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Molecular and Phylogenic Analysis of Bovine Respiratory Syncytial Virus in Nineveh province, Iraq

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ABSTRACT

Bovine Respiratory Syncytial Virus (BRSV) is one of the worldwide distributed infectious agents responsible for diversified clinical disease in cattle populations which causes considerable economic loss due to its negative effects on health and production. In this study, 450 nasal swab samples were collected from cows with different ages and breeds in different areas across Nineveh province, Iraq. Molecular diagnosis using nested RT-PCR and phylogenetic analysis of the G gene were performed. The results indicated a 37.31% prevalence rate of BRSV using specific primers in the PCR technique. The local isolate was submitted in GenBank under the accession number MN129181 Mosul isolate. The phylogenetic tree of local isolates of BRSV was made using the neighbor-joining system after comparison with other GenBank data. In conclusion, phylogenetic analysis of BRSV can provide information about the viral strains present in cattle and subsequently may be useful for infection control programs.

Key words: Bovine respiratory syncytial virus, Cattle, PCR, Phylogenic analysis.

INTRODUCTION

Bovine respiratory disease has an essential economic impact on animal production and cattle industry (Hacioğlu et al., 2019). Bovine Respiratory Syncytial Virus (BRSV) belongs to the *Paramyxoviridae* family and is one of the major etiology of bovine respiratory disease displaying different severities from subclinical features to fatal outcomes (Stott et al., 1980; Easton et al., 2004; Valarcher and Taylor, 2007; Brodersen, 2010).

The BRSV is enveloped and has a negative-sense, single-stranded RNA. The genome encodes 10 major proteins including the Glycoprotein (G protein) that is responsible for the virus entry into the cell (Furze et al., 1994). The G protein has a substantial mutation rate and it is the target of investigations for molecular and phylogenetic analysis (Valarcher et al., 2000).

Shony et al. (2008) isolated the virus from calves using tissue culture in Baghdad. Recently, Hussain et al. (2019) documented the prevalence rate and risk factors of the BRSV in cattle in Nineveh province and found that 83.11 % of animals were antibody positive for BRSV. The present study is the first trial to detect and identify BRSV using nested RT-PCR in cattle in Nineveh province, Iraq.

MATERIALS AND METHODS

Ethical approval

Samples were collected as per the standard sample collection procedure without any stress or harm to the animals.

Study area and study population

The study was conducted in northern, eastern, southern and western regions of Nineveh province, Iraq from September 2017 to September 2018.In this study, 26 herds with different sizes divided into small (≤10 animals) and large herds (≥20 animals) were evaluated. From each herd, 50% of cows displaying respiratory signs were sampled.A total of 450 nasal swab samples were obtained, placed in sterile test tubes, kept in an ice bag and stored at-20 °C until analysis. The animals had no history of vaccination against BRSV. Epidemiological and demographic data (animal origin, age, sex, type of breeding, season and geographical area) were recorded (Table 1).

Laboratory analysis

RNA was extracted from nasal samples using the PrimePrep Viral RNA/DNA Extraction Kit (Genet Bio Inc., South Korea). The concentration and purity of extracted RNA were determined by using Nanophotometer™ P-Class

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(IMPLEN, Germany). The RNA concentration ranged between 54 and 72 ng. The purity of RNA was obtained by computing the ratio of A_{260} nm to A_{280} nm,the purified RNA had an A_{260}/A_{280} nm ratio of 1.9-2.1.
Nested RT-PCR was performed using One Tube RT-PCR System Script RT-PCR Premix Kit (GeNet Bio Inc., South Korea). The RT-PCR mixture consisted of 3.5 µl of PCR-Grade water, 1 µl of forward primers,1 microliter of reverse primers, 1 µl of magnesium chloride, 3.5 µl of purified RNA, and 10 µl of SuPrime Script RT-PCR Premix (2X).
Two pairs of primers B5A (5'-CCA CCC TAG CAA TGA TAA CCT TGAC-'3), B6A (5'-AAG AGA GGA TGC (T/C) TT GCT GTGG'3), B7 (5'-CATCAATCCAAAGCACCACACTGTC-'3) and B8 (5'-GCTAGTTCTGTGGTGGATTGTTGTC'-3) (Vilcek et al., 1994) were used. Reverse transcription was performed at 50 °C for 30 min, followed by the denaturation step at 95 °C for 5 min. The amplification was done in 35 cycles under the following conditions: 30s at 95 °C, 30 s at 57 °C, and 60 s at 72 °C. The reaction was completed by final elongation at 72 °C for 5 min. These steps were performed in the first and second PCR reactions. PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

