: CGGCCACGACGCTGACGA
PR: CGCCGCCGAGTACTACCC

PCR components and cycle temperature and conditions involved during PCR were shown in table (1). The resulting mixture was subjected to optimized thermocycling in a thermocycler.

Table 1 PCR components used for identification of BoHV-1 gC gene

Component	Volume/reaction
Emerald Amp GT PCR master mix (2× premix)	12.5 µl
PCR grade water	5.5 µl
Forward primer (20 pmol)	1 µl
Reverse primer (20 pmol)	1 µl
Template DNA	5 µl
Total	25 µl

Cycling conditions of the primers during PCR
The resulting mixture was subjected to optimized thermo cycling in a thermocycler as follow: pre-denaturing at 94 °C for 3 min; denaturing at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, (35 cycles) followed by a final extension at 72 °C for 5 min.
Agarose gel electrophoreses (Sambrook et al., 1989) with modification was done as 20 µl of each PCR product samples, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel were transferred to UV cabinet. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Sequencing and sequence alignment:
PCR Products were purified from the gel using QIA quick PCR Product extraction kit. (Qiagen Inc. Valencia CA) according to the QIA quik PCR product purification protocol. A purified RT-PCR product was sequenced in the forward and/or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit (Perkinelmer/Applied Biosystems, Foster City, CA), with Cat. No. 4336817. A BLAST® analysis (Basic Local Alignment Search Tool) (Altschul et al., 1990) was initially performed to establish sequence identity to GenBank accessions. The sequence reaction was done according to the instruction of the manufacturer. Purification of the sequence reaction: Using Centrisep (spin column): Cat. No. CS-901 of 100 reaction according to the instruction of the manufacturer. Accession number, locality, and date of collection of reference BoHV-1 isolates published on gene bank data base was illustrated in table 2

Table 2 Accession number, locality, and date of collection of reference BoHV-1 isolates published on gene bank data base

#	Virus isolate	Accession number	Locality and Date of isolation
1	BoHV1 Cooper	KU198480.1	USA, 1966
2	BoHV1.1 NVSL 97-11	JX898220.1	USA, 2012
3	BoHV1 gIII	M27491.1	Canada, 1989
4	BoHV1 ATCC: VR-188	MF421714.1	USA, 2017
5	BoHV1 PA3	MG407792.1	USA, 2017
6	BoHV1 PA2	MG407791.1	USA, 2017
7	BoHV1 PA1	MG407790.1	USA, 2017
8	BoHV1 MN14	MG407788.1	USA, 2017
9	BoHV1 MN13	MG407787.1	USA, 2017
11	BoHV1 MN12	MG407786.1	USA, 2017
12	BoHV1 MN10	MG407784.1	USA, 2017
13	BoHV1 MN6	MG407780.1	USA, 2017
14	BoHV1 MN5	MG407779.1	USA, 2017
15	BoHV1 216 II/1976/India	KC756965.1	India, 2013
16	BoHV1 17-92	KC479144.1	India, 2013
17	BoHV1 7E	JX127195.1	India, 2012
18	BoHV1.1	AJ004801.1	Switzerland, 1997
19	BoHV1 31-kb	Z54206.1	Switzerland, 1995
20	BoHV1.2 B589	KM258881.1	Australia, 2001
21	BoHV1	JN787953.1	China, 2006
22	BoHV1 MN2	MG407776.1	USA, 2017
23	BoHV1.2 SM023	KM258882.1	USA, 1986
24	BoHV1 257	JX127204.1	India, 2012
25	BoHV1.2 K22	KM258880.1	USA, 1958
26	BoHV1.2 SP1777	KM258883.1	USA, 2009
27	BoHV-1.1 Dakahlia 2018	MK541889	Egypt, 2018

A comparative phylogenetic analysis of the isolate sequence and other isolates was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign module of Laser gene DNA Star software Pairwise (Thompson et al., 1994) and maximum likelihood, neighbor joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

3. RESULTS

Virus isolation:
Suspected samples from prepared nasal swabs and lung tissue were inoculated into Specific Pathogen Free-Embryonated Chicken Egg (SPF-ECE) via Chorio-Allantoic membrane (CAM). Characteristic Pock lesions were observed on CAM with only one of 37 nasal swab and 2 of 6 tissue samples. Virus infected CAMs showed pinpoint pock lesions, scattered all over the membrane that were more pronounced after the third passage (Figure 1).

