



## RESEARCH ARTICLE

# Genetic diversity and phylogenetic analysis of Bovine Viral Diarrhea virus in cattle of Nineveh province, Iraq

Sadam, D. Hasan<sup>1\*</sup> and Kamal. M. Alsaad<sup>2\*\*</sup>

<sup>1</sup>Department of Internal and Preventive Medicine, College of Veterinary, Medicine, University of Mosul, Mosul, Iraq

<sup>2</sup>Department of Internal and Preventive Medicine, College of Veterinary, Medicine, University of Basrah, Basrah, Iraq

### Abstract

This is a pioneering molecular report investigating the genetic diversity and phylogenetic analysis of BVDV in cattle of Nineveh province, Iraq. A total of 494 ear notch samples was collected from cattle in five different regions of Nineveh province and tested by multiplex PCR assay. The overall prevalence of BVDV genotypes was 13.96% (69 out of 494), for BVDV1 it was 12.95% and for BVDV2 it was 1.01%. Bovine viral diarrhoea virus 1 was significantly more detected than BVDV2 ( $P < 0.05$ ). The individual sequencing analysis (BLASTn) of 69 sequences of 5'UTR gene, revealed for the first time the existence of three sub genotypes of BVDV1 in Nineveh province: sub genotype A, B and E were 3.44%, 7.89%, and 1.61% respectively. BVDV 1 sub genotype B was the most commonly detected in cattle ( $P < 0.05$ ). In addition, for the first time, two sub genotypes of BVDV2 were found in Nineveh province (sub genotype A 0.60% and sub genotype B 0.40%). Bovine viral diarrhoea virus 2 sub genotype A was commonly detected in cattle. It has been concluded that the current study found the genetic diversity of BVDV which circulating among the cattle population in Nineveh province and this result could have a significant implications for the epidemiology, diagnosis and control of the disease in Iraq.

**Key words:** BVDV, Genetic Diversity, Phylogenetic Analysis, Nineveh, Iraq.

### Introduction

Bovine Viral Diarrhoea virus BVDV affects cattle, regardless of age and also pregnant dam and this can lead to substantial economic losses because of the consequential reproductive failures, calf mortality, and also enteric and respiratory disease [1]. The BVD is a significant pathogen of cattle and globally distributed. In cattle, infections of BVDV may be as persistent infection (PI) or transient infections (TI) disease [2]. Bovine Viral Diarrhoea virus, classical swine fever virus (CSFV) and sheep Border disease virus (BDV), are classified in genus Pestivirus in the Flaviviridae family [3,4]. The genome of BVDV 12.5 kb length is a positive single strand RNA, which encodes a long open reading frame (ORF) that is flanked by 5 and 3 untranslated regions (UTRs). The translated polyprotein is cleaved by cellular and viral proteases to provide the viral structural and non-structural (NS) proteins [5]. Bovine viral diarrhoea transmission occurs either horizontally or vertically from a pregnant cow to its fetus [6]. When infection occurs in the first trimester of gestation the consequence could be immune tolerance, and fetuses that survive are born as PI (persistently infected) calves. PI animals have no detectable antibodies against BVDV, but continuously shed the virus in their excretions and secretions, and are thus primary sources of BVDV infection [7]. Bovine viral diarrhoea virus has been categorized into two genotypes, BVDV1 and BVDV2 based on the antigenic variation and sequence differences in the 5' UTR region [8]. BVDV strains have two biotypes, cytopathic

(cp) and non-cytopathic (ncp), which are distinguishable based on their impact on cultured bovine cells [9]. Both biotypes of the virus can cause disease in cattle, however, high incidence of the disease is caused by the non-cytopathic biotype [10]. The 5'UTR region is a highly-conserved component of the viral genome and consists of an internal ribosome entry site involved in translating the viral polyprotein [11]. The features and the biological roles of the Pestivirus proteins, properties of the 5 and 3'UTR, as well as molecular aspects of the replication and cytopathogenicity of the virus were reviewed recently [12,13]. Three different mechanisms result genetic changes in Pestivirus genomes first accumulation of point mutations, second non-homologous RNA recombination; and third homologous RNA recombination [14]. Different genomic regions 5' UTR, Npro, E2, NS2-3, and NS5B-3'UTR have often been employed to genotype and classify BVDV and other pestiviruses. Partial 5'UTR sequences are being commonly utilized phylogenetic ally analyzer and genetically type BVDV isolates [15]. Subtypes of the two genotypes have also been defined [16]. In recent times, reverse transcription-PCR (RT-PCR) approaches have been used in facilitating the typing of