Sequencing and phylogenetic analysis

Using the PrimePrep™ Gel purification kit (GeNet Bio Inc., South Korea), the RT-PCR products purified were suspended in a total volume of 25 µl of diethylpyrocarbonate-treated water. A total of eight PCR amplicons from cattle nasal swabs that were positive for BRSV were sent to the commercial company for sequencing (Macrogen, South Korea) using primers B5A, B6A, B7, and B8.

Sequences of the cDNA were analyzed by using the Bioedit program version 7.2.5 and blasted against other published BRVS sequences from the GenBank using NCBI BLAST (BLASTn) (available at http://www.ncbi.nlm.nih.gov). Sequences similarity analyses were performed using online multiple sequences alignment-CLUSTALW (GenomeNet) (available at https://www.genome.jp/tools/clustalw). Multiple sequence alignment was done employing ClustalX (NCBI) program and the phylogenetic tree was generated using the same ClustalX (NCBI) and Neighbor-joining (NJ) programs.

Table 1. Epidemiological and demographic data of animal population in the study

Factors	Category	Numbers of animals
Type of breeding	Beef cattle	191
	Dairy cattle	259
Animal origin	Native	191
	Non-native (Imported)	259
Age	≤7 months	85
	7 months -1.5 years	160
	1.5 - 4 years	175
	> 4 years	30
Sex	Male	141
	Female	309
Herd size	Small (≤ 10)	191
	Large (≥ 20)	259
Season	Fall	86
	Winter	143
	Spring	107
	Summer	156
Geographical area (Nineveh province, Iraq)	Western region	42
	Southern region	86
	Eastern region	254
	Northern region	77

RESULTS

Results of the amplification of the RNA of the BRSV isolated from 450 nasal swab samples demonstrated prevalence rate of 37.31% by using specific primers B5A and B6A of G protein gene in the first reaction (Figure 1) and specific primers B7 and B8 of G protein gene in the second reaction (Figure 2).
Finally, one sequence of BRVS was deposited in GenBank using Bankit submission tool (available at http://www.Ncbi.nlm.nih. gov/WebSub/?tool= GenBank), under the accession numberMN129181 (Table 2). In this study, the homology between variable BRSV sequences obtained from nasal swabs and GenBank database

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demonstrated that sequences were highly related (99 % identity) to sequences obtained in Croatia (KY680337.1; KY680336.1 KY680335.1), and found to have a lower identity (84%) with Brazilan sequence (FJ543090.1) (Table 3).
G gene sequences of the local isolate of BRSVwas in a major clad comprising the previously published genotypes of BRSV (KY680337.1, KY680336.1, and KY680335.1) isolated in Croatia were present in the current study (Figure 3). The phylogenetic tree of BRSV demonstrated one genetically distinguishing genotype (MN129181,Mousl,Iraq). The tree was rooted with (NC 038272.1,USA) as an outer group (Figure 3).

