Full-length genome sequencing of a very virulent infectious bursal disease virus isolated in Tunisia

Jihene Lachheb,[*,1] Adam Jbenyeni,[†] Jihene Nsiri,[*] Imen Larbi,[*] Faten Ammouna,[*] Imen El behi,[*] and Abdeljelil Ghram[*]

[*]*Laboratory of Epidemiology and Veterinary Microbiology LR0020, Institut Pasteur of Tunis, Tunis El Manar University, Tunis Belvedere 1002, Tunisia; and* [†]*Veterinary Practice El Intilaka, Canal Street 20, 2097 Ben Arous, Tunisia*

ABSTRACT Infectious bursal disease (**IBD**), an acute, highly contagious, and immunosuppressive avian disease, is caused by infectious bursal disease virus (**IBDV**) and constitutes one of the main threats to the poultry industry, worldwide. This study was performed to isolate and characterize IBDV isolates circulating in Tunisia. Eleven collected bird samples were identified using an SYBR Green–based one-step real-time reverse transcriptase polymerase chain reaction. The full-length genome sequencing of 7 of the 11 IBDV isolates has been realized. *VP2* gene data showed limited sequence variations for all the 7 tested samples. The few nucleotide changes were silent and the deduced amino acid sequences were identical with the exception of a unique and characteristic nonsilent mutation (C_{1203}) detected for the TN37/19 isolate, with a change of amino acid (L) to (F) at position 401. In addition, the serine-rich heptapeptide SWSASGS, characteristic of virulent IBDV, as well the amino acid residues, conserved in most very virulent IBDV (**vvIBDV**) strains, were detected in all the Tunisian tested isolates. Nucleotide sequences of *VP5* gene revealed the presence of 5 substitutions leading to changes in the amino acid sequences of the virus. Two of these mutations were unique and characteristic of the Tunisian isolates. Besides, the alternative AUG start codon, characteristic of vvIBDV, was observed in all obtained *VP5* gene sequences. The Tunisian protein sequences of VP1 showed E242 and the TDN triplet at positions 145, 146, and 147, a motif specific of vvIBDV. Phylogenetic analyses of the 5 genes confirmed the sequence alignment results and showed that the Tunisian strains are closely related to the very virulent Algerian IBDV strains.

Key words: full genome, phylogeny, Tunisia, vvIBDV

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INTRODUCTION

Infectious bursal disease virus (**IBDV**) causes a contagious and immunosuppressive disease and is one of the most economically significant viruses of poultry worldwide (Winterfield et al., 1978; Berg, 2000). The virus belongs to *Avibirnavirus* genus within the *Birnaviridae* family. It has a bisegmented dsRNA genome named A and B. The larger segment A (3.4 kb) contains 2 open reading frames, which encodes a polyprotein designated VP2- VP4-VP3 and a nonstructural protein VP5. The smaller segment B (2.8 kb) encodes VP1 protein, an RNA-dependent RNA polymerase with capping enzyme activities (Spies et al., 1990).

Two serotypes of IBDV have been recognized, of which only serotype 1 is pathogenic and causes mortalities in chickens. This serotype can be categorized into different pathotypes including classical virulent strains (**cvIBDV**), antigenic variant strains (**avIBDV**), and very virulent strains (**vvIBDV**) (Snyder et al., 1988). These antigenic variations were shown to be based on mutation in the hydrophilic hypervariable region of the *VP2* gene, known to be critical for the determination of conformational epitopes, responsible for recognition of the virus neutralizing antibodies (Nagarajan and Kibenge, 1997; Eterradossi et al., 1998). Indeed, characterization of IBDV field strains has been fundamental in the development of preventive measures and epidemiologic campaigns aimed at control of the spread of vvIBDV (Hosseini et al., 2004). Further studied demonstrated the contribution of *VP1* gene in obtaining

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[1]Corresponding author: jihene.lachheb@pasteur.tn

complementary genetic information for more precise characterization of IBDV's virulence (Molini et al., 2019). Recently, it has been proven that molecular analysis of both genome segments is necessary to correctly identify genetic reassortment within the genome segment B and amino acid substitution in the genome segment A, often shown to occur in vvIBDV (Hon et al., 2006; Le Nouën et al., 2006; Wang et al., 2019). Our study was undertaken to study molecular characteristics of the full genome length of the Tunisian IBDV isolated strains, with the aim of better understanding of genetic variations in circulating viruses, in Tunisia.

MATERIALS AND METHODS

Bursa of Fabricius Sampling and Processing

Eleven samples of bursa of Fabricius were collected by the veterinarian from commercial poultry and backyard birds reared in different regional districts of Tunisia (Table 1). Samples were treated as part of routine diagnosis of infectious bursal disease (**IBD**), during 2013–2019 (Table 1). The bursas were collected from birds with clinical signs of IBD, such as depression, prostration, and anorexia. The animals also showed ruffled feathers and diarrhea charged with urates. At necropsy, chickens had gross lesions including enlarged, edematous, and/or hemorrhagic bursa, showing petechial hemorrhages with fibrinous exudate. Small hemorrhages in the skeletal muscles, nephritis, and hemorrhages in the proventriculus were also observed. Theses samples were transported at 4°C in the Dulbecco's minimum essential medium (PANTM Biotech) media then stored at −80°C.

Mortality rates about 1.35 to 4% were recorded in the flocks. Commercial flocks were already vaccinated against (IBDV) either in the hatchery at day one, with live immune complex or recombinant vaccine, or in drinking water, in the farms with Winterfield 2512 intermediate live attenuated vaccine at day 7, followed by a hot live attenuated vaccine at day 14, in drinking water. Backyard birds were not vaccinated. The Fabricius bursas (25 mg) were homogenized using a bead-based homogenizer (PowerLyzer 24) in presence of 1 mL Dulbecco's minimum essential medium (PANTM Biotech) and 30 µL of premade mix of L-glutamine, penicillin, and streptomycin of antibiotics solution (SIGMA). Homogenates were centrifuged at 1,500 rpm for 15 min to remove organ debris and recover viral supernatants. The virus suspensions were then stored at −80°C until use.

