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Molecular evolution of the Newcastle disease virus matrix protein gene and phylogenetic relationships among the paramyxoviridae

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Abstract

Matrix (M) gene sequences for recent field isolates and older reference Newcastle disease viruses (NDV) were examined to determine phylogenetic relationships and population trends among these viruses. Overall, the M gene has a majority of synonymous nucleotide sequence substitutions occurring among NDV isolates. However, several predicted amino acid changes in the M protein of specific NDV isolates have occurred that correlate to phylogenetic relationships. Nucleotide substitutions in these codons have a greater number of nonsynonymous base changes. The NDV isolates arising since the 1970s belong to a population of viruses that expanded worldwide at an exponential rate. These viruses may have their origins in free-living birds, are present worldwide, and continue to circulate causing disease in poultry. A specific NDV lineage composed of virulent isolates obtained in the US prior to 1970 appears to no longer exists among free-living birds or commercial poultry. However, "vaccine-like" viruses are common in the US and continue to circulate among commercial poultry. Based on M protein amino acid sequences, NDV separates as a clade most closely related to morbilliviruses and not with their current designated category, the rubulaviruses among the Paramyxoviridae. Consequently, avian paramyxoviruses should have their own taxonomic subfamily among the Paramyxovirinae. © 2000 Elsevier Science B.V. All rights reserved.

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Newcastle disease virus (NDV) is classified as a member of the superfamily Mononegavirales in the family Paramyxoviridae. This virus family is divided into two subfamilies, the Paramyxovirinae and the Pneumovirinae. In 1993, the International Committee on the Taxonomy of Viruses rearranged the order of the Paramyxovirus genus and placed NDV within the Rubulavirus genus among

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the Paramyxovirinae (Rima et al., 1995). The enveloped virus has a negative-sense single-stranded RNA genome of approximately 15 kilobase pairs, which codes for an RNA-directed RNA polymerase (L), hemagglutinin-neuraminidase protein (HN), fusion protein (F), matrix protein (M), phosphoprotein (P) and nucleoprotein (NP) in the 5′ to 3′ direction (Alexander, 1997). The virus has a wide host range with 27 of the 30 orders of birds reported to have been infected by NDV (Kaleta and Baldauf, 1988). Lentogenic isolates are of low virulence and cause mild respiratory or enteric infections. Viruses of intermediate virulence that cause primarily respiratory disease are termed mesogenic, while virulent viruses that cause high mortality in birds are termed velogenic. Diagnostically velogenic NDV forms are further classified as neurotropic or viscerotropic based on clinical manifestation (Alexander, 1997). Velogenic NDV are List A pathogens that require reporting to the Office of International Epizootes, and outbreaks result in strict trade embargoes (Office International des Epizootes, 1996).

The primary molecular determinant for NDV pathogenicity is the F protein cleavage site amino acid sequence (Nagai et al., 1976; Glickman et al., 1988) and the ability of specific cellular proteases to cleave the F protein of different pathotypes (Gotoh et al., 1992; Ogasawara et al., 1992). Dibasic amino acids surrounding glutamine at position 114 are present in the fusion protein cleavage site of mesogenic or velogenic strains, while lentogenic NDV isolates lack this motif (Nagai et al., 1976; Glickman et al., 1988). Presence of dibasic amino acids in the fusion protein sequence allows for systemic spread of velogenic NDV, whereas replication of lentogenic NDV is primarily limited to mucosal surfaces of avian tissues (Ogasawara et al., 1992). This is also the major factor used in differentiating velogenic and mesogenic NDV from lentogenic NDV isolates in cell culture (King, 1993), presumably due to the presence of a required protease (Ogasawara et al., 1992). Lentogens must have added proteases for replication in avian fibroblasts or mammalian cell types, whereas mesogenic and velogenic NDV do not have this requirement (Nagai et al., 1976; King, 1993). The HN protein is involved with NDV attachment to cells and potentiates fusion by F1, while NP, P and L proteins are intimately involved with viral replication (Lamb and Kolakofsky, 1996).

