Arch Virol (1996) 141: 243–261

Archives of Virology
© Springer-Verlag 1996
Printed in Austria

Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from the F gene

A. Ballagi-Pordány[1], **E. Wehmann**[2], **J Herczeg**[2], **S. Belák**[1], and **B. Lomniczi**[2]

[1] Department of Virology, The National Veterinary Institute, Uppsala, Sweden,
[2] Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary

Accepted October 11, 1995

Summary. A 75% region of the F gene (between nucleotides 334 and 1682) of Newcastle disease virus (NDV) RNA was amplified by reverse transcription-polymerase chain reaction (RT-PCR). PCR products were cleaved by three restriction endonucleases and the positions of thirty cleavage sites were mapped in more than 200 NDV strains. Restriction site analysis established six major groups of NDV isolates and unique fingerprints of vaccine strains. Group I comprised lentogenic strains isolated mainly from waterfowl with some from chickens. "Old" (prior to 1960s) North American isolates of varying virulence including lentogenic and mesogenic vaccine strains belonged to group II. Group III included two early isolates from the Far East. Early European strains (Herts 33 and Italien) of the first panzootic (starting in the late 1920s) and their descendants with some modifications were placed into group IV. NDV strains isolated during the second panzootic of chickens (starting in the early 1960s) were classified into two groups. Group V included strains originating in imported psittacines and in epizootics of chickens at the early 1970s. Group VI comprised strains from the Middle East in the late 1960s and later isolates from Asia and Europe. Pigeon paramyxovirus-1 strains that were responsible for the third panzootic formed a distinct subgroup in group VI. Our grouping of NDV strains has confirmed group differences established by monoclonal antibodies. It is concluded that restriction site analysis of F gene PCR amplicons is a relatively fast, simple and reliable method for the differentiation and identification of NDV strains.

Introduction

Newcastle disease (ND) is a highly contagious infection of poultry that, depending on the virulence of the virus strain, may manifest in a wide range of severity from subclinical infection to lethal disease [2]. Its causative agent is ND-virus

(NDV) the prototype of the *Paramyxovirus* genus of the family *Paramyxoviridae* [19]. The distribution of ND is influenced by the effectiveness of control measures, therefore different geographical regions vary greatly in the incidence of infection. In Africa and Asia large areas are characterised by endemic infections while most European countries are considered free from the disease [3]. Nevertheless, Europe suffered considerable economic losses during three panzootics due to NDV. The first, starting in the mid-1920s, took some 30 years to spread from Southeast Asia to other regions of the world including Europe. The second panzootic might have also originated in Asia in the 1960s but it took only a few years to conquer the same territory as the first [32]. This panzootic seemed in addition to be associated with exotic pet birds, introduced from South America and Indonesia, both to Europe and to the United States. The third panzootic that started in the late 1970s, affected racing pigeons with outbreaks in chickens in England [8].

The effectiveness of control measures can be improved if the origin of disease outbreaks is quickly and unambiguously identified. Strain identification is also of great importance with diseases or in countries where different live vaccines are used, as in the case of ND, to establish vaccine identity. Accordingly, much effort has been made in the past decades to introduce methods of strain identification or at least those of differentiation. These techniques have been based on biological properties of the virus, such as pathogenicity or plaque formation; physicochemical properties, such as thermostability and analyses of structural polypeptides or oligonucleotides (reviewed in [2]). However, epizootiologically meaningful results were provided only by monoclonal antibody (mAb) analysis of NDV strains [6, 7, 41]. This method established 10 antigenic groups of which five included virulent (velogenic) strains. This was the first classification of NDV strains in which strains of a group shared antigenic and epizootiological properties. Based on the comparison of nucleotide sequences of the haemagglutinin-neuraminidase (HN) and fusion (F) genes of 11 NDV strains three distinct evolutionary lineages were identified [42, 47].