Fig. 1 isolation of BoHV-1 on CAM of SPF-ECE showing pinpoint pock lesions

Virus identification:
Suspected isolates gave positive results after PCR amplification using set of primer specific for gC gene as shown by electrophoretic pattern of the amplified products as the specific PCR product was at the correct expected size (575 bp). BoHV-1 reference strain and isolates had the same size of specific fragment without significant differences between them (Figure 2). The isolated BoHV-1 was named BoHV-1_Dakahlia 2018.

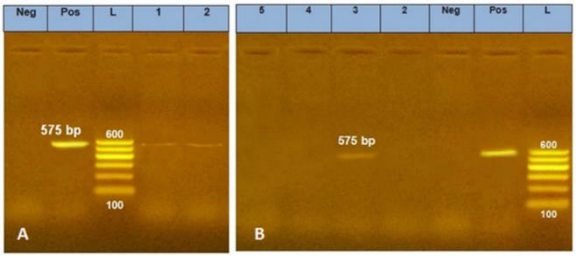

Fig. 2 Agarose gel electrophoresis of PCR amplicons for gC gene of BoHV-1 separated on 1.5% Agarose gel and stained with ethidium bromide (Positive PCR amplicons are at the size of approximately 575 bp). A: Lane L denotes for 100 bp Marker. Lanes 1 and 2: Positive isolated samples, 575 bp size). B: Lane L denotes for 100 bp Marker. Lane Pos: Positive control. Lane Neg: negative control. Lane 3: Positive sample after purification.

Characterization of gC gene of BoHV-1_Dakahlia 2018:
The gC gene of BoHV-1_Dakahlia 2018 was partially sequenced (573 nt). Partial Sequence of gC gene of BoHV-1_Dakahlia 2018 was published on GenBank, the accession No. is MK541889.

Multiple alignments based on gC gene of BoHV-1.1_Dakahlia _2018 indicated that no variation were found between gC gene of BoHV-1.1_Dakahlia_ 2018 and other BoHV-1 isolates (KU198480.1 BoHV1 Cooper, JX898220.1 BoHV1.1 NVSL 97-11, M27491.1 BoHV1 gIII, MF421714.1 BoHV1 ATCC:VR-188, MG407792.1 BoHV1 PA3, MG407791.1 BoHV1 PA2, MG407790.1 BoHV1 PA1, MG407788.1 BoHV1 MN14, MG407787.1 BoHV1 MN13, MG407786.1 BoHV1 MN12, MG407784.1 BoHV1 MN10, MG407780.1 BoHV1 MN6, MG407779.1 BoHV1 MN5, KC756965.1 BoHV1 216 II/1976/India, KC479144.1 BoHV1 17-92, JX127195.1 BoHV1 7E, AJ004801.1 BoHV1.1 and Z54206.1 BoHV1 31-kb) (Table 3). However, between gC gene of BoHV-1.1_Dakahlia_2018 and other BoHV-1 isolates (KM258881.1 BoHV1.2 B589, JN787953.1 BoHV1, MG407776.1 BoHV1 MN2, KM258882.1 BoHV1.2 SM023, JX127204.1 BoHV1 257, KM258880.1 BoHV1.2 K22 and KM258883.1 BoHV1.2 SP1777), the genetic diversity showed little differences.
Based on BoHV-1 gC gene analysis, phylogenetic relationship of reference BoHV-1 isolates in the phylogenetic tree showed that BoHV-1.1_Dakahlia_ 2018 was clustered together with other reference BoHV-1 isolates which have 100% homology, while other reference BoHV-1 isolates that were divergent from BoHV-1.1_Dakahlia_ 2018 were not located in the same subgroup (Figure 3).