Viscerotropic velogenic viruses have entered the US via importation of exotic birds (Bruning-Fann et al., 1992; Panigrahy et al., 1993; Seal et al., 1998) and a virus of psittacine origin caused the early 1970s outbreak in the southern California area of the US (Utterback and Schwartz, 1973). Recent outbreaks of Newcastle disease among cormorants in the north central US and southern Canada were attributed to neurotropic velogenic viruses (Seal et al., 1995; Heckert et al., 1996; Kuiken et al., 1998). Highly virulent viruses continue to circulate among free-living birds and commercial poultry worldwide (Alexander et al., 1992; Collins et al., 1993; Yang et al., 1997; Lomniczi et al., 1998; Oberdorfer and Werner, 1998; Roy et al., 1998; Seal et al., 1998; Stram et al., 1998). Examination of the F and HN protein genes from several NDV isolates resulted in these viruses being divided into three sub-types based on phylogenetic analysis and size of the HN protein (Sakaguchi et al., 1989; Toyoda et al., 1989). The M protein is one of the more conserved proteins among the paramyxoviruses (Rima, 1989) and could be used to help establish a reliable classification of these virus types.

To further examine molecular taxonomy of NDV, the M gene and predicted protein product from recent isolates were analyzed and the relationship of NDV with members of the Paramyxoviridae was determined. Viscerotropic velogenic NDV isolates from imported exotic birds and poultry were provided by the Diagnostic Virology Laboratory, National Veterinary Services Laboratory, Animal Plant Health Inspection Service, and USDA, Ames, IA, and histories are presented in detail elsewhere (Seal et al., 1998). A velogenic viscerotropic NDV isolated from Korean poultry was included for analysis (King, D.J., unpublished work). The viruses analyzed represent all NDV pathotypes that originate from areas worldwide and are represented chronologically from 1932 to as recently as 1997 (Table 1).

Table 1
Newcastle disease virus isolates phylogenetically examined, listed as country of origin and year of isolation with pathotype designation

Isolate[a]	Pathotype[b]	Access[c]
APMV1/Chicken/Australia/AV/32	N	M16622
APMV1/Chicken/UK/Herts/33	V	U25830
APMV1/Chicken/Italy/Milano/45	V	AF124442
APMV1/Chicken/U.S./LaSota/46	L	U25831
APMV1/Chicken/U.S./Kimber/47	M	U25831
APMV1/Chicken/U.S./B1/48	L	U25828
APMV1/Chicken/U.S./GB/48	N	U25835
APMV1/Chicken/U.S./Roakin/48	M	AF124443
APMV1/Chicken/U.S./BeaudetteC/52	N	X04687
APMV1/Chicken/NorthernIreland/Ulster/64	L	U25837
APMV1/Chicken/Australia/QV4/66	L	U25834
APMV1/mixed species/U.S./Largo/71	V	U25832
APMV1/Chicken/U.S./CA1083(Fontana)/72	V	U25829
APMV1/Duck/Japan/D26/78	L	X04687
APMV1/Cockatiel/U.S.(FL)/FL/80	V	AF124452
APMV1/Turkey/U.S./VGGA/89	L	U25838
APMV1/Chicken/Korea/12a/89	V	AF124449
APMV1/Cockatoo/Indonesia/14698/90	V	AF124446
APMV1/Parakeet/Myanmar/11592/91	V	AF124445
APMV1/Parrot/U.S.(IL)/27994/91	N	AF124447
APMV1/Cormorant/U.S.(MI)/40068/92	N	AF124451
APMV1/Turkey/U.S.(ND)/43084/92	N	U25836
APMV1/R.F.Parakeet/Tanz,Belg,China/28710/93	V	AF124448
APMV1/Anhinga/U.S.(FL)/44083/93	M	AF124450
APMV1/Chicken/Mexico/37821-550-1/96	V	AF124453
APMV1/Cormorant/U.S.(SaltonSea)/CWI/97	N	AF124444

[a] Isolate is given as bird type/country of origin/accession number/year of isolation, as designated by regulatory agencies or by historical reference.