The purpose of the present investigations was two-fold: i. to find a relatively simple way of NDV strain grouping that would lead to epizootiologically meaningful categories and/or shed light on the phylogenetic relationship of strains; ii. to provide a reliable method for the identification of virus isolates that is paramount to epizootiological investigations.

In this paper we describe restriction enzyme (RE) analysis of reverse transcription-polymerase chain reaction (RT-PCR) amplicons of a 1349 nucleotide (nt) long region of the F-gene. Physical maps of fragments were constructed after determining their lengths in agarose gel electrophoresis and by using data of published sequences. Location of new cleavage sites, relative to those found in previously sequenced strains were determined by sequencing (to be published). This approach allowed a quick screening and a grouping of a large number of strains into six groups. Within these groups the strains shared epizootiological properties and/or presumably a common ancestor.

Materials and methods

Viruses

NDV strains were obtained from the following laboratories listed in the order as on Table 1: Weybridge, UK, Avian Virology, Central Veterinary Laboratory (from D. J. Alexander); VMRI, Bp., Hungary, Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest. Strains for the collection maintained in this institute (by B. Lomniczi) were provided by the Central and Regional Veterinary Institutes in Budapest (from E. Sághy), in Debrecen (from J. Tanyi), in Miskolc (from Zs. Máté) and Békéscsaba (from V. Palya); Phylaxia, Bp., Hungary, commercial vaccine stocks; London, UK, St. Thomas' Hospital Medical School (from T. H. Pennington); Giessen, FRG, Institut für Geflügelkrankheiten (from E. F. Kaleta); München, FRG, Institut für Geflügelkrankheiten, Ludwig-Maximilians-Universität (from M. Shakal); P.T.E. Singapore, Primary Industries Enterprise (PTE) Ltd., commercial vaccine stock; Hong Kong, HK, Department of Microbiology, University of Hong Kong (from K. F. Shortridge); Vienna, Austria, Institut für Pathologie und Gerichtsmedizin. Vet. Med., Vet. Med. Universität (from H. Burtscher); Novi Sad, Yugoslavia, Veterinary Institute (from I. Oláh); Zagreb, Croatia, Veterinary Institute (from Z. Čač). NDV strains were grown in 9–11-day-old chicken embryos as described [33]. Aliquots were stored in Eppendorf tubes at −70 °C until use.

Preparation of viral RNA

RNA was extracted from 500 µl of allantoic fluid with Proteinase K (Sigma, St Louis, USA) followed by phenol and chloroform. The final pellet was dissolved in 25 µl double distilled (dd) DEPC treated H_2O and stored at −70 °C [48].

Reverse transcription

Synthesis of cDNA was carried out in 25 µl reaction volume containing 8 µl DEPC treated ddH_2O, 5 µl 5× first-strand-buffer (Gibco, Bethesda, Maryland, USA), 0.02 U random hexamers (Pharmacia, Uppsala, Sweden), 1 µl of each dNTP (each 10 mM), 1 µl RNAguard (Pharmacia), 1 µl Moloney murine leukemia virus reverse transcriptase (Gibco) and 5 µl sample. The reaction mixture was incubated at 37 °C for 90 min, followed by enzyme inactivation at 98 °C for 5 min [48].

Primers

Sequence data of F gene of 18 NDV strains which were available in the GenEMBL databank were aligned by applying the Pileup program of the GCG program-package. Primers were selected from the consensus parts by applying the Oligo 4.0 computer program (National Biosciences, Inc., Plymouth, MN, USA). A 1349 bp PCR product was produced with ONDV 1a and ONDV 4a primers from restriction enzyme analysis.