**Correspondence to:** Kamal. M. Alsaad, Department of Internal and Preventive Medicine, College of Veterinary, Medicine, University of Basrah, Basrah, Iraq, Mobile: 00964 7709161432, Email: kamalsad58[AT]yahoo[DOT]com

**Received:** April 17, 2018; **Accepted:** April 19, 2018; **Published:** April 23, 2018

BVDV at the genotype level directly from blood samples of PI cattle [17]. Several of these sequences of BVDV which have normally been carried out utilizing genetic typing sequences from the mention regions are available in the Gen Bank [18]. Phylogenetic analysis shows the presence of sub genotypes BVDV-1 and BVDV- 2, 12 for genotype BVDV-1 (1a, 1b, 1c, 1d, 1e, 1f, 1g, 1h, 1i, 1j, 1k, 1l), five further sub genotypes of BVDV1 have been suggested (1l, 1m, 1n, 1o and 1p) and two for genotype BVDV-2 (2a, 2b) sub genotypes [19, 20]. No less than 21 BVDV-1 sub genotypes (BVDV-1a to-1u) and four BVDV-2 sub genotypes (BVDV-2a to-2d) have been defined to date [21]. Serological and molecular approaches have been employed to detect and identify genotypes and sub-genotypes of BVDV [15]. The use of molecular techniques has increased because of their speed and accuracy. The nucleic acid-based techniques are useful tools for detecting and simultaneous genotyping of BVDV without isolation and propagation in cell cultures [21]. Several researchers have used a different type of PCR technique, including reverse transcriptase polymerase chain reaction (RT-PCR) [22], multiplex RT-PCR [21]. And nested RT-PCR [23] to detect and type BVDV. The aim of the present study was to explore the genetic diversity and phylogenetic analysis of BVDV circulating in cattle populations in Nineveh region north of Iraq.

**Materials and Methods**

**Animal and sample collection**

From Jan 2017 to Aug 2017, a total of 494 ear notch samples (1 centimeter square) were obtained from the external ear pinna by utilizing sterile disposable surgical blades. The samples were placed in a sterile test tube, and kept in an ice bag and taken to the laboratory to be stored at -20 °C until testing.

**RNA extraction and amplification from cattle ear notches**

The RNA of BVDV was extracted from 494 ear notches employing the QIAamp® Viral RNA kit (RNA extraction from ear notches without purification) (ADIAGENE, BIOX DIGNOSTIC. FRANCE). The procedure was adapted from the literature that came with the extraction kit. Amplifying the highly conserved region 5’ UTR gene of BVDV from ear notch samples (n=494) was a target in multiplex PCR technique utilizing QIAGEN One-step RT-PCR Kit (QIAGEN, GERMANY) for BVDV detection and determination of genotypes. The oligonucleotides of specific primers were designed by [17] and provided by FIRST BASE LABORATORIES SDN. BHD. MALAYSIA (Table 1).

In this PCR process, two positive cDNA derived from persistently infected calve were used as positive control. The

GenBank accession numbers for cDNA positive controls were (MF347399, MF491394) for BVDV1 and BVDV2 respectively. Further, cDNA extracted from non-infected calve were used as negative control for each PCR amplification. In this study, all the animals tested positive were re-sampled 30 days following the first round of testing to differentiate PI animals. Multiplex PCR reactions were conducted in a total volume of 50 µl, composed of 10µl 5XQaigen RT PCR buffer, 3µl of each primer, 2µl of dNTP mix, 2µl of RT-PCR enzyme, 10µl of template (RNA sample) and 17µl dH2o. The mixture was centrifuged briefly and reverse transcription was carried out at 55 °C for 30 min in the thermo-cycler machine, then subjected to initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 20 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s and final extension at -72 °C for 15 min, The samples were removed at the end of the program when the temperature on the screen showed 4°C