[b] Initial virus pathotype following biological characterization in chickens and embryonated chicken eggs. L, lentogen; M, mesogen; V, viscerotropic velogenic; N, neurotropic velogenic.

[c] GenBank accession number for the matrix protein gene.

Isolates of NDV were replicated in embryonated chicken eggs (Alexander, 1997) and RNA was extracted (Chomzcynski and Sacchi, 1987) directly from allantoic fluid as described (Seal et al., 1995). Degenerate oligonucleotide primer pairs were derived by alignment of published NDV nucleotide sequences followed by analysis of conserved nucleotide sequences using the PRIMER2™ (Scientific & Educational Software, Stateline, PA) computer program. Oligonucleotide reverse transcription-polymerase chain reactions (RT-PCR) primers were designed within the P, M and F genes to amplify regions of the NDV genome that spanned intergenic regions and overlapped respective M gene coding regions (Seal, 1996). A single-tube RT-PCR for genomic NDV RNA was completed as described (Lewis et al., 1992; Seal et al., 1995), using Superscript™ (Kotewicz et al., 1988; Life Technologies, Gaithersburg, MD) and Amplitaq™ (Perkin-Elmer) polymerase (Saiki et al., 1985).

Amplification products were purified using Microcon™ (AMICON) spin filters and cloned using the TA cloning system™ (Mead et al., 1991) according to the methods described by the manufacturer (Invitrogen, San Diego, CA). Direct double-stranded nucleotide sequencing (Sanger et al., 1977) of amplification products and cloned material was completed (Applied Biosystems, Inc.) with NDV-specific oligonucleotide primers or universal amplification primers, fluorescent-labeled dideoxynucleotides and an automated nucleic acid sequencer (Smith et al., 1986).

Nucleotide sequence editing, analysis, prediction of amino acid sequences and alignments were conducted using INTELLIGENETICS GENEWORKS 2.5.1™ (IntelliGenetics, Mountain View, CA) and DNASTAR™ (Madison, WI) software. Phylogenetic relationships among NDV isolates presented were constructed in the Phylogenetic Analysis Using Parsimony (PAUP*4.0b) (Swofford, 1998) and the Molecular Evolutionary Genetics Analysis (MEGA) (Kumar et al., 1993) software. All phylogenetic relationships generated were evaluated by 2000 bootstrap replicates (Hedges, 1992). Nucleotide sequence analyses were also completed in MULTICOMP with a 90 base pair sliding window (Reeves et al., 1994) using the algorithm of Li

(1993) for determining synonymous and nonsynonymous substitutions. These data were prepared for graphical presentation using EXCEL98 (Microsoft Corp., Seattle, WA). A statistical test for recombination among homologous nucleotide sequences was also completed (Sawyer, 1989). Phylogenetic trees generated by Neighbor-joining utilizing a gamma distribution were subjected to End-Epi analysis to infer population dynamics from aligned sequences (Rambaut et al., 1997). All oligonucleotide primer sequences used are available from the author upon request.

