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# Molecular and phylogenetic characterization based on the complete genome of a virulent pathotype of Newcastle disease virus isolated in the 1970s in Brazil

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ABSTRACT

Newcastle disease (ND) is caused by the avian paramyxovirus type 1 (APMV-1) or Newcastle disease virus (NDV) that comprises a diverse group of viruses with a single-stranded, negative-sense RNA genome. ND is one of the most important diseases of chickens, because it severely affects poultry production worldwide. In the 1970s, outbreaks of virulent ND were recorded in Brazil, and the strain APMV-1/Chicken/Brazil/SJM/75 (SJM) of NDV was isolated. This strain was characterized as highly pathogenic for chickens but not pathogenic for other bird species. Here we present the complete genome of NDV strain SJM and investigate the phylogenetic relationships of this virus with other NDV strains in terms of genome and proteins composition, as well as characterizing its evolution process. The NDV strain SJM is categorized as a velogenic virus and the complete genome is 15,192 nucleotides in length, consisting of six genes in the order 3′-NP-P-M-F-HN-L-5′. The presence of the major pathogenic determinant of NDV strains ($^{112}$R-R-Q-K-R↓F$^{117}$) was identified in the Fusion protein of the NDV strain SJM. In addition, phylogenetic analysis classified the NDV strain SJM as a member of class II, genotype V, and indicates that this virus help us in the understanding of the evolutionary process of strains belonging to this genotype. This study contributes to the growing interest involving the characterization of NDV isolates to improve our current understanding about the epidemiology, surveillance and evolution of the pathogenic strains.


## 1. Introduction

Newcastle disease (ND) is a highly contagious and widespread disease, which causes severe economic losses in domestic poultry, especially in chickens (Alexander, 2000; Sinkovies and Horvath, 2000). The World Organization for Animal Health (OIE) lists it as a notifiable disease and imposes restrictions and trade embargoes on countries and areas where outbreaks occur (OIE, 2009).

The causative agent of the disease is Newcastle disease virus (NDV) or avian paramyxovirus serotype 1 (APMV-1), which belongs to the genus *Avulavirus* within the subfamily *Paramyxovirinae* and family *Paramyxoviridae* (Lamb et al., 2005).

The NDV genome comprises a single-stranded, negative-sense RNA genome of ~15,200 nucleotides (nt) that contains six genes, which encode seven proteins. The nucleoprotein (NP gene), the phosphoprotein (P gene), a V protein resulting from mRNA editing of the P gene (Steward et al., 1993), the matrix (M gene), the fusion (F gene), the haemagglutinin–neuraminidase (HN gene) and the RNA-dependent RNA polymerase (L gene) proteins (Samson, 1988; Alexander and Senne, 2008).

Based on the analysis of the nucleotide sequence of the F gene, 19 different genotypes of NDV have been identified and classified into two classes. Class I, genotype I and class II, I–XVIII genotypes. Class I viruses are distributed worldwide and have been isolated mainly from waterfowl and shorebirds. Class II virus are typically found circulating within wild-bird and poultry species and have been divided into 18 genotypes (I–XVIII), with genotypes V–VIII being the predominant genotypes circulating in the world (Miller

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et al., 2009b, 2010; Diel et al., 2012a, 2012b; Courtney et al., 2013; Snoeck et al., 2013). Since ND was first described in 1926, three worldwide panzootics have occurred. The first panzootic (1926–1960) was caused by viruses belonging to genotypes II–IV, and the second (1960–1973) and the third (1970–1980) by genotypes V and VI (Alexander, 2003).