RNA Extraction and IBDV Detection

RNA extraction was carried out from 200 µL of organ supernatant, using the QIAamp cador Pathogen Mini Kit QIAGEN extraction kit, automated on the QIAcube system. Infectious bursal disease virus detection was performed in a Swift spectrum 48 Thermal Cycler (ESCO), using KAPA SYBR FAST One-Step qRT-PCR Kit in accordance with the manufacturer's instructions and primers as described by Kong et al. (2009). The melting curve analysis was conducted by raising 0.5°C between 55°C and 95°C.

Full Genome Amplification

All 5 genes corresponding to the full length of IBDV genome were targeted by a conventional RT-PCR, using primer pairs specific for each gene (Table 2). The RT-PCR reaction was carried out using EasyScript One-Step RT-PCR (TransGen Biotech) kit in accordance with the manufacturer's instructions. The RT-PCR was performed in Bio-Rad T100 thermocycler under the following conditions: reverse transcription at 45°C for 30 min, denaturation at 94°C for 5 min, 36 cycles of denaturation at 94°C for 30 s, annealing at 52-55-55-53-55-52°C for 30s, for *VP2*, *VP5*, *VP3*, *VP4*, and *VP1*(a, b, e) and *VP1*(c, d) genes, respectively, extension for 72°C (1-2 kb/min), depending on the gene length, and final extension at 72°C for 10 min. The RT-PCR products were analyzed in a 1-2% agarose gel electrophoresis and visualized by ultraviolet illumination.

Sequencing and Phylogenetic Analysis

The full-length genome of all the 7 Tunisian isolates, designated TN160/17, TN19/19, TN20/19, TN37/19, TN46/19, TN57/19, and TN74/19, were successfully

Table 1. Summary table of flock sample history.

Sample code/years of isolation	Production type	Age (days)	All number	Region
TN575/13	B[1]	24	NS	NS
TN54/17	B	30	24,000	Nadhour
TN160/17	B	37	24,000	Sfax
TN106/18	B	23	NS	Zaghouan
TN19/19	B	30	6,400	Morneg, El Ksibi
TN20/19	B	25	10,000	Ghobet Legha, Korba
TN37/19	B	26	15,000	Soliman
TN46/19	B	28	17,000	Soliman
TN57/19	B	33	7,100	Ben Arous
TN74/19	B	26	19,000	Boumhel
TN95/19	BC[2]	NS	50	Ben Arous

[1]B= Broilers.
[2]BC= Backyard Chicken.

Table 2. Primers used for the RT-PCR amplification of all genes.

Target regions	Primers 5′-3′	Positions	Length (bp)	References
VP5 and the beginning of *VP2*	F: GGATACGATCGGTCTGAC R: TCAGGATTTGGGATCAGC	1-1,263	1,263	Hernández et al., 2010
The following of *VP2*	F: GCCCAGAGTCTACACCAT R: CCCGGATTATGTCTTTGA	736-1,478	743	Sreedevi and Jackwood, 2007
VP1 (a)	F: GGATACGATGGGTCTGAC R: ATCCTTGACGGCACCCTT	1-695	695	Rudd et al., 2002
VP1 (b)	F: GCATAGCCCAGCTACTTGA R: GGGCAATGTTCATCGC	662-1,384	722	Jackwood et al., 2008
VP1 (c)	F:CGGTGAGGATGACAAGCCCC R: GGCACGATGAGTCCACCAC	756-1,522	767	He et al., 2014
VP1 (d)	F:ACCCTTGTGCTAGACCAGTG R: GAACCCCTTTGCCTCCAAG	1,518-1,997	480	Tiwari et al., 2003
VP1 (e)	F: ATACAGCAAAGATCTCGGG R: CGATCTGCTGCAGGGGGCC CCCGCAGGCGAAGG	1,839-2,827	988	Mundt and Vakharia 1996
VP3	F:GGTCTAGAAAGTTGGCTGGTCCCGGAGCATT R:GGTCTAGAAGCCTCACTCAAGGTCCTCATCAG	2,233-3,172	939	Wang et al., 2007
VP4	F: GCAGGAGCATTCGGCTTC R: CCACGTTGGCTGCTGC	1,446-2,466	1,020	Jackwood et al., 2008

amplified and sequenced. Their respective GenBank Accession Numbers are MN480305-MN380311 for *VP2*, MN447297-MN447303 for *VP5,* MN539750-MN539755 and MN563601 for *VP*3, MN652173-MN652175 and MN696992-MN696994 for *VP*4, and MN746269-MN746275 for *VP1* genes. Multiple alignments of IBDV strain sequences with downloaded reference sequences were performed, using BioEdit (version 7.2.5.0) and ClustalW program. Phylogenetic analyses using the maximum likelihood method with the Hasegawa-Kishino-Yano-parameter model were conducted by MEGA (Molecular Evolutionary Genetics Analysis, version 7) and assessed statistically by analyzing 100 bootstrap replications.

Analysis of Recombination Events

Recombination Detection Program 4 (RDP v.4.97) software was used to detect potential recombination sites in segment A and B genome of IBDV sequences using default settings. Only recombination events supported by no fewer than 5 independent methods will be regarded as positive.

RESULTS

Detection of IBDV by One-Step Real-Time RT-PCR

All 11 suspected bursa samples analyzed were positive for IBDV by RT-PCR that target a conserved region of *VP4* gene. The specific amplicons showed a melting peak at 85°C; primer-dimers or nonspecific products were not detected.