Nucleotide sequences of M protein genes for NDV isolates are as reported previously (Chambers et al., 1986; McGinnes and Morrison, 1987; Sato et al., 1987; Seal, 1996) or have accession numbers AF124442–AF124453 (Table 1). Sequences for M protein alignments among the Paramyxoviridae were obtained from GenBank as the following: avian pneumovirus subtype A (APV/A) (Yu et al., 1992), avian pneumovirus subtype B (APV/B) (Randhawa et al., 1996), bovine parainfluenza virus 3 (bPIV3) (Sakai et al., 1987), bovine respiratory syncytial virus (bRSV) (Samal and Zamoram, 1991), dolphin morbillivirus (DMV) (Bolt et al., 1994), human parainfluenza virus 1 (hPIV1) (Power et al., 1992), human parainfluenza virus 2 (hPIV2) (Kawano et al., 1990), human parainfluenza virus 4A and 4B (hPIV4A and hPIV4B) (Kondo et al., 1991), human respiratory syncytial virus 1 (hRSV1) (Elango et al., 1985), human respiratory syncytial virus 2 (hRSV2) (Satake and Venkatesan, 1984), measles virus and canine distemper virus (MV and CDV) (Bellini et al., 1986), mumps virus (MuV) (Afzal et al., 1994; Saito et al., 1998), measles virus subacute subsclerosing panencephalitis (MV-SSPE) (Haga et al., 1992), ovine respiratory syncytial virus (ORSV) (Alansari and Potgeiter, 1994), porcine morbillivirus (LPMV) (Berg et al., 1991), pneumonia virus of mice (PnVM) (Easton and Chambers, 1997), rinderpest virus (RPV) (Baron et al., 1994), Sendai virus (SeV) (Blumberg et al., 1984; Willenbrink and Neubert, 1990), simian virus 5 (SV5) (Sheshberadaran and Lamb, 1990), simian virus 41 (SV41) (Tsurudome et al., 1990), with Ebola virus (Sanchez et al., 1993) as an outgroup (Smith, 1994).

The synonymous substitution rate (K_S) for the entire M gene (1092 bases) among NDV isolates was 0.33874 and the nonsynonymous substitution rate (K_A) was 0.05033. This results in a nonsynonymous to synonymous base substitution ratio of 0.14. There were 469 polymorphic sites, 314 of which were informative. The average substitution distance was 124.24 bases (11.38%). Sliding window analysis of K_S and K_A (Fig. 1) illustrates that variation occurs throughout the M protein gene, with the majority of nonsynonymous changes occurring in the 5′ region of the gene. Increased numbers of nonsynonymous changes are reflected in the variability of the predicted amino acid sequences in this portion of the predicted M protein (data not shown).

This suggests that NDV has been undergoing purifying selection, resulting in disadvantageous nonsynonymous mutations being eliminated from the population (Kimura, 1977). This has occurred in areas of the gene coding for conserved regions of the M protein. However, certain portions of the gene do have increased numbers of nonsynonymous base changes. This agrees with other viral systems, wherein specific areas of a gene may have regions where the rate of nonsynonymous substitutions exceeds that of synonymous substitution rate regardless of the overall K_A/K_S ratios (Ina and Gojobori, 1994; Seibert et al., 1995). Although the M protein of paramyxoviruses is relatively conserved (Rima, 1989), antigenic differences may be detected among NDV isolates by monoclonal antibody reactivities to the M protein (Faaberg and Peeples, 1988; Panshin et al., 1997).

Following alignment of M gene nucleotide coding sequences, distance matrix methods were applied to determine phylogenetic relationships among NDV isolates (Fig. 2A). The unrooted phylogram was generated using absolute distances. The same topological relationships resulted using Kimura two-parameter, Jukes–Cantor or gamma-corrected distances. Parsimony analysis of M protein gene sequences among NDV isolates also produced a tree with a congruent topology (data not shown). Among all isolates examined, the average pairwise M gene nucleotide sequences shared 57% identity, while the predicted amino acid sequences shared 63% identity.