ONDV 1a: 5′ TGACTCTATCCGTAGGATACAAGAGTCTG 3′.
ONDV 4a: 5′ GATCTAGGGTATTATTCCCAAGCCA 3′

PCR

The reaction was carried out in 100 µl volume which comprised 10 µl 10× PCR buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl and 1 mg/ml BSA), 10 µl $MgCl_2$, 1 µl of each dNTP (each 10 mM, Pharmacia), 30 pmole of each primer, 2 U *Taq* polymerase (Perkin-Elmer

Table 1. Origin and properties of NDV strains and source of F sequences

NDV	Origins			Virulence	mAb binding group [6, 7, 41]	Sequences of F genes	Source of viruses
strains	year	country	host				
Ulster 2C	1967	Northern Ireland	fowl [37]	L	G	D00243[a] [39]	Weybridge
V4 (Queensland)	1966	Australia	fowl [45]	L	G	M24693[a] [47]	Weybridge
D26/76	1976	Japan	duck [40]	L		M24692[a] [43]	–
H/6/65	1965	Hungary	fowl [34]	L, vaccine	–	–	VMRI, Bp.
LaSota,	1946	USA	fowl [21]	L, vaccine	E	M24696[a] [47]	Phylaxia, Bp.
H/5L/70	1970	Hungary	fowl	L	–	–	VMRI, Bp.
B1	1947	USA	fowl [26]	L, vaccine	E	M24695[a] [47]	Phylaxia, Bp,
F	1948	Great Britain	fowl [9]	L, vaccine	F	–	London
Roakin	1946	USA	fowl [11]	M, vaccine	D	–	Ames
Komarov	1945	Palestine	att. in duck [31]	M, vaccine	D		Phylaxia, Bp.
Lederle	194?	USA	fowl	M, vaccine		–	Phylaxia, Bp.
Beaudette C	1945	USA	fowl [22]	M	D	X04719[a] [14]	Weybridge
TexasGB	1948	USA	fowl [13]	V	D	M24698[a] [44]	London
CA 11914	1943	USA	fowl [10]	V		–	Giessen
IN/Purdue 1250	1949	USA	fowl [23]	V	–	–	Ames
AUS Victoria	1932	Australia	fowl [1]	V		M21881[a] [38]	–
Miyadera	1951	Japan	fowl [29]	V		M18456[a] [46]	–
Hertfordshire	1940	England	att. in eggs [27]	M, vaccine	B	–	Phylaxia, Bp.
Mukteswar	1941	India	att. in eggs	M, vaccine	B	–	PTE, Singapore
Herts 33	1933	Great Britain	fowl [4]	V	B	M24702[a] [47]	London
Italien	1945?	Italy	fowl	V		M17710[a] [18]	–
H/219/50	1950	Hungary	fowl	V		–	VMRI, Bp.
H/221/54	1954	Hungary	fowl	V		–	VMRI, Bp.
H/1/70	1970	Hungary	fowl	V		–	VMRI, Bp.
H/5/70	1970	Hungary	fowl	V		–	VMRI, Bp.
A/78/60	1960	Austria	fowl	V		–	Vienna
A/82/60	1960	Austria	fowl	V		–	Vienna
D/194/78	1978	Germany	falcon	V		–	Giessen
D/195/79	1979	Germany	fowl	V		–	Giessen
YU/246/83	1983	Yugoslavia	fowl	V		–	Novi Sad
Essex 70	1970	Great Britain	fowl [4]	V	A	–	Weybridge
NY 70181	1970	USA	parrot [4]	V	A	–	Weybridge