Virulent NDV (vNDV) strains are enzootic in several countries and have been responsible for outbreaks in at least six of the seven continents of the world (Miller et al., 2010). In Brazil, the first known outbreak of ND was described in 1953 (Santos, 1954), and the disease was endemic for nearly 20 years, with the occurrence of sporadic and isolated outbreaks. However, in the 1970s the disease re-emerged in the form of a highly pathogenic NDV (Flores et al., 2006). Over the next 20 years, vNDV was responsible for several outbreaks in different regions of Brazil until implementation of stricter control measures, including extensive vaccination with attenuated NDV strains (LaSota and B1 strains), reduced the number and severity of ND outbreaks (Orsi et al., 2010). Indeed, in 2003 the OIE recognized Brazil as free of pathogenic NDV in industrial poultry (Orsi et al., 2010). However, serological studies detected the activity of NDV infection among wild birds in 2003 and 2006 (Oliveira Júnior et al., 2003; Silva et al., 2006) and backyard birds in 2005 (Oliveira Júnior et al., 2005) and 2006 (Flores et al., 2006).

The strain APMV-1/Chicken/Brazil/SJM/75 (SJM) of NDV is the only vNDV strain available from the 1970 outbreaks in Brazil. This strain produces severe lesions and high mortality in experimentally infected chickens, whereas in other non-galliform birds species it replicates and is excreted without pathological effects (Campioni et al., 2012; Martins et al., 2012; Denadai et al., 2011; Carrasco et al., 2008; Nishizawa et al., 2007; Paulillo et al., 2005; Lima et al., 2004). These findings suggest that wild birds may still be reservoirs of this virus and contribute to virus dissemination. Indeed, vNDV strains that emerged in South and Central America in the 1970s have been linked to the outbreaks in Europe and the United States in the same period, through imported exotic birds (Ballagi-Pordany et al., 1996; Miller et al., 2010).

Therefore, although it is plausible that an epidemic outbreak of NDV is unlikely to occur in Brazil in the short term, the threat in the medium and long term must not be underestimated. This requires not only constant epidemiological vigilance but also efforts to increase the current understanding about the relationships of NDV strains relevant to Brazil and other worldwide circulating strains. To this end, accurate molecular characterization and phylogenetic analysis of NDV isolates, including the SJM strain, are warranted. Indeed, extensive molecular and phylogenetic study of this strain as well as its relationship with other worldwide circulating strains and its possible role in the evolution of NDV genotypes remain undefined.

Here we present the complete genome of NDV strain SJM and investigate the phylogenetic relationships of this virus with other NDV strains in terms of genome organization and proteins signatures, as well as characterizing its evolution process.

## 2. Materials and methods

### 2.1. Isolation and propagation of virus

The SJM, a highly pathogenic NDV strain for chickens, was isolated in 1975 from a poultry farm located in the state of Rio de Janeiro, Brazil. This virus has an Intracerebral Pathogenicity Index (ICPI) of 1.78 and a Mean Death Time (MDT) of 48 h (Lima et al., 2004; Carrasco et al., 2008). The NDV strain SJM isolate was propagated by inoculation in nine-day-old embryonated SPF chicken eggs. The embryos were incubated at 37 °C for 40 h; subsequently, a sample of allantoic fluid was extracted, clarified by centrifugation and stored at −70 °C (Sousa et al., 2000).

### 2.2. RNA isolation and sequencing

Total RNA was extracted from allantoic fluids using TRIzol LS (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. *De novo* sequencing of SJM was carried out using the Illumina HiscanSQ. The library was constructed using TruSeq® RNA Sample Prep kit v2 (Illumina®), and cluster formation of the library cDNA templates was performed with the TruSeq PE Cluster kit v3 (Illumina®) and the Illumina cBot workstation, using conditions recommended by the manufacturer. Paired end 100 base pair ($2 \times 100$ bp) sequencing by synthesis was performed with TruSeq SBS kit v3 (Illumina®) on an Illumina HiscanSQ using protocols defined by the manufacturer.

Base call conversion to sequence reads was performed using CASAVA 1.8.3 (Illumina®). Virus assembly was performed using CLC Genomics Workbench 6.5.1. The genome annotation was performed using Prokka 1.5.2 (Prokka: Prokaryotic Genome Annotation System, http://vicbioinformatics.com/).