**cDNA sequencing**

A total of 69 PCR amplicons from cattle ear notches that were positive for BVDV1 and BVDV2 by multiplex PCR, were sent to a commercial company for purification and sequencing (My TACG Bioscience Enterprise, Kuala Lumpur, Malaysia). Sequences of the cDNA were subjected to analysis using Bio edit program version 7.2.5 [24] and blasted against other published BVDV1 and BVDV2 sequences from the GenBank using NCBI BLAST (BLASTn) from NCBI (available at <http://www.ncbi.nlm.nih.gov>)

**Statistical analysis**

The Statistical analysis in this study was done by using computed 2 by 2 tables in Epi-InfoTM 7 software (version 7).

**Results**

In the current study, a total of 494 ear notch samples were tested by Multiplex PCR technique. The overall prevalence of BVD in Nineveh province was 13.96% (69 out of 494), for BVDV1 it was 12.95% and for BVDV2 it was 1.01%. BVDV 1 was significantly more detected than BVDV2 (P< 0.05) (Table 2). For the first time, three sub genotypes of BVDV1 were detected in Nineveh province: sub genotype A 3.44%, sub genotype B 7.89%, and sub genotype E 1.61%. BVDV 1 sub genotype B was the most commonly detected in cattle (P<0.05) (Table 3) In addition, for the first time two sub genotypes of BVDV2 were detected in Nineveh province: sub genotype A 0.60% and sub genotype B 0.40%. Bovine viral diarrhea virus 2 sub genotype A was commonly detected in cattle (Table 3). In this study, a total of 69 amplicons obtained from the extracted cattle ear notch using multiplex PCR was sequenced. Nine variable sequences of BVDV1 from cattle were deposited in the GenBank under accession numbers MF347398-MF347406 (Table 4). Moreover, four variable sequences of BVDV2 obtained from cattle were also deposited in the GenBank under accession numbers MF491394- MF491397 (Table 4). In this study, phylogenetic tree analysis using the neighbor-joining BVDV1 and BVDV2 yielded a tree with almost identical topologies and high bootstrap or nodal support values.

Type of PCR technique	primer	Genotypes detected	Product size (bp)	No. positive (%)
Multiplex PCR	Specific	BVDV1	360	64 (12.95)a
		BVDV2	604	5 (1.01)b
		Overall %		69 (13.96)

**Table 1:** The Oligonucleotide primers used to amplify the 5’UTR gene.

Type of BVDV	No. of samples tested	Sub genotypes	No. of sequences	Percentage (%)
BVDV1	494	A	17	3.44a
		B	39	7.89b
		E	8	1.61c
Total			64	12.95
BVDV2	494	A	3	0.60a
		B	2	0.40a
Total			5	1.01

Value significantly different ( $P < 0.05$ ) are labeled with different letters (a, b or c).