CA 1085/71	1971	USA	fowl [4]	V	A	–	Weybridge
Northants 72	1972	Great Britain	fowl [4]	V	B	–	Weybridge
H/10/72	1972	Hungary	fowl	V		–	VMRI, Bp.
H/242/84	1984	Hungary	fowl	V		–	VMRI, Bp.
H/133/76	1976	Hungary	fowl	V		–	VMRI, Bp.
H/143/79	1979	Hungary	fowl	V		–	VMRI, Bp.
Pa/WA/75	1975	West Africa	parrot	V		–	Giessen
HR/271/84	1984	Croatia	fowl	V		–	VMRI, Bp.
D/372/86	1986	Germany	fowl	V		–	Giessen
Warwick 66	1966	Great Britain	fowl [4]	V	B	–	Weybridge
Iraq AG68	1968	Iraq	fowl [4]	V	B	–	Weybridge
Kuwait 256	1968	Kuwait	fowl [4]	V	C	–	Weybridge
Lebanon 70	1970	Lebanon	fowl [4]	V		–	Weybridge
Israel 70	1970	Israel	fowl [4]	V		–	Weybridge
Greece 68	1968	Greece	fowl	V		–	VMRI, Bp.
Pi/IRQ/78	1978	Iraq	pigeon [28]	V	P	–	Giessen
Pi/M/82	1982	Malta	pigeon		P [30]	–	Giessen
Pi/I/82	1982	Italy	pigeon [12]		P [30]	–	Perugia
Pi/GB/561/83	1983	Great Britain	pigeon [5]	V	P	–	Weybridge
Pi/B/83	1983	Belgium	pigeon		P [30]	–	Weybridge
Pi/GB/454/84	1984	Great Britain	chicken[b]	V	P[b]	–	Weybridge
Pi/H/83	1983	Hungary	pigeon	M	P [30]	–	VMRI, Bp.
Pi/DK/83	1983	Denmark	pigeon [5]		P	–	Weybridge
Pi/HR/84	1984	Croatia	pigeon		P [30]	–	Zagreb
Pi/H/87	1987	Hungary	pigeon	M	P [30]	–	VMRI, Bp.
Pi/J/89	1989	Japan	pigeon			–	Giessen
Pi/H/89	1989	Hungary	pigeon	M	P [30]	–	VMRI, Bp.
Pi/ET/86	1986	Egypt	pigeon	M	P [30]	–	München
HK/4/79	1979	China	partridge	V		–	Hong Kong
HK/109/78	1978	Hong Kong	healthy duck	V		–	Hong Kong
H/310/82	1982	Hungary	fowl	V	B	–	VMRI, Bp.
H/386/85	1985	Hungary	fowl	V		–	VMRI, Bp.
H/345/86	1986	Hungary	fowl	V		–	VMRI, Bp.
H/514/86	1986	Hungary	fowl	V		–	VMRI, Bp.

L Lentogenic, *M* mesogenic, *V* velogenic, *att.* attenuated

[a] GenEMBL accession number

[b] pers. comm.

Cetus, Norwalk, CT, USA), 5 µl cDNA, ddH_2O up to 100 µl and 1 drop of mineral oil (Sigma). Amplification was carried out in a Genetic Thermal Cycling System, GTC-2 (Precision Scientific, Chicago, IL, USA) using 5 cycles of 94 °C for 45 sec, 55 °C for 30 sec, 72 °C for 1 min and 30 cycles of 94 °C for 45 sec, 48 °C for 1 min, 72 °C for 3 min and finally 72 °C for 7 min. The primers for PCR were synthetised in our laboratory using a PCR-MATE DNA synthetiser (Applied Biosystem, Warrington, UK).

Restriction enzyme cleavage and electrophoresis

After amplification the entire volume of PCR (without oil) was transferred into 1.5 ml Eppendorf tube and mixed with 1 ml n-butanol by vortexing for 15 sec. The pellet was collected by centrifugation at 12500 **g** for 2 min and dried after removing the supernatant. The pellet was dissolved in 15 µl ddH_2O, divided into three parts subsequently 2 µl respective restriction enzyme buffer and 8–12 U of *Hinf*I, *Bst*OI or *Rsa*I restriction enzyme (Promega, Madison, WI, USA) were added. The digestion of the PCR product was carried out at 37 °C for overnight or at least 4 h. The reaction mixture was mixed with loading buffer containing glycerol [36] and loaded in 2.5% agarose gel, which was prepared by dissolving 2:1 ratio of MetaPhor agarose (FMC BioProducts, Rockland, ME, USA) and DNA grade agarose (Sigma) in 0.5 × TBE Buffer [36]. A one hundred base pair ladder (Gibco) was applied to estimate the size of the bands. The fragments were separated at 185 V for 2 h, stained in 1.5 mg/ml EtBr for 10 min and visualised on a UV-transilluminator. If double digestion was necessary for mapping, the Core buffer was used (Promega) instead of the specific ones.