### 2.3. Phylogenetic analysis and estimating evolutionary distances

Alignment and comparison of the nucleotide and amino acid sequences between SJM and selected strains representing established NDV genotypes were performed using the software ClustalW 2.2 with iteration in each alignment step (Thompson et al., 1994).

Phylogenetic analysis was performed using the MEGA5 software (MEGA, version 5.2.2) (Tamura et al., 2011). To select the best-fit models of DNA evolution the jModelTest 2 software were used (Darriba et al., 2012). The SJM genomic sequence was compared against 129 complete and near-complete reference genome sequences (only sequences >15,180 nt) of viral strains from class I and II (genotypes I–XIII and XVI) available at GenBank. The F gene sequence of SJM was analyzed with 100 sequences of the full F gene from class I and II (genotypes I–XVIII) published in GenBank in order to construct the Fusion gene tree. The evolutionary history was inferred by the Maximum Likelihood method based on the General Time Reversible (GDR) model (Tavaré, 1986), with standard errors being calculated based on 1000 bootstrap replicates and expressed based on the number of nucleotide substitutions per site. The codon positions included in the analysis were the 1st, 2nd, 3rd, and non-coding. All positions containing gaps and missing data were eliminated from the data set (the "complete deletion" option). The name and numbers used in the phylogenetic trees represent the name and accession numbers in GenBank. Estimation of the evolutionary distances between strains of NDV was performed by the maximum composite likelihood method in MEGA5.

### 2.4. Nucleotide sequence accession number

The annotated sequence of strain APMV-1/Chicken/Brazil/SJM/75 (SJM) has been deposited at DDBJ/EMBL/GenBank under the accession number KJ123642.

## 3. Results

### 3.1. Genome analysis and deduced proteins

A summary of the genomic features of NDV strain SJM is presented in Table 1. These characteristics are similar to those presented by other virulent APMV-1. Comparisons of nucleotide and amino acid sequences between NDV strain SJM and selected class II reference strains representing genotypes I–XIII, XVI e XVIII of NDV are shown in Table 2. The name and numbers used in

**Table 1**
Genomic features of Newcastle disease virus isolate APMV-1/Chicken/Brazil/SJM/75.

| Protein[a] | Intergenic sequence (IS) | Nucleotide length (nt) | 5′ UTR[b] (nt) | ORF length (nt) | % G+C | 3′ UTR[b] (nt) | Deduced amino acid length (aa) |
|---|---|---|---|---|---|---|---|
| NP | 2 | 1752 | 66 | 1470 | 50.5 | 216 | 490 |
| P | 1 | 1451 | 83 | 1188 | 52.7 | 180 | 395 |
| M | 1 | 1241 | 34 | 1095 | 48.2 | 112 | 364 |
| F | 31 | 1792 | 46 | 1662 | 44.9 | 84 | 553 |
| HN | 47 | 2002 | 91 | 1716 | 46.1 | 195 | 571 |
| L | – | 6703 | 11 | 6615 | 44.2 | 77 | 2204 |
| Genome | – | 15,192 | | – | 46.2 | – | – |

[a] NP, nucleoprotein; P, phosphoprotein; M, matrix protein; F, fusion protein; HN, haemagglutinin–neuraminidase protein; L, RNA-dependent RNA polymerase.
[b] UTR, untranscribed region.

Table 2 represent the name and accession numbers in GenBank used for each genotype.

Alignment of the complete genome sequence revealed that SJM shares 97% nucleotide identity with NDV strain Largo/71 (genotype V). Notably, the lowest nucleotide identity was observed between SJM and the vaccine strain LaSota/46 (84%; genotype II).