**Table 2:** Detection rate of BVDV genotypes in ear notch cattle samples using multiplex PCR techniques (n= 494).

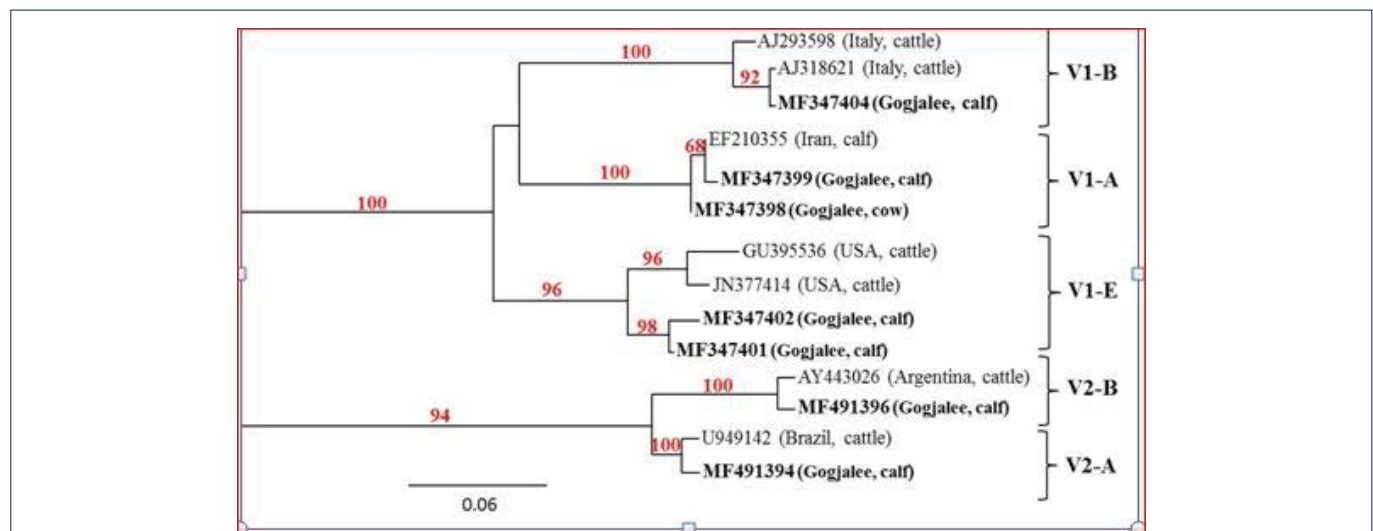
BVDV1 Cattle No.	Accession No. of 5'UTR gene from cattle	Sub genotype	BVDV2 Cattle No.	Accession No. of 5'UTR gene from cattle	Sub genotype
Co -20	MF347398	A	Ca -110	MF491394	A
Ca -110	MF347399	A	Ca -221	MF491395	A
Ca -260	MF347400	A	Ca -226	MF491396	B
Ca -6	MF347401	B	Ca -233	MF491397	B
Co -58	MF347402			B	
Co -130	MF347403			B	
Ca -11	MF347404			E	
Ca -13	MF347405			E	
Ca -223	MF347406			E	

Value significantly different at ( $P < 0.05$ ) between genotypes are labeled with different letters (a, b or c).

**Table 3:** Detection rate of BVDV1 and BVDV2 sub genotypes in cattle base on individual BLASTn analysis of positive samples.

Primer	Sequences 5'-3'	Amount of oligo(n Moles)	Size(bp)
BVD_F	TGG AGA TCT TTC ACA CAA TAG C	30.7	
V1-R	GGG AAC CTA AGA ACT AAA TC	33.7	360
V2-R	GCT GTT TCA CCC AGTT (A/G)TA CAT	27.8	604

**Table 4:** GenBank accession numbers of Nineveh BVDV1 and BVDV2 sub genotypes.



**Figure 1:** Phylogenetic tree of BVDV1 and BVDV2 obtained with partial sequences of the 5'UTR gene. The numbers on the branches indicate bootstrap supports (100 replications). Sequences in bold indicate those obtained sequences in this study. Cattle and locations are in between brackets. V1-A, V1-B and V1-E indicate to Sub genotypes of BVDV1, V2-A, V2-B indicate Sub genotypes of BVDV2 and used as out group.

Nineveh BVDV1 of 5'UTR gene sequences exhibited three major classes comprising the previously published sub genotypes of BVDV1 [49], were present in the current study (Figure 1). BVDV 1 tree demonstrated three genetically

distinguishing sub genotypes represented by cladding (sub genotype A, sub genotypes B and sub genotype E). Nineveh BVDV2 of 5'UTR gene sequences showed two major clads comprising the previously published sub genotypes of

BVDV2, were present in the current study (Figure 1). Bovine viral diarrhea virus 1 tree demonstrated three genetically distinguishing sub genotypes represented by cladding (sub genotype A and sub genotypes B). The tree was rooted with BVDV2 (U949142A, AY443026, MF491394 and MF491396) as an out group. In the phylogenetic tree of BVDV1 and BVDV2, the samples were obtained from cattle ear notch, from different regions in Nineveh province.