Restriction enzyme analysis and mapping

Physical maps of restriction fragments were constructed for all strains. The positions of the enzyme cleavage sites were determined by using the length of the fragments and the restriction maps of the strains available in GenEMBL databank. Their maps were constructed by using Map and Mapsort programs of GCG program-package (Genetic Computer Group, Inc., Madison, WI, USA). The locations of new cleavage sites not present in strains in the databank were determined by double digestion and direct sequencing of the relevant regions (to be published).

Results

Restriction fragment profiles of the PCR products of representative NDV strains generated by three restriction enzymes (*Hinf*I, *Bst*OI, *Rsa*I) are shown in Fig. 1 (A, B and C, respectively). Strains represent genetic groups or subgroups, detailed below, that were established on the basis in differences of cleavage site distribution. Physical maps of restriction fragments and cleavage sites of the region of F gene between nucleotides 334 and 1682 of the same strains are shown in Fig. 2. The restriction site patterns of further 59 NDV strains based on the presence or absence of 30 cleavage sites are summarised in Table 2. Eleven were strains whose restriction site positions were taken from the databank, while the remainder represent over 200 strains examined to date that had been isolated from different geographical regions and/or with different epizootiological backgrounds in the past decades.

Group I corresponds to lineage A of Toyoda et al. [47] based on complete F or HN sequences of NDV strains. We have looked at a number of duck and