4. DISCUSSION

BoHV-1 is a herpesvirus responsible for infectious bovine rhinotracheitis, which is a disease of major economic concern (Thiry et al., 2006). BoHV-1virus infected host is persistently infected and established life-long latency upon recovery, so it represents the virus reservoir that may be reactivated at intervals and spread the virus infection (Ackermann and Engels, 2006).
In Egypt, BoHV-1 was isolated from cattle suffering from a respiratory syndrome, the majority of animals' population is not subjected to vaccination for BoHV-1, but few number of private farms used cattle master vaccine for controlling viral infection (Taha, 2011; Zeedan et al., 2017).
Pock lesions were shown on CAM which became thick, edematous, and congested at the 1st passage level, but typical pocks lesion was observed on CAM on later passages (Figure 1). This result agreed with Zeedan et al. (2017), who isolated BoHV-1 from nasal and ocular swabs and the virus was adapted in CAM of 11-day-old embryonated chicken eggs. More severe changes were observed in CAM after serial passages, indicating adaptation of BoHV-1 on to CAM and this result came in agreement with Thakur et al. (2017).
Glycoprotein C gene of BoHV1 is highly conserved in all the isolates and it can be used as a target for designing of primers for diagnosis of BoHV-1 infection (Sobhy et al., 2014). Viral isolates from CAM were examined by PCR for detection of BoHV-1 DNA using primer specific for gC gene of BoHV-1 (Esteves *et al*., 2008) and compared with positive and negative BoHV-1 control. PCR amplicons from gC sequences were obtained only with addition of 5% glycerol to the reaction mixture. This is due to the high G+C content (71-72%) of BoHV-1 sequences (Majumder et al., 2013). The results were in accordance with the previous reports of use of glycerol for amplification of BoHV1 genes (Gupta et al., 2006; Nandi and Kumar, 2011). The sensitivity

of the designed gC specific primers was 75 % and compared with the published primers targeting gB gene whose sensitivity was 66 % (Ranganatha et al., 2013).

Differentiation between BoHV-1subtypes 1.1. and 1.2. is possible by sequence analysis of the gC amplified PCR products, and that is why we picked the primers described by Esteves et al. (2008). It was reported that the amino acid position 76 of gC is a marker for differentiating BoHV1.1 (valine) and BoHV1.2 (glycine) (Rijsewijk et al., 1999). Multiple alignments based on gC gene of BoHV-1_Dakahlia_ 2018indicatedthat no variation was found between gC gene of BoHV-1_Dakahlia_ 2018 and other BoHV-1 isolates as USA (1966, 2012, 2017), Canada (1989), India (2012, 2013) and Swizerland (1997)

Table3 Pair wise comparison showing identity and divergence for partial sequence of gC gene between BoHV-1.1_Dakahlia_ 2018 and the other reference BoHV-1 isolates published on gene bank data base

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
1		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	1
2	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	2
3	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	3
4	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	4
5	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	5
6	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	6
7	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	7
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	8
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	9
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	10
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	11
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	12
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	13
14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	14
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	15
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	16
17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	17
18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		99.7	99.7	99.3	99.3	99.3	99.1	99.0	100.0	18
19	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4		99.3	99.7	99.7	99.7	99.5	99.3	99.7	19
20	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.7		99.0	99.0	99.0	98.8	98.6	99.7	20
21	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.4	1.1		100.0	99.7	99.1	99.0	99.3	21
22	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.4	1.1	0.0		99.7	99.1	99.0	99.3	22
23	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.4	1.1	0.4	0.4		99.5	99.3	99.3	23
24	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.5	1.2	0.9	0.9	0.5		99.1	99.1	24
25	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	0.7	1.4	1.1	1.1	0.7	0.9		99.0	25
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.7	0.7	0.7	0.9	1.1		26
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	