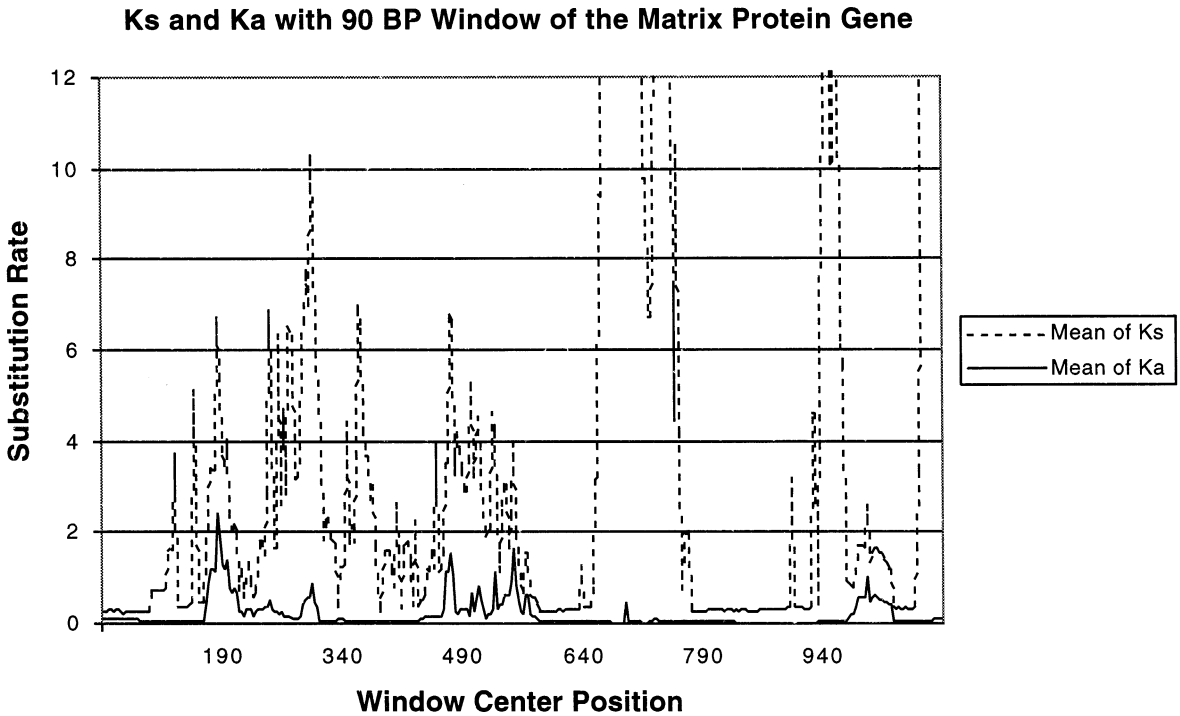

Fig. 1. Sliding window analysis of synonymous and nonsynonymous substitutions in the matrix protein gene among Newcastle disease virus isolates. Dashed line represents the rate of synonymous (K_S) substitutions and the solid line represents the rate of nonsynonymous (K_A) substitutions in a 90 base pair window.

Phylogenetic relationships based on M protein gene sequences further support the hypothesis that there are two major groups of NDV isolates (Seal et al., 1995; Lomniczi et al., 1998). Isolates restricted to the US and obtained prior to the last major outbreak during the 1970s form a clade, with lentogens separating from virulent viruses. This collection of older US NDV isolates represents a lineage wherein virulent forms of the virus are possibly no longer present among free-living birds or poultry. No virulent viruses of these types have been reported (Yang et al., 1997; Lomniczi et al., 1998; Seal et al., 1998), and NDV currently circulating among US poultry are most likely of vaccine origin (Marin et al., 1996). This accounts for the vaccine virus isolate turkey/U.S./VGGA/89.

Virulent viruses with chicken/Australia/AV/32 as a possible progenitor have a relatively continuous step-wise (chaining) evolutionary pattern in the phylogenetic tree. These isolates share 72% nucleotide sequence identity among one another and originate worldwide (Table 1). These virulent NDV isolates share only 66% nucleotide sequence identity with the closely related lentogenic NDV isolates D26/78, QV4/66 and Ulster/64 that share 92% sequence identity among themselves. These virus groups separate most closely to one another relative to the US viruses primarily isolated prior to 1970.

In contrast, among those NDV strains available, those that were isolated from the US prior to 1970 have a more balanced topology with little sequence variation among these viruses. The La-Sota/46, VGGA/89 and B1/48 vaccine viruses share 95% sequence identity, while the closely related virulent NDV isolates are 92% similar among one another. Overall, these two groups of relatively older viruses limited to the US share 86% sequence identity and separate from viruses isolated from other parts of the world or virulent viruses isolated more recently within the US.