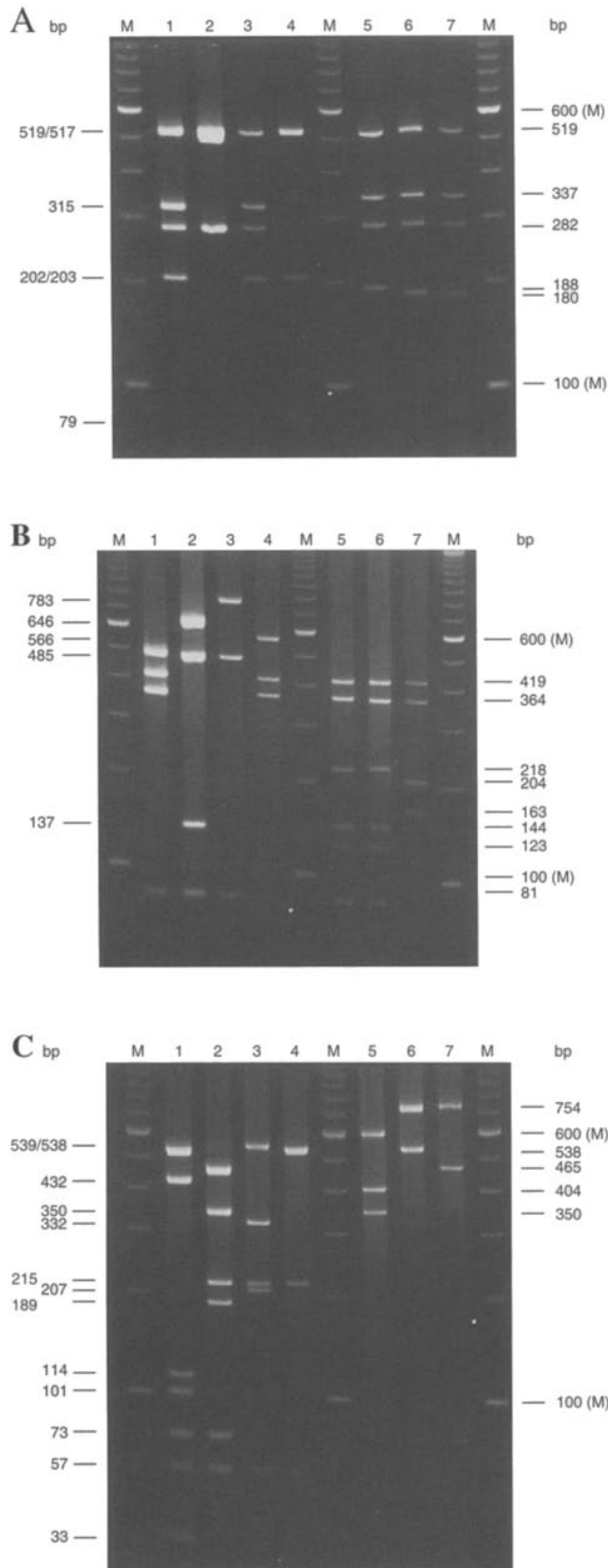

Fig. 1. Restriction fragment profiles of the PCR products of representative NDV strains electrophoresed in a 2.5% agarose gel and stained with ethidium bromide. Restriction enzymes *Hinf*I (**A**), *Bst*OI (**B**) and *Rsa*I (**C**) were used. *M* 100 bp molecular weight markers (Gibco); *1* NDV strain Ulster 2C (group I); *2* Komarov (group II); *3* Hertfordshire (group III); *4* H/219/50 (group IV); *5* Essex 70 (group V); *6* Iraq AG68 (group VIa); *7* Pi/GB/561/83 (group VIb). VIc profile is similar to VIa (not shown)

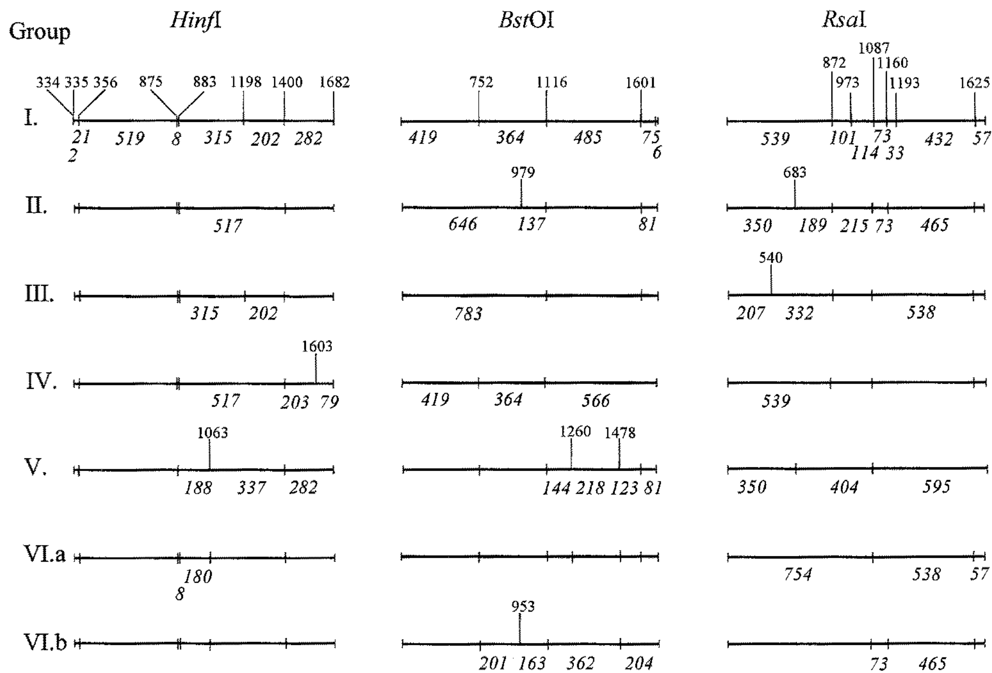

Fig. 2. Physical maps of restriction fragments and cleavage sites of the PCR products of representative NDV strains shown in Fig. 1. Numerals above the line designate the last nucleotide of a fragment except nt 334 that is the starting bp of the amplified region. Italics below the line mark fragment length in bp. Unmarked fragments have the same length as the corresponding marked one above

geese lentogenic isolates from Hong Kong and China (to be published) and although they showed some restriction site variations they were very close to the V4 (Queensland) and D26/76 branch. H/6/65, a lentogenic strain isolated in Hungary from chickens, also belongs to this group. Group I strains are characterised by a unique *Rsa*I cutting site at nt position 973.