Full Genome Sequences Analyses

The studied Tunisian strains showed high percentage of similarity varying between 99 and 100% for the 5 genes and highlighted the probability of similar strains circulating in 2017 as compared with those present in 2019. However comparing with vvIBDV strains isolated in Algeria and Morocco, the similarity is between 97-99.2% and 95-96%, respectively.

VP1 *gene* A high similarity of 90 and 96% was observed between the Tunisian and other reference strains. The results showed silent and specific mutations in single, some, or all strains were seen at positions T336, C687, and A2364. Nucleotide modifications shown in one, some, or all Tunisian isolates are reported in Table 3.

VP2 *gene* The percentages of similarity between the Tunisian isolates is much higher than that calculated when comparing with the old Tunisian strain (AY665672 isolate PO7) (97.1–97.4%). Unique and characteristic silent mutations T630 and A1104 were shown in TN74/19 and TN57/19 isolates, respectively. The substitution of nucleotide A1203>C, in TN37/19 isolate, was unique and change aa L401 > F. The result of protein sequence alignments showed that the aa sequences of the Tunisian isolates possess conserved residues, characteristic of vvIBDV strains such as A222, I242, Q253, I256, D279, A284, I294, S299. The pattern (SWSASGS) from aa 326 to 332, a serine-rich heptapeptide next to the second hydrophilic region of the *VP2* gene, specific for vvIBDV strains, was identified in the 7 Tunisian isolates.

VP3 *gene* Analysis of the nucleotide sequences of the *VP3* genes made it possible to note specific substitutions representing nonsilent mutations reported in Table 3. Other variations such as G198 > C/T, T582 > C, G849 > A were characteristics of certain Tunisian strains and were considered as silent mutations. In addition, similarity percentage of 94 to 97% was also observed between the Tunisian isolates and the reference strains from different regions of the world.

VP4 *gene* A similarity score between 95 and 97% was observed between the Tunisian isolates and the considered reference strains. Analysis of the nucleotide sequences of the *VP4* genes of the Tunisian isolates revealed that variations at positions G30 > A, G300 >

Table 3. Nucleotide and amino acid substitutions in all genes between Tunisian and other IBDV strains.

	Nucleotide mutations									
	VP1							VP5		VP2
Strains	275	807	823	1195	1490	2209	2621	15	16	1200
TN	G	T	T	A	A	A	C	A	A	C
Others	T	A	A	G	G	C/T	A	T	C	A/G

	VP4								VP3					
	67	68	104	393	533	534	914	915	22	77	370	877	904	905
TN	A	G	G	-	-	-	C	C	C	A	A	G	G	A
Others	G	A	C	T	T	A	G	A/T	G	C	C	A	C	G

	Amino acid substitutions								
	VP1						VP5		VP2
Strains	92	275	399	497	737	874	5	6	400
TN	G	L	I	S	I	P	E	K	F
Others	V	I	V	N	L	Q	D	Q	L

	VP4						VP3				
	23	35	131	178	305	321	8	26	124	293	302
TN	R	G	-	-	C	A	R	H	K	A	D
Others	E	A	I	I	S	E	G	P	Q	T	R

T, A492 > G, T540 > C, C555 > T, A709 > C, G750 > A, C762 > T, T/C939 > G are silent mutations, found either in all, some, or single Tunisian isolates. Thus, specific and missense mutations within the Tunisian isolates are reported in Table 3.

VP5 *gene* The results of nucleotide sequence alignment of *VP5* genes demonstrated that the Tunisian isolates show an AUG alternative initiation codon, characteristic of vvIBDV. It was then shown that the unique substitutions at positions A15 in TN46/19 isolate and A16 in TN37/19 isolate are different from those observed in other Tunisian isolates as well as isolates from other countries. These mutations represented a nonsilent mutations resulting in aa sequence changes (Table 3).

Phylogeny

Phylogenetic trees, based on the nucleotide sequences of the 5 genes, are illustrated in Figures 1–5. The Tunisian isolates being part of the vvIBDV strains, distinct from variant, classical and attenuated IBDV strains which composed 2 clusters (Figures 1–5). Phylogenetic studies, based on the nucleotide sequence of each one of the 5 genes among the 43, 44, or 45 sequences analyzed, were classified in 2 distinct phylogenetic branches in the vvIBDV cluster. The first branch contained the 7 Tunisian isolates along with vvIBDV strains from Algeria. A heterogeneous second branch grouped vvIBDV, isolated from different other countries namely Malaysia, China, America, Nigeria. The similarity scores, calculated from the sequence alignments, supported the phylogenetic results and revealed scores of 98.7 to 99.2% for (VP2), 99.3 to 100% for (VP5), 95 to 99% for (VP1 and VP3), and 89 to 98% for (VP4). It should be noted that the Tunisian isolates have the highest percentages of similarities between each other and with the Algerian strains, reaching a 100%. Nevertheless, this percentage, although it is lower than that of vvIBDV reference strains from other countries and attenuated strain of the second branch, remains relatively high with a reading between 96.5-97.8% and 96% with Morocco strains.

Analysis of Recombination Events

The RDP4 was run to identify putative recombinant events of genome sequences for segment A and B. It was shown that no recombination events were identified for Tunisian isolates as compared with different sequences in the GenBank database. This result supports the novelty of the strains by showing the absence of probable positions of break and recombination points.