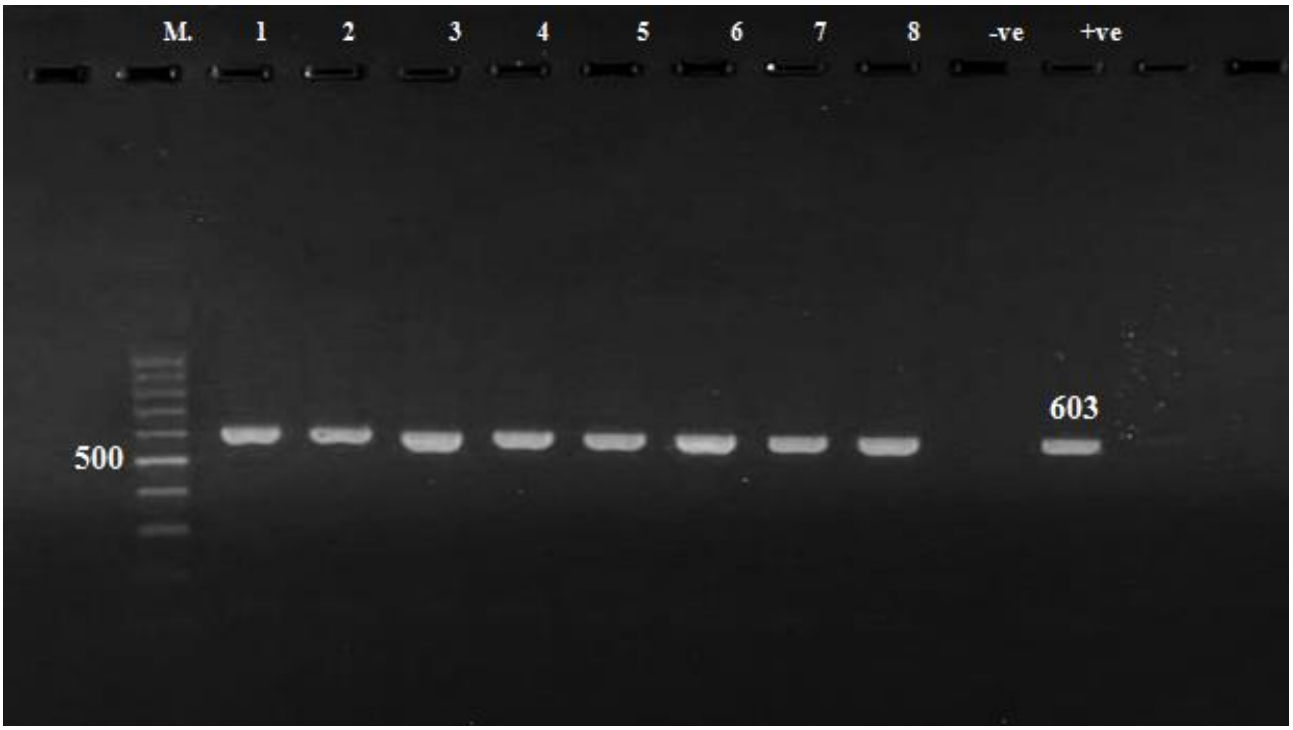

Figure 1. Gel electrophoresis of first RT-PCR reaction products using specific primers B5A and B6A of G protein gene for detection of Bovine Respiratory Syncytial Virus (BRSV) from nasal swabs of cows. lane M) Exact Mark 100-1000bp DNA ladder; Lane 1-8) positive samples 603 bp; Lane –ve) cDNA extracted from BRSV-free calf used as negative control; Lane +ve) cDNA extracted from BRSV-infected calf used as positive control.