Group II comprises mostly North American isolates, irrespective of their virulence. In addition to the four strains (LaSota, B_1, Beaudette C and Texas GB) that were classified into genetic lineage B by Toyoda et al. [47], group II included natural mesogenic isolates Roakin and L (Lederle) and an attenuated strain Komarov. Of the virulent strains California 11914 and Indiana/Purdue 1250 were placed in group II. This group is characterised by the presence of *Rsa*I cutting site at nt position 1160 and by the simultaneous absence of *Bst*OI site at nt 752. The absence of *Bst*OI site at 979 is exceptional with this group. All these strains were isolated prior to the mid-1950s. The lentogenic strain F isolated from chickens in England [9] has a very similar restriction site pattern to the American mesogenic strains, but is distinct from the American lentogenic vaccines.

Restriction site analysis has divided lineage C of Toyoda et al. [47] and separated the two early isolates of the Far East (Australia Victoria and Miyadera)

from early European strains (Herts 33 and Italien). The former was designated group III. The parallel presence of *Hinf*I site at nt 1198 and *Rsa*I site at position 540 distinguishes this group from group I, II and IV. Vaccine strain H is more closely related to this group, than to group IV where Herts 33 was placed. Herts 33 was reported to be the parent of strain H [27].

Group IV appears to be a heterogeneous cluster. The lack of restriction site by *Hinf*I at nt 1198 distinguishes this group from group III. *Rsa*I site at nt 540 is missing from strains with the exception of H/5/70 and this is the only group where *Rsa*I site at position 1593 appears at all in some members. Apart from old European strains (Herts 33, Italien) later isolates belong to here, too. It seems, however, that strains isolated in 1970 in Hungary and in 1960 in Austria are more closely related and tend to separate from the remainder. Some of recent isolates from Germany and Yugoslavia are identical with strain Herts 33.

NDV strains isolated during the second panzootic of chickens (starting in the early 1960s in Asia) were classified into two main categories. These strains were isolated after the mid-1960 and they can be recognised by two novel cutting sites at nt positions 1063 and 1478 by restriction enzymes *Hinf*I and *Bst*OI, respectively.

A distinct group (V) comprises isolates deriving from imported psittacines and also from chickens during the 1970–72 epizootics in Great Britain and the United States. Some of these viruses like Essex 70 and NY 70181 were previously classified into mAb binding group A [41]. We find here strains isolated in Hungary, Croatia and Germany. In addition to the two novel cutting sites, group V is distinguished from all the others by the loss of *Hinf*I cutting site at nt position 883. Similarity the absence of *Rsa*I cutting sites at nt 1625 and a 872 strengthens the uniqueness of this group.

Group VI is quite a heterogeneous cluster. Strains originating in the Middle East and Greece from 1968 to 1970, Hong Kong and China, England and Hungary were examined and placed here. Two restriction sites (*Hinf*I at nt 883 and *Rsa*I at nt 1625), missing in combination only in group V are present in group VI but the absence of *Rsa*I sites at nt 683 and 872 is also characteristic of all members. It has been demonstrated that the loss of a particular cleavage site in two different groups involved different changes. For instance, the *Rsa*I site CTAC has been lost due to a change to ATAT in group V, while the change is to GTAT in group VI (A. Ballagi, unpubl.). Restriction site analysis and epizootiological considerations have warranted the subdivision of this group into subgroups.