DISCUSSION

This work was developed to characterize Tunisian isolates for a better knowledge on circulating viral strains in the country by means of studying their full genome. Indeed, the virulence of IBDV can be related to several genes such as the *VP2* gene, which encodes the major structural protein of the capsid (Kim et al., 2010). This protein is involved in the pathogenicity, the virulence and the tropism of the virus (Pikula et al., 2018). It should be noted that substitution A1200 > C in TN37/19 isolate, which is unique with respect to other Tunisian isolates as well as other strains from different countries, leading to a change of aa L400 > F

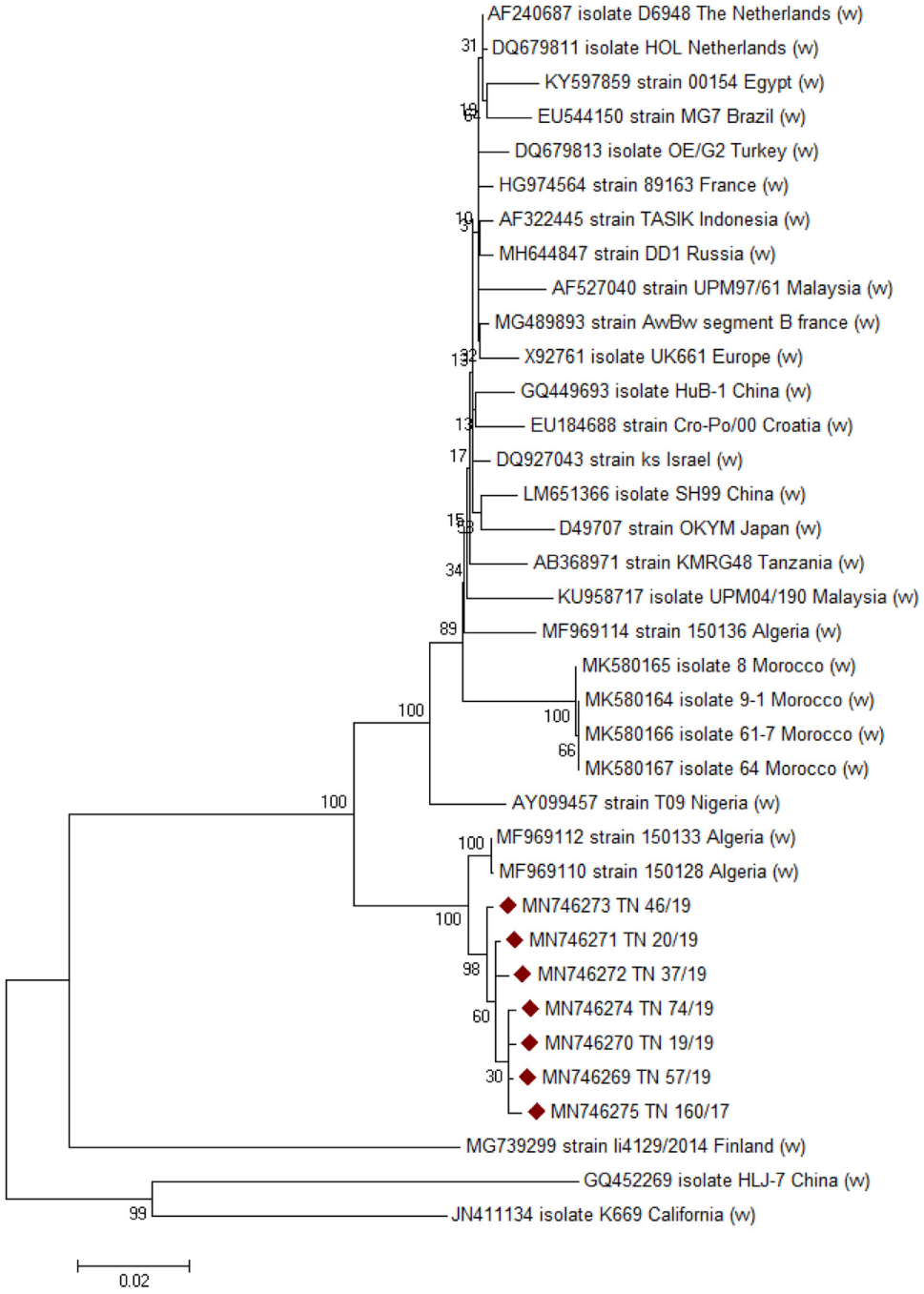

Figure 1. Phylogenetic tree of the Gumboro disease virus based on the alignment of the nucleotide sequences of the *VP1* gene. Tunisian isolates are marked with a red diamond.

(Table 3). This mutation was located nearby the hypervariable region of *VP2* gene, and its impact on the pathogenicity is hard to anticipate. The results of the aa sequence analyses of the Tunisian strains showed that they share the conserved residues A_{222}, I_{242}, Q_{253}, I_{256}, D_{279}, A_{284}, I_{294}, S_{299}, characteristic of vvIBDV (Ndashe et al., 2016; Drissi Touzani et al., 2019; Yilmaz et al., 2019). Indeed, the residues I_{242}, I_{256}, I_{294}, and S_{299} are thought to be involved in virulence, cellular tropism, and pathogenicity (Jackwood et al., 2008). However, the residues Q253 > H, D279 > N, and A284 > T participate in both cell culture adaptation and virus attenuation (Shehata et al., 2017). The Tunisian isolates showed the aa Q249, which is present in several vvIBDV strains. Indeed, Qi et al. (2013) have shown that mutations R249 > Q and V256 > I increase IBDV virulence, indicating their significant contribution to the replication and the virulence of IBDV. These 2 residues are located in the PDE domain of VP2; their positions are very close and belong to the minor hydrophilic peak A (aa 247-254 of VP2), which is suspected of having a strong antigenic activity. They also surround residue 253 and are next to residue 284 (Qi et al., 2013), which are molecular determinants of virulence (Mwenda