The highest similarities between the SJM and Largo/71 strains were identified at the NP and L genes, both showing 98.0% similarity in the nucleotide sequence and 99.0% in the deduced amino acid sequence. The gene showing more differences respect to Largo/71 strain and SJM strain encodes for protein P (96.0% similarity in nucleotide sequence and 95.0% in amino acid sequence). The deduced amino acid sequence of F and HN proteins of the SJM strain displayed 96.0% similarity to the Largo/71 strain. The amino acid sequence similarity rates were the same for F protein in the Fontana/72 (genotype VI) and QH4 (genotype VIII) strains (96.0 and 95.0%, respectively) (Table 2).

Comparisons of the deduced amino acid sequences of both antigenic proteins (F and HN) of SJM and strains representative of genotypes of class II showed the lowest similarity with the LaSota strain (F:89.0% and HN: 88.0%),Ulster strain (F: 91.0% and HN:90.0%) and NDV poultry/Peru/1918-03/2008 strain (F: 90% and HN: 88%) (Table 2).

### 3.2. Phylogenetic analysis and estimate of evolutionary distances

In order to localize the NDV strain SJM into the phylogenetic trees and to compare the isolate with others viruses isolated worldwide, two analyses were performed. One tree was done using 129 complete and partial genome nucleotide sequences of representative viruses belonging to class I and class II (genotypes I–XIII and XVI) available at GenBank, are shown in Fig. 1. The results indicate that the NDV strain SJM strain belongs to genotype V, supported by a maximum bootstrap value at the node that groups this with the remaining strains of this genotype.

The other phylogenetic analysis was done with the full length of the F gene sequence of NDV strain SJM and compared with 100 complete sequences of F gene of viruses pertaining to class I and II (genotypes I–XVIII) available at GenBank, are shown in Fig. 2. The full fusion region was used to confirm the classification and identity of NDV strain SJM.

The full fusion gene sequence analysis confirmed that all the virulent isolates continue to be clustered within the sub-genotype Vb together with other NDV isolates that have been obtained from commercial poultry.

### 3.3. APMV-1/Chicken/Brazil/SJM/75. belongs to the velogenic NDV pathotype

The pathogenicity of APMV-1/Chicken/Brazil/SJM/75 was assessed by the analysis of the F protein cleavage site and by the standard ICPI test. The F protein cleavage site revealed the presence of three basic amino acid residues at positions 113, 115, and 116 and a phenylalanine at position 117 ($^{112}$R-R-Q-K-R↓F$^{117}$).

Furthermore, the predicted amino acid sequence of SJM's complete F gene was compared with 117 different strains pertaining to class I and II (genotypes I–XVIII) (Table 3). In this analysis, we found four specific amino acid residues located between 1 and 553 amino acids that are not found in any other NDV genotype (Table 3). Two of these amino acids are located in the N-terminus of the F protein Q$^{28}$→P/L/R/S/E/M/W and N$^{107}$→T/S. The two remaining substitutions G$^{509}$→I/V/A/T and L$^{510}$→T/I/V/A/M are located in the tertiary structure of the F protein.

The HN gene of NDV strain SJM is 1,716 nucleotides in length and is composed of 571 amino acids, characteristic of virulent NDV strains. In this study, 59 sequences of HN strains pertaining to class I and II (genotypes I–XVIII) with different lengths were aligned with the NDV strain SJM (Table 3).

We found that in the SJM strain, all the previously described NDV neutralizing epitopes are conserved (Yusoff et al., 1988). The regions involved in hemagglutinating activity are partially conserved with only one change at position S$^{400}$→A (Lamb and Lolakofsky, 1996). Analysis of HN amino acid sequence also revealed that the SJM strain contains four specific amino acid residues: D$^{47}$→S/G/I/T, P$^{111}$→S, A$^{328}$→T/I/N/K and N$^{437}$→T/S/A, all of which are different from those displayed by other NDV strains used in this study (Table 3).