## Discussion

BVDV is a widely spread virus that causes various clinical syndromes leading to very substantial economic losses. Furthermore, the two species, BVDV-1 and BVDV-2, are dissimilar and differentiated based on genetic and antigenic variances, and are categorized in the genus Pestivirus within the Flaviviridae family and found on all continents of the world [11]. The results illustrated that the overall prevalence of BVDV in Nineveh was 13.96% compared to previous studies in Iraq and other countries that used the PCR technique to diagnose this disease. The prevalence was 6% in cattle in regions around Baghdad [15]. In Basrah and Nasiriya cities in Iraq, the prevalence of BVDV was 10 % [26]. In Tunisia, it was 2.65% [27]. In Egypt it was 10.4% [28]. Regional differences within the same country could be attributed to the different management practices, and how sensitive the diagnostics methods used are, incidence of competent vectors, cattle activity, presence and efficacy of control programs, the climatic variations, extensive cattle trade and uncontrolled animal movement as well as variations in the cattle population size, Biosecurity, and persistence of BVDV [29]. In the current study, based on conserved untranslated regions 5'UTR, multiplex polymerase chain reaction (PCR) allow for the detection and differentiation between BVDV genotype 1, 2. These results suggest that multiplex PCR can be used to amplify two or more DNA fragments in a single reaction. In comparison with single template PCR, multiplex PCR can lower the time, number of tubes, the cost of the reagents, and possible contamination. This finding was consistent with [21, 30, 31], who showed that the BVDV could be typed directly from blood samples by using multiplex polymerase chain reaction (PCR).

This study has demonstrated that the 5'-UTR region of the genome is very conservative and can be utilized as a target sequence for diagnostic RT-PCR. This finding agrees with [32, 33]. Moreover, the 5'UTR gene is highly important in the epidemiological genetic diversity and phylogenetic studies of pestiviruses [34, 35]. The results in this study revealed that the overall detection of both genotypes was 13.96%, with a detection rate of 12.95% and 1.01% for BVDV1 and BVDV2 respectively in Nineveh province. This may be due to the fact that BVDV1 and BVDV2 is globally distributed. This finding corresponds with other studies in different countries such as in Iran [36], Turkey [36], Tunisia [27], Spain [38] and Italy [39]. It should be noted though that these results differ from those documented for another province of Iraq by [27], who detected BVDV genotype 1 only in Basrah and Nasiriya cities in Iraq.

The results of the current study indicated that that BVDV1 was significantly more detected than BVDV2 in Nineveh province. This result is consistent with previous studies in other countries [38, 40, 41]. In addition, Yesilbag [27] mentioned that the global distribution rate of BVDV-1 was 88.2%, considerably higher than for BVDV-2, which was 11.8% based on phylogenetic of BVDV in different countries. In the current study regarding sequencing BLASTn of PCR amplicons of the 5'UTR gene for BVDV1 and BVDV2 were obtained from cattle ear notch samples. These findings suggest that there is extensive genetic diversity found within BVDV type 1. Three sub genotypes of BVDV1 were detected for the first time in Nineveh province, including sub genotypes A, B and E. This is almost similar to previously known genotypes in other parts of the world such as reported by [42, 43]. The absence of possible BVDV1 sub genotypes C, F, G, H and U, in this current study reflects the findings of other studies by Factor et al. [44, 45]. In addition, two sub genotypes of BVDV2 were detected for the first time in Nineveh province including sub genotypes A and B. This result is similar to previously reported sub genotypes in other countries such as China, Brazil, Argentina, Italy and USA [39, 46-49].

BVDV2 sub genotype A was more commonly detected in cattle in Nineveh province compared to sub genotypes B. This is similar to previous studies in other parts of the world as reported by [25]. These variations in the distribution of BVDV sub genotypes can be interpreted based on the insertion or deletion in the 5'UTR gene sequences, which can be valuable to the virus by way of modifying the gene expression, which supports their adaptation to a new environment such as re-occurring PI, a different host (sheep, goats and cattle) and climatic changes [50].

## Acknowledgements

This work was supported by the Faculty Veterinary Medicine, University of Mosul, Iraq.