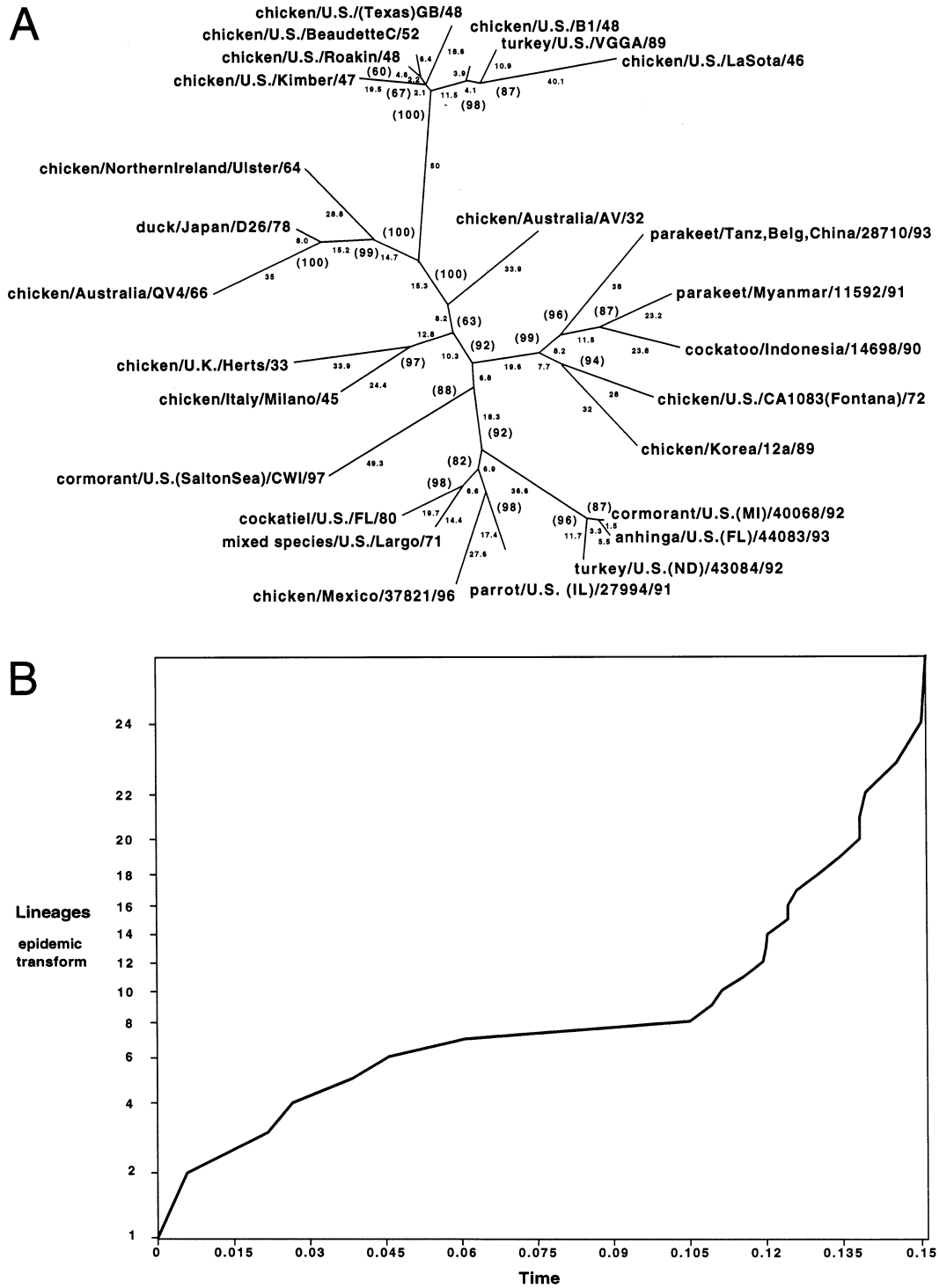

Fig. 2. Phylogenetic relationships and population analysis among Newcastle disease virus isolates. (A) An unrooted phylogram was generated by neighbor-joining following alignment of Newcastle disease virus matrix protein gene nucleotide coding sequences. Distances are provided with bootstrap confidence levels in parentheses. (B) Epidemic and endemic transformation analysis of Newcastle disease virus isolates. Analysis is based on matrix protein gene nucleotide coding sequence analysis.