## 4. Discussion

Numerous outbreaks of ND throughout Central and South America are consistently reported to OIE, as ND is endemic to many of the countries located in these regions (OIE, 2012). In this study, we present the molecular characterization and phylogenetic analysis of the SJM strain of NDV, isolated from the outbreak in Brazil in 1975.

We found that NDV strain SJM has a close genetic relationship with Largo/71 strain that belongs to class II of genotype V in terms of the full genome analysis. Interestingly, the NDV strain SJM induced outbreak nearly coincided with the second NDV panzootic, including the outbreaks recorded in the USA in the early 1970s, when the Largo/71 strain was isolated (Walker et al., 1973).

ICPI of APMV-1/Chicken/Brazil/SJM/75 in day-old chicks resulted in 1.78, and a Mean Death Time (MDT) of 48 h, which is typical of velogenic NDV strains (Lima et al., 2004; Carrasco et al., 2008). NDV strain SJM contains the F protein cleavage site motif sequence $^{112}$R-R-Q-K-R↓F$^{117}$, which is the major determinant of virulence for NDV strains (Rott and Klenk, 1988). This high proportion of basic amino acids and the presence of a phenylalanine at position 117 (↓F$^{117}$) are characteristic of highly virulent strains, as previous described in other vNDV strains (Li et al., 1998; Peeters et al., 1999; Panda et al., 2004; Kattenbelt et al., 2006; Lamb and Lolakofsky, 1996).

The predicted amino acid sequence of the complete F gene of SJM was compared with those from different strains pertaining

**Table 2**
Nucleotide and amino acid comparison between Newcastle disease virus isolate APMV-1/Chicken/Brazil/SJM/75 and viruses representing other genotypes within class II.[a]

| Genotype | I | | II | | III | | IV | | V | | VI | | VII | | VIII | | IX | | X | | XI | | XII | | XIII | | XVI | | XVIII | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain | Ulster | | LaSota/46 | | JS/7/05/Ch | | Herts/33 | | Largo/71 | | Fontana/72 | | ZJ1 | | QH4 | | JS/1/02 | | Mallard/US(OH)/04-411/2004 | | MG_1992 | | Poultry/Peru/1918-03/2008 | | Sterna/Astr/2755/2001 | | Dominican Republic (JuanLopez)/49931/2008 | | NDV/chicken/Togo/AKO18/2009 | |
| | AY562991 | | AY845400 | | FJ430159 | | AY741404 | | AY562990 | | AY562988 | | AF431744 | | FJ751919 | | FJ436306 | | GQ288377 | | HQ266603 | | JN800306 | | AY865652 | | JX119193 | | JX390609 | |
| Gene[b] | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) | (nt) | (%) (aa) |
| NP | 90 | 94 | 87 | 93 | 89 | 94 | 91 | 96 | 98 | 99 | 92 | 97 | 90 | 96 | 92 | 96 | 89 | 93 | 87 | 93 | 87 | 93 | 90 | 95 | 90 | 95 | 89 | 94 | 88 | 94 |
| P | 85 | 83 | 84 | 84 | 87 | 85 | 88 | 88 | 96 | 95 | 90 | 89 | 88 | 86 | 89 | 88 | 85 | 83 | 83 | 81 | 84 | 82 | 87 | 84 | 87 | 85 | 84 | 84 | 85 | 86 |
| M | 88 | 90 | 85 | 89 | 88 | 90 | 91 | 94 | 97 | 98 | 91 | 95 | 88 | 94 | 89 | 93 | 88 | 91 | 86 | 92 | 86 | 92 | 88 | 93 | 88 | 95 | 86 | 93 | 85 | 93 |
| F | 88 | 91 | 86 | 89 | 88 | 92 | 90 | 94 | 96 | 96 | 91 | 96 | 89 | 93 | 90 | 95 | 88 | 92 | 86 | 92 | 85 | 90 | 88 | 94 | 88 | 94 | 87 | 92 | 87 | 93 |
| HN | 86 | 90 | 84 | 88 | 87 | 88 | 89 | 90 | 96 | 96 | 91 | 94 | 89 | 92 | 89 | 93 | 87 | 90 | 84 | 90 | 85 | 88 | 88 | 90 | 89 | 91 | 87 | 90 | 86 | 91 |
| L | 89 | 95 | 86 | 92 | 89 | 95 | 91 | 96 | 98 | 99 | 92 | 97 | 90 | 95 | 92 | 97 | 89 | 95 | 87 | 95 | 87 | 94 | 89 | 96 | 90 | 96 | 88 | 95 | 89 | 95 |
| Genome | 87 | – | 84 | – | 87 | – | 89 | – | 97 | – | 91 | – | 88 | – | 90 | – | 87 | – | 85 | – | 85 | – | 87 | – | 88 | – | 86 | – | 87 | – |