## References

1. Segura-Correa JC, Zapata-Campos CC, Jasso-Obregón JO, Martínez-Burnes J, López-Zavala R (2016) Seroprevalence and risk factors associated with bovine herpes virus 1 and bovine viral diarrhea virus in North-Eastern Mexico. *Open Vet J* 6: 143-149. [[View Article](#)]
2. Passler T, Walz P, Ditchkoff S, Givens M, Maxwell H. et al. (2007) Experimental persistent infection with bovine viral diarrhea virus in white-tailed deer. *Vet Microbiol* 122: 350-356. [[View Article](#)]
3. Loneraga GH, Thomson DU, Montgomery DL, Mason GL, Larson RL (2005) Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. *J Am Vet Med Assoc* 226: 595-601. [[View Article](#)]
4. Simmonds P, Becher P, Collet MS, Gould EA, Heinz FX, et al (2012) Family Flaviviridae. In: *Virus Taxonomy. Ninth report of the International Committee on Taxonomy of Viruses*. Elsevier, Academic Press. Amsterdam, Boston, Heidelberg etc., pp. 1003-1020. [[View Article](#)]
5. Lindenbach BD, Thiel HJ, Rice, CM (2007) Flaviviridae: the

- viruses and their replication. In *Fields Virology*, (5<sup>th</sup> Edn) pp. 1101-1151. [[View Article](#)]
6. Tremblay R (1996) Transmission of bovine viral diarrhoea virus. *Vet Med* 91: 858-866. [[View Article](#)]
  7. Baker J (1995) The Clinical Manifestations of Bovine Viral Diarrhea Infection. *Vet Clin North Am Food Anim Pract* 11: 425-445. [[View Article](#)]
  8. Ridpath I, Bolin S, Dubovi E (1994) Segregation of bovine viral diarrhoea virus into genotypes. *Virology* 205: 66-74. [[View Article](#)]
  9. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005) Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses. *Elsevier Academic*. [[View Article](#)]
  10. Kelling CL (2004) Evolution of bovine viral diarrhoea virus vaccines. *Vet Clin North Am Food Anim Pract* 20: 115-129. [[View Article](#)]
  11. Yesilbag K, Förster C, Ozyigit MO, Alpaya G, Tuncer, P, et al. (2014) Characterization of bovine viral diarrhoea virus BVDV isolates from an outbreak with haemorrhagic enteritis and severe pneumonia. *Vet Microb* 169: 42-49. [[View Article](#)]
  12. Becher P, Tautz N (2011) RNA recombination in pestiviruses, cellular RNA sequences in viral genomes highlight the role of host factors for viral persistence and lethal disease. *RNA Biol* 8: 216-224. [[View Article](#)]
  13. Tautz N, Tews BA, Meyers G (2015) The molecular biology of pestiviruses. *Adv Virus Res* 93: 47-160. [[View Article](#)]
  14. Luzzago C, Bandi C, Bronzo V, Ruffo G and Zecconi A (2001) Distribution pattern of bovine viral diarrhoea virus strains in intensive cattle herds in Italy. *Vet Microb* 83: 265-274. [[View Article](#)]
  15. Ridpath JF, Fulton RW, Kirkland PD and Neill JD (2010) Prevalence and antigenic differences observed between bovine viral diarrhoea virus sub genotypes isolated from cattle in Australia and feedlots in the southwestern United States. *J Vet Diagn Invest* 22: 184-191. [[View Article](#)]
  16. Vilcek S, Paton DJ, Durkovic B, Strojny L, Iyata G, et al. (2001) Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. *Arch Virol* 146: 99-115. [[View Article](#)]
  17. Gillespie JH, Coggins L, Thompson J, Baker JA (1961) Comparison by neutralization tests of strains of virus isolated from virus diarrhoea and mucosal disease. *Cornell Vet* 51:155-159. [[View Article](#)]
  18. Vilcek S, Herring AJ, Herring JA, Nettleton PF, Lowings JP, et al. (1994) Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three gene groups using polymerase chain reaction and restriction endonuclease analysis. *Arch Vir* 136: 309-323. [[View Article](#)]
  19. Alpaya G, and Yeşilbağ K (2015) Serological relationships among subgroups in bovine viral diarrhoea virus genotype 1 (BVDV-1). *Vet Microbiol* 175: 1-6. [[View Article](#)]