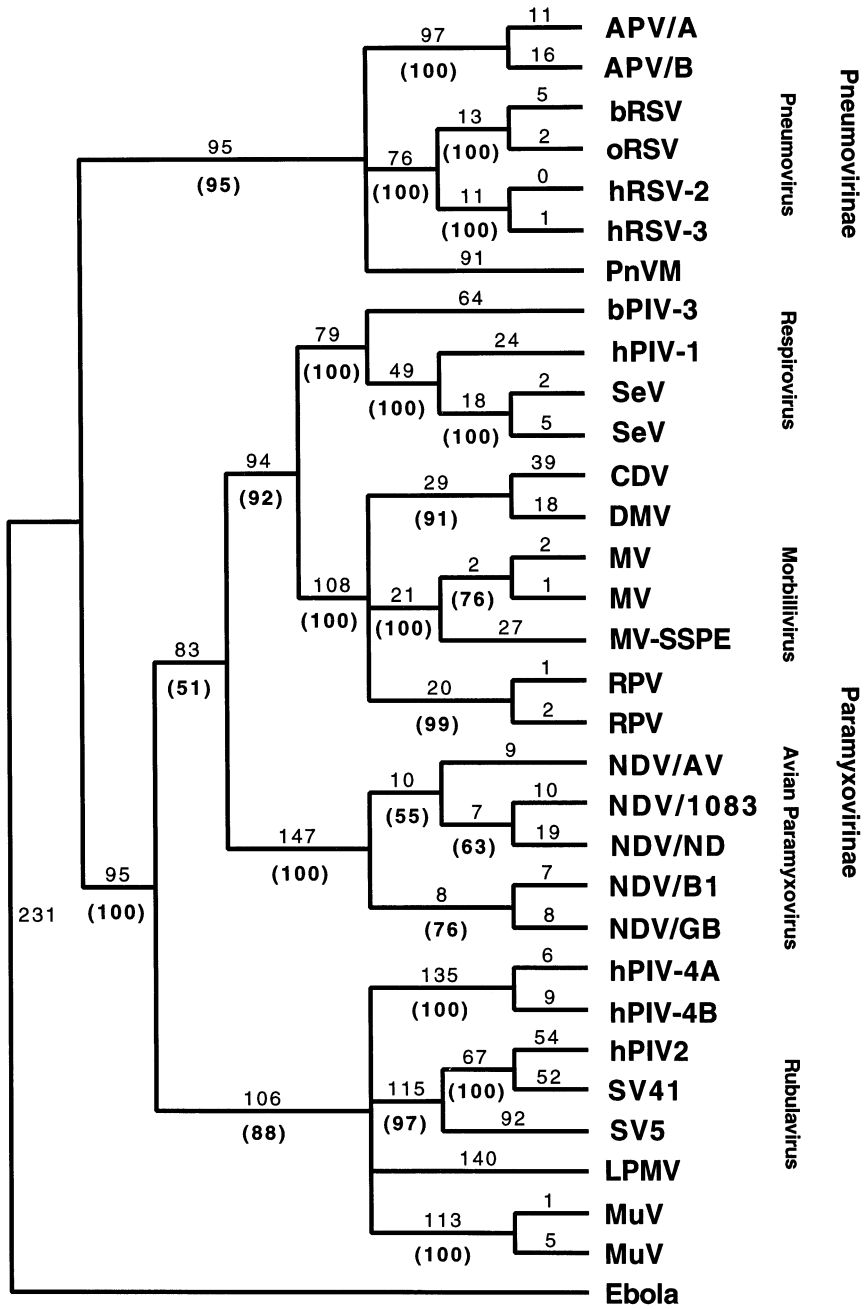

Fig. 3. Phylogenetic relationships among the Paramyxoviridae based on matrix protein amino acid sequences. A rectangular cladogram was generated by maximum parsimony analysis following clustal alignment of matrix protein amino acid sequences. Ebola virus, a member of the Filoviridae, was used as an outgroup during analysis. Distances are provided, with bootstrap confidence levels presented in parentheses.

Following sequence analysis, no evidence of recombination was detected among the NDV M gene sequences examined. Consequently, phylogenetic trees generated from these nucleotide sequences were subjected to population analysis utilizing a lineage through-time plot (Fig. 2B). An epidemic transformation of these data resulted in a roughly horizontal line with a recent upward curve. This indicates that among the NDV sequences examined, a population of genetically distinct but related NDV isolates has expanded recently.

The phylogenetic relationships among NDV isolates have been confirmed by independent investigators (Seal et al., 1998; Lomniczi et al., 1998) and indicate that the NDV population may be expanding. This recent exponential increase most likely occurred during the 1970s. Several outbreaks of highly virulent Newcastle disease occurred worldwide at this time (Alexander, 1997). During the US outbreak, a virus of pet bird origin was epidemiologically implicated as the causal agent (Utterback and Schwartz, 1973), which was subsequently confirmed by nucleotide sequence analysis (Seal, 1996; Seal et al., 1995). These virus types continue to plague the poultry industry and have been identified from many geographic areas (Yang et al., 1997; Alexander et al., 1998; Lomniczi et al., 1998; Seal et al., 1998). Increase in poultry production and international trade have been the most likely contributors to the current situation (Alexander, 1997).