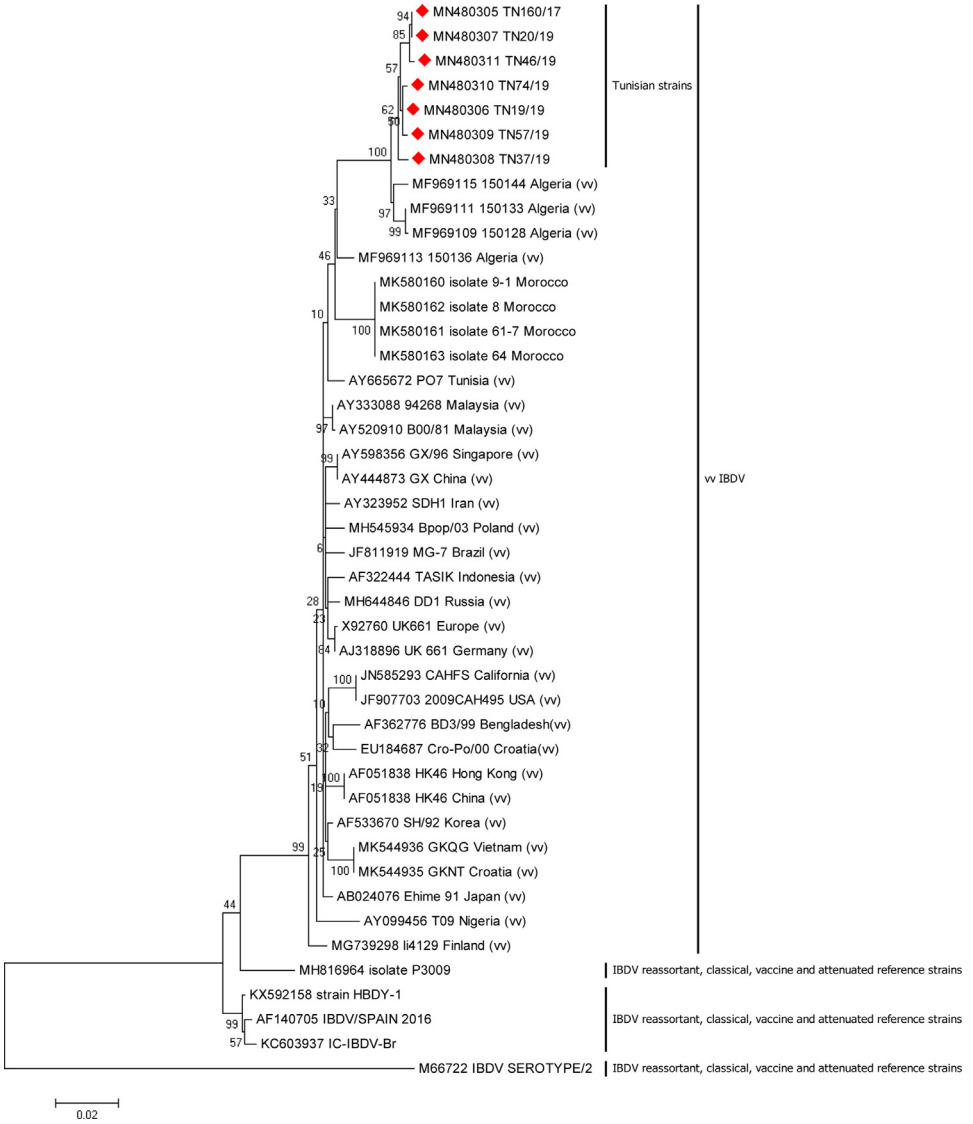

Figure 2. Phylogenetic tree of the Gumboro disease virus based on the alignment of the nucleotide sequences of the *VP2* gene. Tunisian isolates are marked with a red diamond.

et al., 2018). Isolates containing Q_{253} and A_{284} had increased pathogenicity, whereas those with H253 and T284 are less virulent (Mwenda et al., 2018). Qi et al. (2013) and Abed et al. (2018) also reported that aa mutations Q253 > H and A284 > T, separately or simultaneously, define virulence of vvIBDV. Qi et al. (2009) and Ben Abdeljelil et al. (2014) have also found that the double mutations D279 > N/A284 > T and Q253 > H/A284 > T are sufficient to confer cell tropism and IBDV replication efficiencies; but this does not necessarily lead to attenuation of the virus pathogenicity. It should be noted that several studies have shown that those residues are involved in cell tropism and virulence of vvIBDV. Protein sequence alignment results have also shown that the aa sequences of the Tunisian isolates have residues I_{272}, M_{290}, Q_{324}, and S_{330} that are common and characteristic of vvIBDV strains as reported in different countries (Hernández 2006; Patel et al., 2016; Abed et al., 2018). The sequence SWSASGS—aa 326 to 332, a serine-rich heptapeptide specific for vvIBDV strains, was identified in the genome of the 7 Tunisian isolates. This correlates well with data from studies conducted by Hernández et al. (2006), Dormitorio et al. (2007) and Felice et al. (2017). It seemed that the most virulent strains are those having region with the highest number of serine residue. Indeed, the hydrogen bonds present at the level of the serine-rich motif, allowed intra- and inter-molecule interactions, decisive for viral virulence. Such interactions are not possible in apathogenic or low pathogenic viruses, as the substitution of one or 2 serines would take up more space in the molecular structure (Lombardo et al., 2000). This confirms our results of alignment of nucleotide sequences of *VP2* gene, suggesting a high virulence of the studied isolates.