  20. Flores E, Ridpath J, Weiblen R, Vogel F, Gil L (2002) Phylogenetic analysis of Brazilian bovine viral diarrhoea virus type 2 (BVDV-2) isolates: evidence for a sub genotype within BVDV-2. *Virus Res* 87: 51-60. [[View Article](#)]
  21. Bazid AI, Nayel MA, Ahmed M, Elsify ZAH, Khalil SA (2015) Molecular diagnosis and genotyping of Bovine viral diarrhoea virus. *Minufiya Vet. J* 9: 1-13. [[View Article](#)]
  22. Hooft van Iddeking BJJ, van Wamel JLB, van Gennip HGP, Moorman RJM (1992) Application of the PCR to the detection of BVDV infections in cattle. *Vet Microbiol* 30: 21-34. [[View Article](#)]
  23. Sullivan DG, Akkin RK (1995) A nested polymerase chain reaction assay to differentiate pestiviruses. *Virus Res* 38: 231-239. [[View Article](#)]
  24. Hall TA (1999) Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98. [[View Article](#)]
  25. Al-Ajeeli KSA, Hasan ASH (2011) Detection of Bovine Viral Diarrhoea Virus by Conventional RT-PCR: A comparative Study. *Al-Anbar J Vet Sci* 4: 121-128. [[View Article](#)]
  26. Jarullah BA, Aed Gati J, and Saleh A (2012) Prevalence of Bovine viral diarrhoea virus in cattle herds from Basrah and Nassirya Provinces by direct and indirect Elisa and Real time qPCR. *AL-Qadisiya J Vet Med Sci* 11: 1-7. [[View Article](#)]
  27. Thabti F, Bakkali L, M'zah A, Ben Romdane S, Russo BEN (2005) First detection and genetic characterization of bovine viral diarrhoea viruses (BVDV) types 1 and 2 in Tunisia. *Rev Med Vet* 156: 419-422. [[View Article](#)]
  28. Soltan MA, Wilkes RP, Elsheery MN, Elhaig MM, Riley MC, et al. (2015) Circulation of bovine viral diarrhoea virus – 1 (BVDV-1) in dairy cattle and buffalo farms in Ismailia Province, Egypt. *J Inf Dev Ctri* 9: 1331-1337. [[View Article](#)]
  29. Graham DA (2001) Bovine viral diarrhoea virus (BVDV) on cattle farms-disease and control. *Cattle pract* 9: 111-118. [[View Article](#)]
  30. Kurćubić V, Đoković R, Vidanović D, Šekler M, Matović K, et al. (2013) Bovine respiratory disease complex (BRDC): viral and bacterial pathogens in Serbia. *Biot Ani Husb* 29: 37-43. [[View Article](#)]
  31. Gafer JA, Hassaneen TK, Salem HA, and Madboly M (2015) Genetic detection and pathological finding of BVDV and BHV-1 in camel calves. *Assiut Vet Med J* 61: 34-45. [[View Article](#)]
  32. Letellier C, Kerkhofs P (2003) Real-time PCR for simultaneous detection and genotyping of bovine viral diarrhoea virus. *J Vir Method* 114: 21-27. [[View Article](#)]
  33. Roman M, Pogranichni M, Schnur E, Eran A, Raizman D, et al. (2011) Isolation and Genetic Analysis of Bovine Viral Diarrhoea Virus from Infected Cattle in Indiana. *Vet Med Int*: 925910. [[View Article](#)]
  34. Robesova B, Kovarcik K and Vilcek S (2009) Genotyping of bovine viral diarrhoea virus isolates from the Czech Republic. *Vet Med* 54: 393-398. [[View Article](#)]
  35. Baki S, Ahmet KA, Serkal G, Muhammet EA (2012) Genetic Variability of Bovine Viral Diarrhoea Virus in the 5'-UTR in the Central Anatolia of Turkey. *Acta Sci Vet* 40: 1-7. [[View Article](#)]
  36. Khodakaram-Taft A, Mohammadi A, Farjani GK (2016) Molecular characterization and phylogenetic analysis of bovine viral diarrhoea virus in dairy herds of Fars province, Iran. *Iran J Vet Res* 17: 89-97. [[View Article](#)]
  37. Huseyin Y, Ed A, Julia R, Nuri T (2012) Genetic Diversity and frequency of bovine viral diarrhoea virus (BVDV) detected in cattle in Turkey. *Comp Immu* 35: 411-416. [[View Article](#)]
  38. Aduriz G, Atxaerandio R, and Cortabarria N (2015) First detection of bovine viral diarrhoea virus type 2 in cattle in Spain. *Vet Rec Open* 2: e000110. [[View Article](#)]