Matrix proteins were aligned for representative members of the Paramyxoviridae and subjected to maximum parsimony analysis to determine relationships among these virus types (Fig. 3). The Pneumovirinae, composed of human, animal and avian pneumoviruses, separate distinctly from members of the Paramyxovirinae. Among the Paramyxovirinae, individual groups separate into the respiroviruses, morbilliviruses and rubulaviruses. However, NDV isolates separate as a cluster most closely related to morbilliviruses and not with rubulaviruses. Consequently, the avian paramyxoviruses form an individual clade among the Paramyxovirinae supported by distance measurements and relatively high bootstrap support.

The International Committee for Taxonomic Virology has classified NDV as a rubulavirus (Rima et al., 1995). However, NDV differs from the rubulaviruses in several characteristics such as the lack of a small hydrophobic protein gene (Chambers et al., 1986; Lamb and Kolakofsky, 1996; de Leeuw and Peeters, 1999). Phylogenetic analysis of the M proteins from all members of the Paramyxoviridae indicate that NDV is more closely related to morbilliviruses than to rubulaviruses. Therefore, avian paramyxoviruses should be placed in their own taxonomic category among the Paramyxovirinae, similar to what was suggested for the avian pneumoviruses among the Pneumovirinae (Pringle, 1996). This proposal is supported by phylogenetic analysis of full-length genomes for those members of the Paramyxoviridae that are available (de Leeuw and Peeters, 1999).

Paramyxovirus M proteins are considered to be relatively conserved among isolates of different virus types (Rima, 1989). This is substantiated by the low number of nonsynonymous base substitutions, such as for the data reported in this article. Consequently, these data can be applied to population analysis and viral taxonomy. The results from our investigations indicate that a virus of veterinary importance, such as NDV, has had an exponential population expansion similar to that reported for tick- and mosquito-borne flaviviruses infecting humans (Zanotto et al., 1996). Finally, use of these data supports the conclusion that NDV is not a rubulavirus (de Leeuw and Peeters, 1999) and should be placed in its own taxonomic group among the Paramyxovirinae.

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