The *VP5* gene encodes a nonstructural VP5 protein involved in the pathogenesis and the *in vivo*

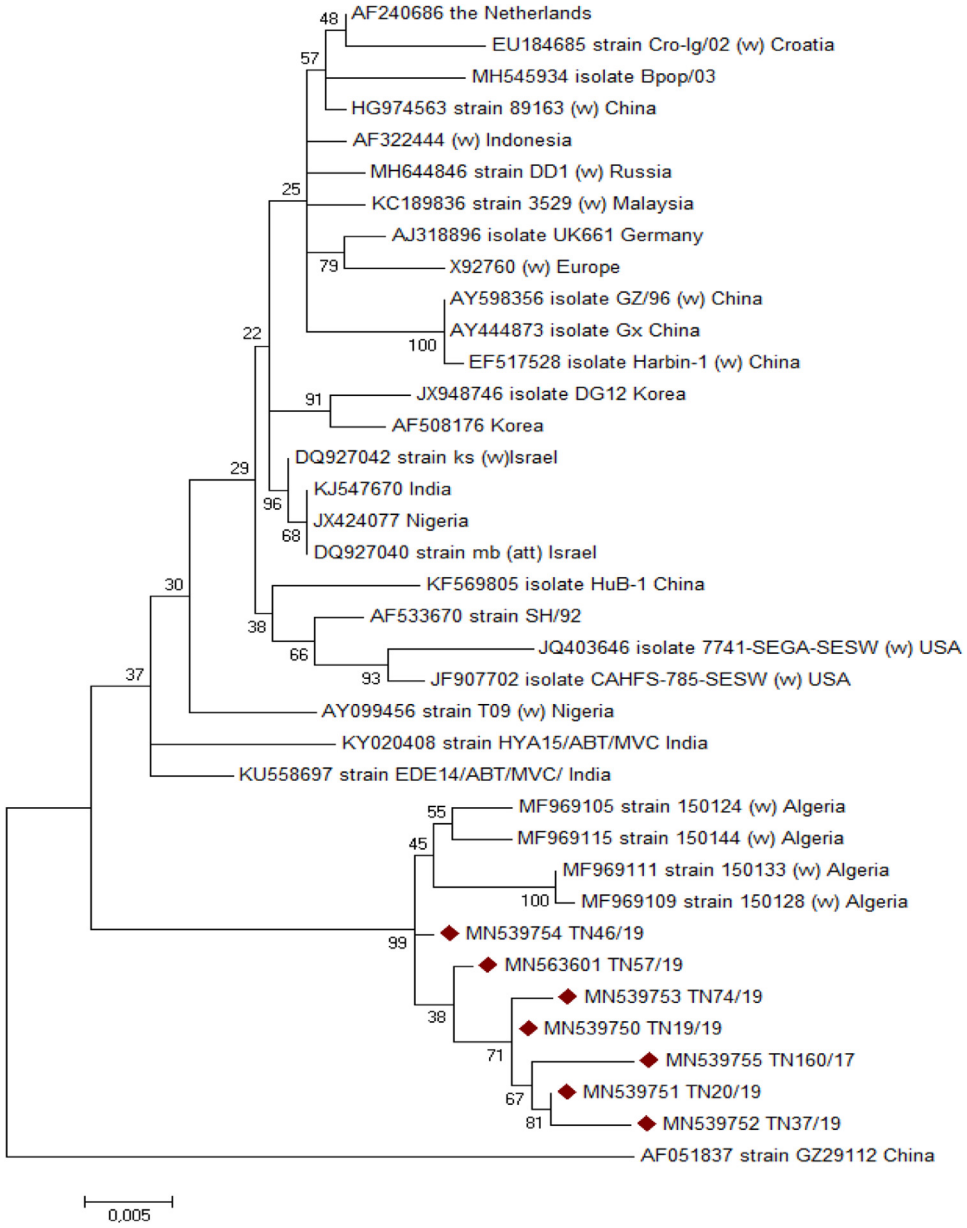

Figure 3. Phylogenetic tree of the Gumboro disease virus based on the alignment of the nucleotide sequences of the *VP3* gene. Tunisian isolates are marked with a red diamond.

dissemination of the virus from infected cells (Ganguly and Rastogi, 2018). This protein is known to prevent apoptosis of infected host cells during the early stages of IBDV infection. However, it promotes induction of the programmed cell death process in later stages and is a major virulence factor required in the beginning of the appearance of clinical signs of IBDV infection and the development of lesions in the bursa of Fabricius (Fan et al., 2019). The multiple alignment of the nucleotide sequences of the *VP5* gene indicated that the Tunisian isolates showed the AUG alternative initiation codon that characterizes vvIBDV (Hernández et al., 2010). The results of these alignments have also shown that the sequences of the Tunisian strains contain substitutions involve aa sequence changes when compared with each other and to the reference strains. Indeed, 2 substitutions A15 and A16 are unique and characteristic of the Tunisian isolates, as substitutions A15 changes aa to E5 and A16 changes aa Q6>K (Table 3). The 3 other mutations found in *VP5* gene were present in several vvIBDV (Abed et al., 2018; Pikuła et al., 2018). The results of such protein sequence alignments have also shown that the aa sequences of the Tunisian isolates do not possess the conserved and characteristic residues of highly virulent strains (vvIBDV): E18, R49, F78, P129, and W137 (Hernández et al., 2010). However, they all shared S18, S49, N78, L129, and R137 residues simultaneously with several vvIBDV isolates as well as the residues R45, F74, P125, W133 with isolates from Algeria and Poland which are highly virulent strains (Abed et al., 2018; Pikuła et al., 2018), thus confirming the virulence of Tunisian isolates.