nt, nucleotide; aa, amino acid.
[a] Alignment was performed by using ClustalW 2.2.
[b] NP, nucleoprotein; P, phosphoprotein; M, matrix protein; F, fusion protein; HN, haemagglutinin–neuraminidase; L, RNA-dependent RNA polymerase.

to class I and II (genotypes I–XVIII). All seven neutralizing epitopes critical for both structure and function of the protein are conserved (Toyoda et al., 1987; Yusoff et al., 1989; Liu et al., 2003). Other conserved features include the 12 cysteine residues (Scal, 2004) and the predicted N-glycosylation sites (Chen et al., 2001; Panda et al., 2004), with the exception of residue $^{191}$N-N-T$^{193}$, which presents an insertion of an N at position 192 of the F protein. These F protein conserved structures are common in the genotypes V–VIII and X, which emerged after 1960 (Czeglédi et al., 2002).

Comparisons of HN protein sequences of class I and II (genotypes I–XVIII) showed that all major neutralizing epitopes of this protein are conserved (Yusoff et al., 1988). The three amino acid motifs involved in receptor binding site (Crennell et al., 2000; Connaris et al., 2002), neuraminidase activity, and hemagglutinating activity (Lamb and Lolakofsky, 1996) were well conserved, except for the change at position S$^{400}$→A. In addition, the SJM strain has preserved 13 cysteine residues in the linear sequence of the ectodomain (Scal, 2004) and 11 sialic acid receptor binding sites of HN protein (Connaris et al., 2002).

Although the unique mutations detected in the F and HN proteins of the SJM strain were not located in residues of virus-neutralizing sites, their effects on the conformation of these proteins and possible consequences in the interaction with the host cells and in evasion of immune response cannot be ruled out. In fact, Gravel and Morrison (2003) have demonstrated that amino acid substitutions in non-neutralizing epitopes of the N-terminus region domain of the HN protein are responsible for an enhanced or diminished virus attachment activity and may result from immune pressures that can contribute to increase phylogenetic distance between NDV strains.

Phylogenetic analysis revealed that the SJM strain constitutes a separate branch of the cluster of strains of genotype V. In addition, SJM is 15,192 nucleotides in length and shows the substitution V$^{118}$→I, characteristic of recent genotypes of NDV and specific of genotype V (Lomniezi et al., 1998; Wehmann et al., 2003; Czeglédi et al., 2006). Another important change identified in the SJM strain was the substitution E$^{104}$ → G, in the F protein, which is considered a marker in the evolution of the old towards the recent genotypes (Yu et al., 2001). It is worth noticing that the genotypes V (to which SJM belongs) and VI, VII, and VIII predominate worldwide and contain only virulent viruses (Miller et al., 2009a).