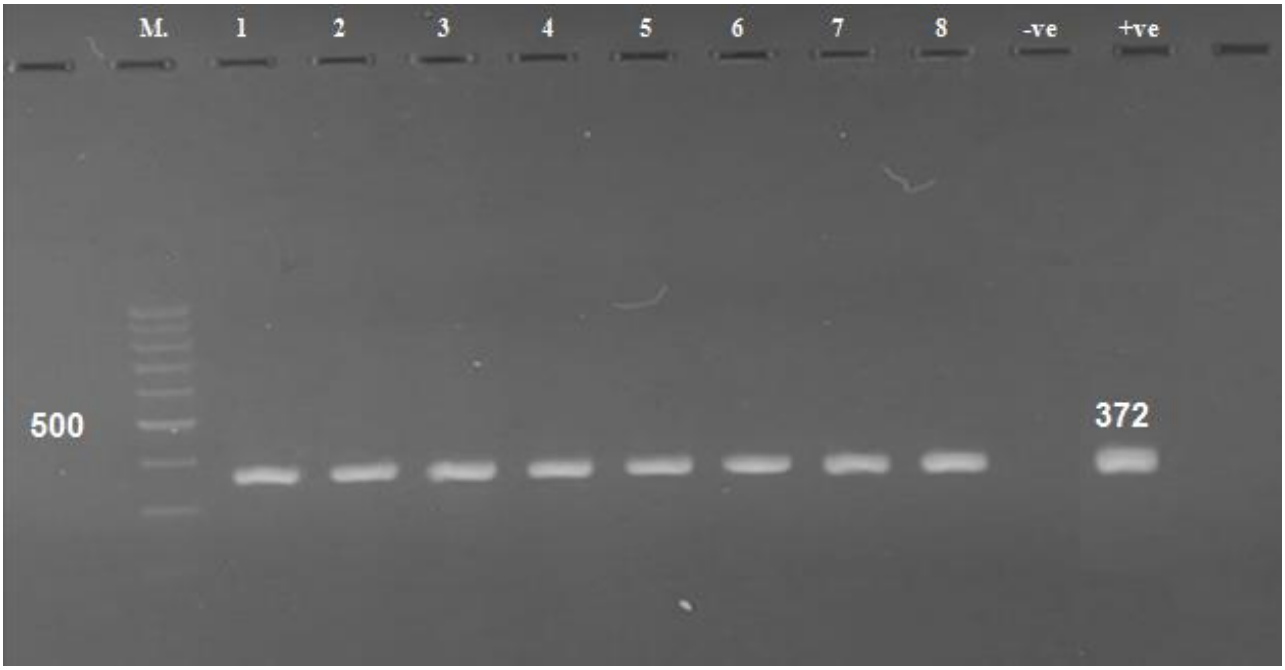

Figure 2. Gel electrophoresis ofsecond RT-PCR reaction products using specific primers B7 and B8 of G protein genefor detection of Bovine Respiratory Syncytial Virus (BRSV) from nasal swabs of cows. lane M) Exact Mark 100-1000 bp DNA ladder; Lane 1-8) positive 372 bp; Lane –ve) negative control; Lane +ve) positive control for BRSV.

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Table 2. Nucleotide sequencing of G gene of bovine respiratory syncytial virus isolated from cattle in Nineveh province, Iraq

Accession number in GenBank	Size (base pair)	Gene sequencing (5' to 3')	Gene name
MN129181	545	ATTTACATTAGTGTGGGTTTGCCAAAGCCAAGCCCACATCT AAACCAACCATCCAACAAACACCACAGCCCCTAAACCATAC CTTATCATTTTTCACAAAGCACAACCCCTAATCAACTCACAC ATCATTTCAAAGCACCACACTGTCCCGACCACCAAACATAG ACACCACTAGAGGAGCTACACACAGTCACTCAACCAATGA AACCCAAAACAGAAAAACCAAAAGCCAATCCACTCTACCT GCCACCAAAAAACCACTAACCTACTCATCAGGAAGCAATCC CTCCAAAAACCATCAAGACCAAACTAACCTCCAAACACTCC CCCATGTGCCTTGTAGTACTTGTGAAGGTAATCTTGGTTTGC TTATGACTCTGCCAGATTGGGCTAGAGAGAGCATCAAGCAG AGCCCCCACAATCACCCTTAAAAAGACACCAAAACTCAAA ACCACTAAAAAGCCAATCAAGGCAACAATCCACCACAGAA CCAGCCCCGAAGCTAAAATGCAACCTAAAAACAACACGGC AACTCCACAGCAAGG	Glycoprotein (G)

Table 3. Homology between glycoprotein (G) gene of local bovine respiratory syncytial virus obtained in this study and GenBank database using online sequence BLASTn

GenBank accession number	Country	Sequence identity	GenBank accession number	Country	Sequence identity
KY680337.1	Croatia	99%	MH133327.1	Turkey	90%
KY680336.1	Croatia	99%	MH133326.1	Turkey	90%
KY680335.1	Croatia	99%	M58307.1	United Kingdom	90%
L08414.1	USA	93%	AF188582.1	France	90%
AY910756.1	Czech Republic	93%	AF188584.1	France	90%
AY910755.1	Czech Republic	92%	Y08719.1	United Kingdom	89%
L08416.1	USA	91%	Y08717.1	United Kingdom	89%
L08415.1	USA	91%	KF501149.1	Norway	89%
U24716.1	Belgium	91%	FJ555202.1	Brazil	88%
U24715.1	Belgium	90%	Y11205.1	United Kingdom	86%
NC_038272.1	USA	90%	FJ543090.1	Brazil	84%
AF092942.1	Germany	90%			