The segment B codes for *VP1* gene play an important role in viral replication and genetic evolution (Fan et al.,

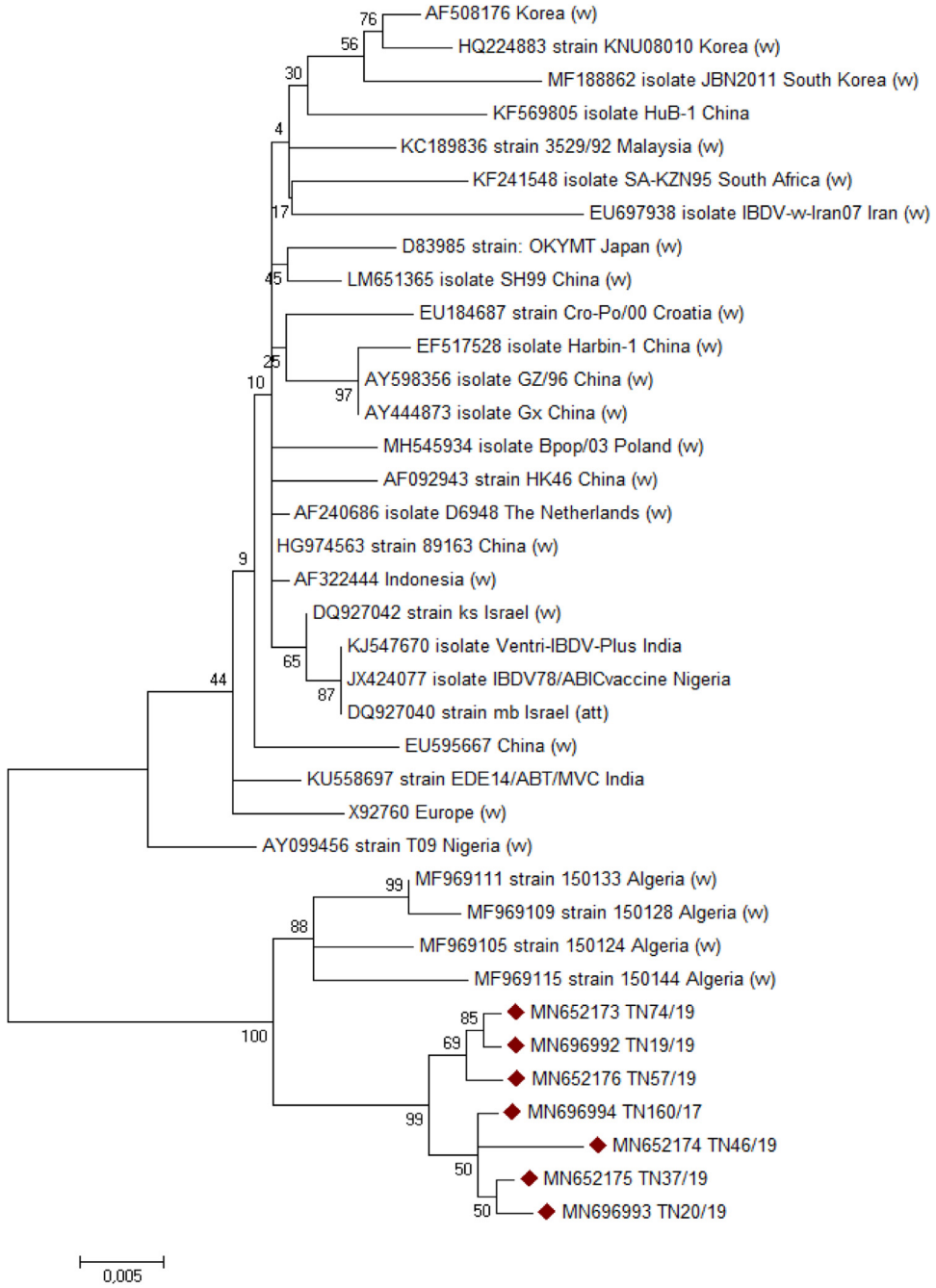

Figure 4. Phylogenetic tree of the Gumboro disease virus based on the alignment of the nucleotide sequences of the *VP4* gene. Tunisian isolates are marked with a red diamond.

2019). Thus, research suggested that *VP1* gene contributes to Gumboro's virulence (Escaffre et al., 2013; Drissi Touzani et al., 2019b). Tunisian isolates share aa $V_4 > I$, $T_{295} > A$, $A_{391} > T$, $D_{393} > E$ which exist also in vvIBDV strains from Algeria (Abed et al., 2018), as well as Moroccan (Drissi Touzani et al., 2019b) and many other country. Based on the analyses of the VP1 protein sequences of the various isolates studied, several nonsilent mutations were shown to be characteristic of the Tunisian isolates (Table 3). In fact, substitution of $V_{92} > G$, $S_{497} > N$ were present in some isolates where substitution of $I_{275} > L$, $V_{399} > I$ were shown for all strains. Finally, substitutions of $L_{739} > I$ present in TN57/19 strain and $Q_{876} > P$ in only TN37/19 were unique and specific to each viral sample.

The protein sequences of VP1 of the Tunisian strains showed the presence of E_{242} residue and TDN triplet at positions 145, 146, and 147, a specific motif for vvIBDV, and allowed distinction of various IBDV pathotypes (Gao et al., 2014). It has already been shown that the existence of such motif associated with the E_{242} increases the virulence of the IBDV (Gao et al., 2014). By contrast, the NEG triplet, located in the N-terminal domain of VP1, is highly conserved in non-vvIBDV strains (Wang et al., 2019). Besides, the Tunisian aa sequences possessed the residues A287, M390, S511, P562,