Interestingly, genotype V viruses emerged in South and Central America in the 1970s and were linked to outbreaks in Europe in that same period (Ballagi-Pordany et al., 1996). These viruses have also been linked to North American outbreaks in Florida (1971, 1993) and California (1971, 2002) (Wise et al., 2004) and are still circulating in Mexico (Absalón et al., 2012; Perozo et al., 2008). For this reason, we made a particular analysis of full fusion gene with sequences available in GenBank (majority of viruses isolated in Central and North America). Our results are similar to those previously reported by Perozo et al. (2008), Absalón et al. (2012), Garcia et al. (2013) and Susta et al. (2014), which found the existence of three lineages of viruses belonging to genotype V. Two of them, have been identified circulating in commercial poultry (Perozo et al., 2008; Garcia et al., 2013) and a third include wild birds.

The first group (Va) corresponds to isolates between 2008 and 2010 in Mexico from poultry, captive wild birds and free-living wild birds. The second group (Vb) corresponds to isolates found between 1970 and 2008 with similarities between them greater than 98%. This group contains the SJM strain and is more closely related to Largo/71 strain, which is representative of the outbreak in North America in the early 1970s. Our phylogenetic analysis of full fusion gene shows that all the vNDV isolated from poultry and


**Fig. 1.** Phylogenetic analysis based on complete genome sequence of 129 viruses representing Newcastle disease virus class I and II available in GenBank. The SJM strain position on the tree is shown in a black diamond and the virulent strains (vNDV) are highlighted with an asteristic (*). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (2 categories (+G, parameter = 0.7732)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 37.2812% sites). There were a total of 11,794 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