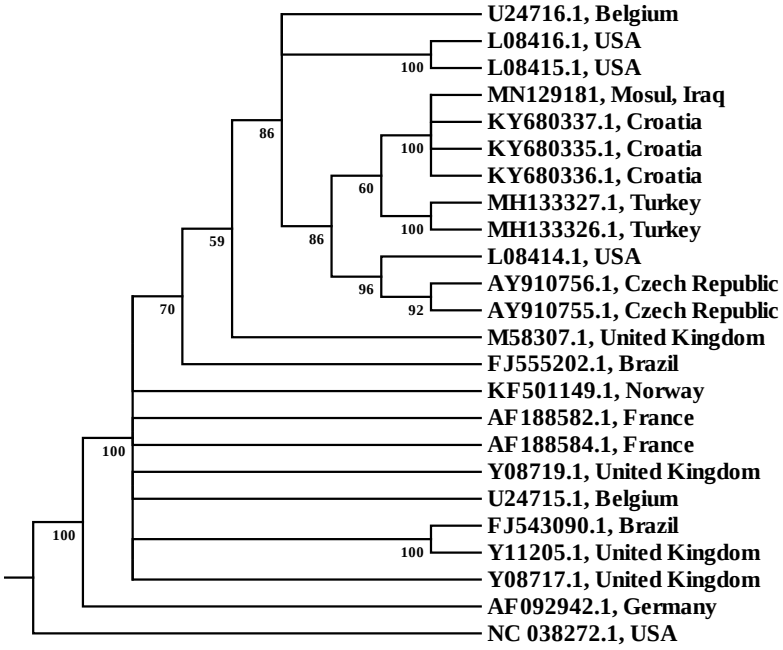

Figure 3. Phylogenetic tree of bovine respiratory syncytial virus obtained with partial sequences of G gene. The numbers on the branches indicate bootstrap supports (100 replications). GenBank accession numberMN129181 was obtained in this study.

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DISCUSSION

Diagnosis of BRSV isolated from nasal samples was determined using nested RT-PCR technique through a couple of primers target against (G) gene in nasal samples which indicate the accuracy and efficacy of the PCR technique for identification of BRSV in naturally infected cattle. This method can be used as a powerful technique in epidemiological studies, as reported in previous studies (Vilcek et al,1994; Valentova et al., 2003; Almeida et al., 2006).

The result obtained in this study demonstrated that the G gene is very conservative and can be utilized as a target sequence for the identification of BRSV through RT-PCR. This result is in agreement with Socha and Rola (2013).

This study indicated that several nucleotide sequences have a great similarity with the local isolation virus. These results are consistent with the results of other researchers as they observed a high percentage of similarity in their isolation compared to what is recorded in the GenBank (Socha, et al., 2009; Klem et al., 2014; Bertolotti et al., 2018; Krešića et al.,2018).

The phylogenetic tree for the local isolate of BRSV showed the high degree of genetic linkage between isolates, indicating the geographical location, as well as the importation of animals from different parts of the world, plays an important role in the re-distribution of genetic bonds between the isolates in different geographical regions. This is consistent with the findings of Yaegashi et al. (2005) and Hacioğlu et al. (2019).

CONCLUSION

In conclusion, the current study provides information on the molecular characterization of BRSV in Mosul, Iraq, which may be useful for future studies on the epidemiology of this infection and the selection of effective vaccines.

DECLARATIONS

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Authors' contributions

Maab Ibrahim AL-Farwachi designed the study. The collection of samples was done by Khder Jassiem Hussain and Sadam Dhahir Hassan. All samples were analyzed in a laboratory by Khder Jassiem Hussain. Maab Ibrahim AL-Farwachi and Khder Jassiem Hussain interpreted the results. Finally, after careful consideration, the manuscript was approved by all authors.

Competing interests

The authors declare that they have no competing interests.

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