39. Luzzago C, Ebranati E, Sasserà D, Lo Prest A, Lauzi S, et al (2012) Spatial and temporal reconstruction of bovine viral diarrhea virus genotype 1 dispersion in Italy. *Infect Genet Evol* 12: 324-331. [[View Article](#)]
40. Byung CA, Walz PH, Kennedy GA, Kapil S (2005) Biotype, Genotype, and Clinical Presentation Associated With Bovine Viral Diarrhea Virus (BVDV) Isolates From Cattle. *Intern J Appl Res Vet Med* 3: 319-325. [[View Article](#)]
41. Tao J, Zhu Q, Lin YQ, Zhu GQ (2011) Identification and characterization of 16 recent Chinese isolates of bovine viral diarrhea virus genotype 2 (BVDV-2). *Afr J Microb Res* 5: 1272-1276. [[View Article](#)]
42. Jackova A, Novackova M, Pelletier C, Audeval C, Gueneau E, et al. (2008) The extended genetic diversity of BVDV-1: Typing of BVDV isolates from France. *Vet Res Commu* 32:7-11. [[View Article](#)]
43. Booth RE, Thomas CJ, El-Attar LMR, Gunn G, Brownlie J (2013) A phylogenetic analysis of Bovine Viral Diarrhea Virus (BVDV) isolates from six different regions of the UK and links to animal movement data. *Vet Res* 44:43-57. [[View Article](#)]
44. Deng M, Ji S, Fei W, Raza S, He C, et al. (2015) Prevalence study and genetic typing of bovine viral diarrhea virus (BVDV) in four bovine species in China. *PLoS ONE* 10: e0121718. [[View Article](#)]
45. Giammarioli M, Ceglie L, Rossi E, Bazzucchi M, Casciari C, et al (2015) Increased genetic diversity of BVDV-1: recent findings and implications thereof. *Virus Genes* 50:147-151. [[View Article](#)]
46. Topliff CL, Kelling CL (1998) Virulence markers in the 5' untranslated region of genotype 2 bovine viral diarrhea virus isolates. *J Virol* 25: 164-172. [[View Article](#)]
47. Jones R, Cigliano MM, Zandomeni RO, Weber EL (2004) Phylogenetic analysis of bovine pestiviruses: testing the evolution of clinical symptoms. *Clad* 20: 443-453. [[View Article](#)]
48. Zhong F, Li N, Huang X, Guo Y, Chen H, et al (2011) Genetic typing and epidemiologic observation of bovine viral diarrhea virus in Western China. *Virus Gen* 42: 204-207. [[View Article](#)]
49. Silveira S, Weber MN, Mósena AC, da Silva MS, Streck AF, et al. (2017) Genetic Diversity of Brazilian Bovine Pestiviruses Detected Between 1995 and 2014. *Transb Emerg Dis* 64: 613-623. [[View Article](#)]
50. Grooms DL (2004) Reproductive consequences of infection with bovine viral diarrhea virus. *Vet Clin North Am Food Anim Pract* 20: 5-19. [[View Article](#)]

**Citation:** Hasan SD, Alsaad KM (2018) Genetic diversity and phylogenetic analysis of Bovine Viral Diarrhea virus in cattle of Nineveh province, Iraq. *Vet Sci Med* 1: 001-006.

**Copyright:** © 2018 Hasan SD, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.