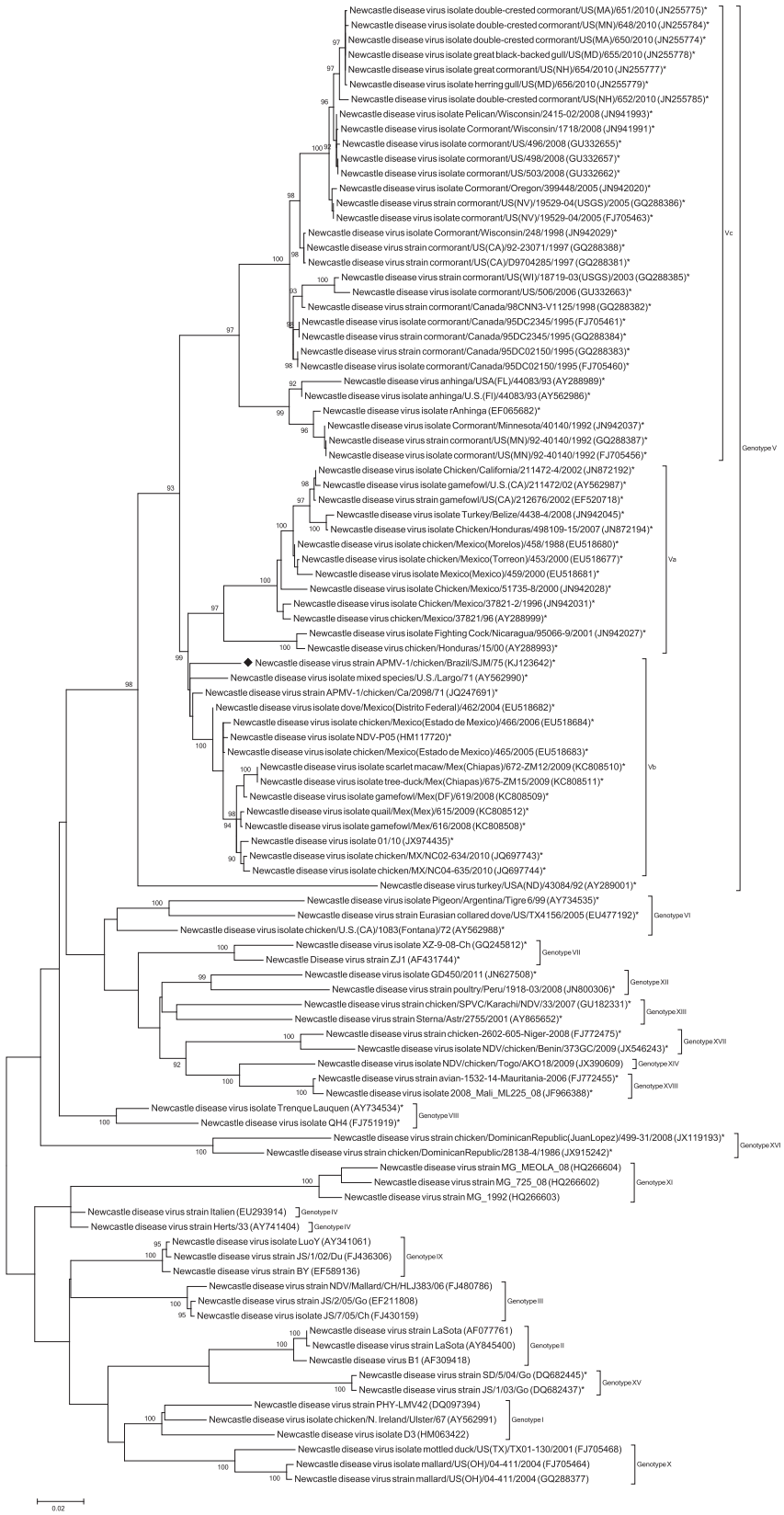


**Fig. 2.** Phylogenetic analysis based on the full length sequence of 100 fusion genes from Newcastle disease virus class I and II available in GenBank. The SJM strain position on the tree is shown in a black diamond and the virulent strains (vNDV) are highlighted with an asteristic (*). The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (2 categories (+G, parameter = 0.6884)). There were a total of 1624 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

**Table 3**
Residue substitutions specific in deduced $F_0$ protein and HN protein sequence of the APMV-1/Chicken/Brazil/SJM/75 and viruses representing other genotypes within class I and II (genotypes I–XVIII).[a]

| | Consensus residue and its position | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | In deduced $F_0$ protein sequence | | | | In deduced HN protein sequence | | | |
| | 28 | 107 | 509 | 510 | 47 | 111 | 328 | 437 |
| APMV-1/Chicken/Brazil/SJM/75 | Q | N | G | L | D | P | A | N |
| Class I and II (genotypes I–XVIII) | L | T | V | T | S | S | T | T |
| | R | S | I | I | G | | I | S |
| | P | | A | V | I | | N | A |
| | S | | T | A | T | | K | |
| | E | | | M | | | | |
| | M | | | | | | | |
| | W | | | | | | | |

[a] Alignment was performed by using ClustalW 2.2.

wild birds continue to be part of sub-genotype Vb. In addition, the phylogenetic tree identifies a third lineage (Vc) which houses wild viruses isolated in the USA from cormorants and is poorly correlated with lineages Va and Vb.

Interestingly, the SJM strain and strains of genotype V isolated in North America (Largo/71) present a close phylogenetic relationship. Altogether, it is possible to infer that the NDV strain SJM help us in the understanding of the evolutionary process of strains belonging to genotype V, mainly sub-genotype Vb, using dated phylogeny, including the old strains isolated in the 1970s in the USA and those currently circulating in North America and responsible for the outbreaks referred to (Absalón et al., 2012; Miller et al., 2009b).

In summary, complete genome analysis of the NDV strain SJM presented herein revealed that it belongs to a genotype that was important in the second NDV panzootics in the 1970s. Despite the fact that this strain is no longer circulating among poultry production in Brazil, some phylogenetically related vNDV strains have been sporadically isolated in North America. Our findings contribute to the growing interest involving the characterization of NDV isolates to improve our current understanding about the molecular epidemiology and evolution of the pathogenic strains. Such knowledge may be valuable for future studies aiming to develop improved control and diagnostic strategies for the disease.

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