1	KU198480.1 BoHV1 Cooper
2	JX898220.1 BoHV1.1 NVSL 97-11
3	M27491.1 BoHV1 gIII
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5	MG407792.1 BoHV1 PA3
6	MG407791.1 BoHV1 PA2
7	MG407790.1 BoHV1 PA1
8	MG407788.1 BoHV1 MN14
9	MG407787.1 BoHV1 MN13
10	MG407786.1 BoHV1 MN12
11	MG407784.1 BoHV1 MN10
12	MG407780.1 BoHV1 MN6
13	MG407779.1 BoHV1 MN5
14	KC756965.1 BoHV1 216 II/1976/India
15	KC479144.1 BoHV1 17-92
16	JX127195.1 BoHV1 7E
17	AJ004801.1 BoHV1.1
18	Z54206.1 BoHV1 31-kb
19	KM258881.1 BoHV1.2 B589
20	JN787953.1 BoHV1
21	MG407776.1 BoHV1 MN2
22	KM258882.1 BoHV1.2 SM023
23	JX127204.1 BoHV1 257
24	KM258880.1 BoHV1.2 K22
25	KM258883.1 BoHV1.2 SP1777
26	MK541889 BoHV-1.1 Dakahlia 2018

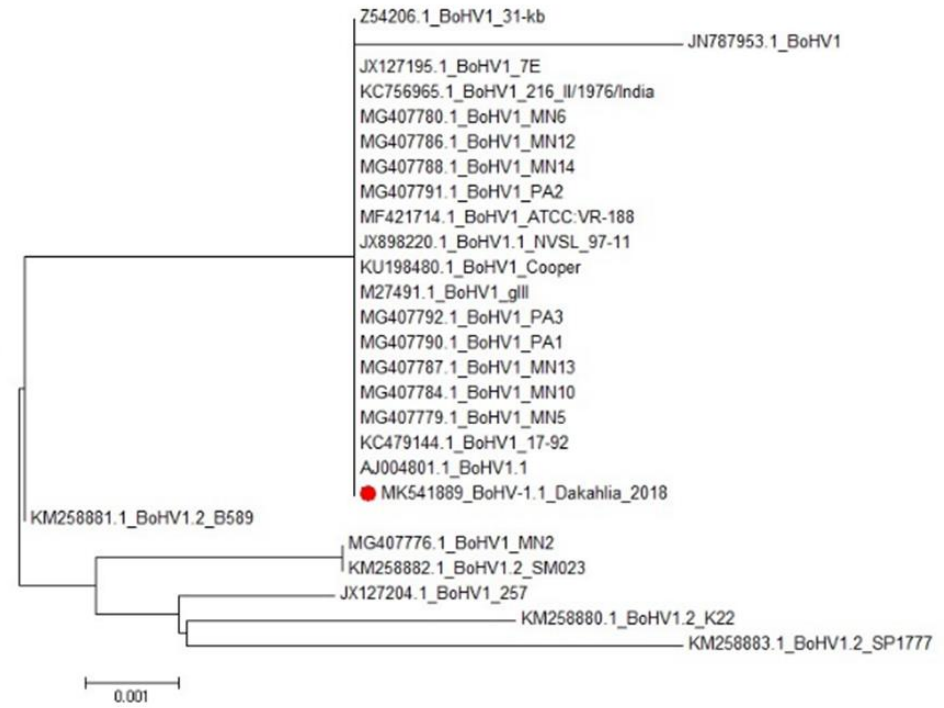

Fig. 3 Phylogenetic relationship of reference BoHV-1 in the phylogenetic tree based on gC gene sequence.

However, divergence was observed between gC gene of BoHV-1_Dakahlia _2018 and other BoHV-1 isolates as isolates from USA (1958, 1986, 2000, 2017), China (2006), India (2012), Switzerland (1993) and Australia (2001). Phylogenetic analysis of nucleotide and amino acid gC sequence clustered our isolate with reference BoHV-1 isolates have 100% homology but other reference BoHV-1 isolates that showing divergence with our isolate were in a separate subgroup (Figure 3). Results obtained herein coincide with recent reports regarding the presence and

circulation of BoHV-1among cattle herds in Egypt as well as molecular importance of the gC gene based PCR assays for typing, subtyping and epidemiological studies ofBoHV-1 infections (Vilcek et al, 1994; Yason et al., 1995; Rocha et al., 1998; Moore et al., 2000; El-Kholy, 2005).

Finally, the isolated virus strain was identified as BoHV-1.1_Dakahlia _2018 that was circulating among cattle suffering from respiratory disease.

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