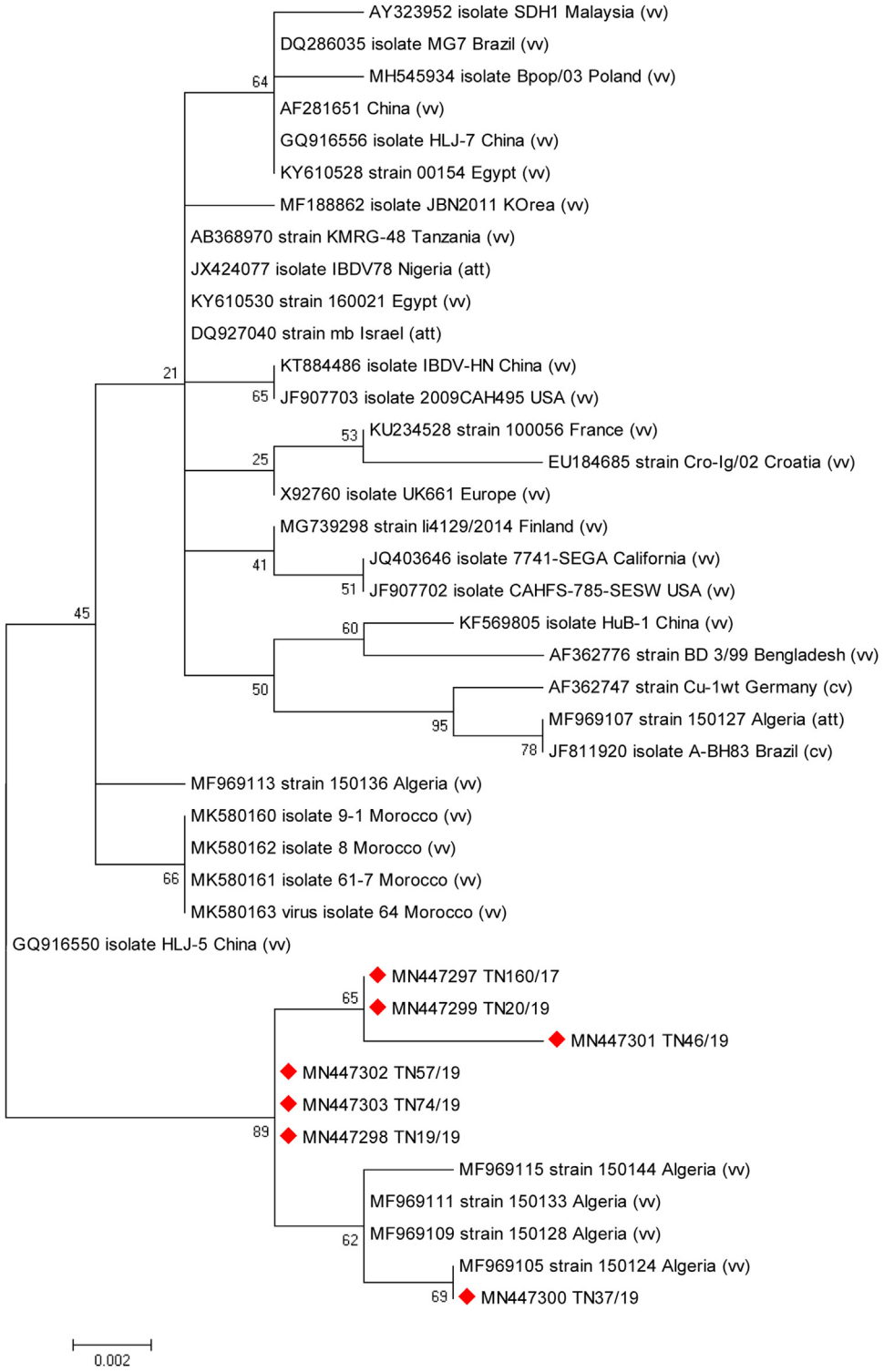

Figure 5. Phylogenetic tree of the Gumboro disease virus based on the alignment of the nucleotide sequences of the *VP5* gene. Tunisian isolates are marked with a red diamond.

which are common in vvIBDV (Kong et al., 2004); the residues A287 being identified as a possible determinant of IBDV virulence (Molini et al., 2019).

The multiple alignment of studied protein sequences of *VP3* gene made it possible to distinguish 4 residues: Q_{84}, E_{220}, P_{283}, and A_{306} that are specific for vvIBDV which corresponded to Q_{783}, E_{919}, P_{981}, and A_{1005} in the polyprotein sequence (VP2-VP4-VP3) (Wang et al., 2007; Drissi Touzani et al., 2019b). Although the results of *VP1, VP2, VP5,* and *VP3* gene sequences confirmed their involvement in the virulence of IBDV, they were not the only determinants. Indeed, *VP4* gene that encodes for viral VP4 protease is responsible for treating the polyprotein (Wang et al., 2015); suggesting that variations within the protein sequences of VP4 may also contribute to the virulence (Rudd et al., 2002). Like

for Moroccan strains, we have shown that the 4 residues Y_{228}, N_{233}, S_{263}, and D_{299}, which correspond to Y_{680}, N_{685}, S_{715}, and D_{751} in the polyprotein sequence, are specific for vvIBDV (Kong et al., 2004; Drissi Touzaniet al., 2019b). Therefore, it is necessary to determine the value of all new aa substitutions characteristic of Tunisian isolates since their implication in the virulence of IBDV is not yet endorsed and suggesting the need for further investigations to explore their role.

Phylogenetic analyzes based on the 5 gene sequences revealed that the Tunisian isolates are closely related to vvIBDV Algerian strains and much less to the Moroccan ones which are not placed in the same tree branch despite their very close geographic area (Figures 1, 2, and 5). Indeed, they were closer to the French and the Malaysian strains (Drissi Touzani et al., 2019b). The calculated similarity scores supported the phylogenetic results and revealed that IBDV circulating in Tunisia could be then of Algerian origin. The corresponding Algerian strains were isolated between September 2014 and September 2015 and its introduction could be explained by its transmission through in particular unformal trade (Abed et al., 2018). Given the high stability of the virus, which may persist for at least 4 mo in the environment and its resistance to usual disinfectants, along with the limited biosecurity measures applied to fight the disease in the farms, the spread of the virus has been facilitated. Vaccination is therefore unavoidable, but its results remain inconsistent, notably because of the neutralization of live virus vaccines by maternal antibodies if vaccination is applied in the early bird life, besides the antigenic and pathotypic variabilities of wild-type viruses.

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DISCLOSURES

The authors declare that they have no conflict of interest.

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