Subgroup VIa comprises strains of the second panzootic that reached the Middle East and Southern Europe by 1970. However, Warwick 66, a prepanzootic isolate in England, also belongs here. A distinct branch of this group includes pigeon PMV-1 strains, that we designated VIb. This subgroup is clearly distinguishable from the remainder of the branch by the loss of *Bst*OI restriction sites at nt 1601 and at nt 1260 (this latter, however, is present in Pi/IRQ/78, that might be the first pigeon PMV-1 isolate as proposed by Kaleta et al. [28]). It is interesting that this recently emerged group already shows a considerable genetic variation. A majority of the strains show the presence of *Rsa*I site at nt

Table 2. Distribution of restriction enzyme cleavage sites on the 1349 nt long region of F genes of NDV strains

Group	NDV strains	*Hinf* I 632	734[b]	875	883[b]	925	1063[b]	1198	1350	1400	1603
I.	Ulster 2C[a]	−	−	+	+	−	−	+	−	+	−
	V4 (Queensland)[a]	−	−	+	+	+	−	−	−	+	−
	D26/27[a]	−	−	+	+	+	−	−	−	+	−
	H/6/65	−	−	+	+	−	−	−	−	+	−
II.	LaSota[a]	−	−	+	−	−	−	−	−	+	+
	H/5L/70	−	−	+	−	−	−	−	−	+	+
	B1[a]	−	−	+	+	−	−	−	−	+	+
	F	−	−	+	+	−	−	−	−	+	−
	Roakin	−	−	+	+	−	−	−	−	+	−
	Komarov	−	−	+	+	−	−	−	−	+	−
	Lederle	−	−	+	+	−	−	−	−	+	−
	Beaudette C[a]	−	−	+	+	−	−	−	−	+	−
	TexasGB[a]	+	−	+	+	−	−	−	−	+	−
	CA 11914	−	−	+	+	−	−	−	−	+	−
	IN/Purdue 1250	−	−	+	+	−	−	−	−	+	−
III.	AUS Victoria[a]	−	−	+	+	−	−	+	−	+	−
	Miyadera[a]	−	−	+	+	−	−	+	−	−	−
	Hertfordshire	−	−	+	+	−	−	+	−	+	−
	Mukteswar	−	−	+	+	−	−	+	−	+	−
IV.	Herts 33[a]	−	−	+	+	−	−	−	−	+	−
	Italien[a]	−	−	+	+	−	−	−	+	+	−
	H/219/50	−	−	+	+	−	−	−	−	+	+
	H/221/54	−	−	+	+	−	−	−	−	+	−
	H/1/70	−	+	+	+	−	−	−	+	+	+
	H/5/70	−	+	+	+	−	−	−	+	+	+
	A/78/60	−	+	+	+	−	−	−	−	+	−
	A/82/60	−	+	+	+	−	−	−	+	+	−
	D/194/78	−	−	+	+	−	−	−	−	+	−
	D/195/79	−	−	+	+	−	−	−	−	+	−
	YU/246/83	−	−	+	+	−	−	−	−	+	−

Group	NDV strains	*Bst*OI 629	752	799[b]	953	979	1116	1260	1478[b]	1601	1676
I.	Ulster 2C[a]	−	+	−	−	−	+	−	−	+	+
	V4 (Queensland)[a]	−	+	−	+	−	+	−	−	+	−
	D26/27[a]	−	+	−	+	−	+	−	−	+	−
	H/6/65	−	+	−	+	−	+	−	−	+	−
II.	LaSota[a]	−	−	−	−	+	+	−	−	−	−
	H/5L/70	−	−	−	−	+	+	−	−	−	−
	B1[a]	−	−	−	−	+	+	−	−	−	−
	F	−	−	−	−	+	+	−	−	+	−
	Roakin	−	−	−	−	+	+	−	−	+	−
	Komarov	−	−	−	−	+	+	−	−	+	−
	Lederle	−	−	−	−	+	+	−	−	+	−
	Beaudette C[a]	−	−	−	−	+	+	−	−	+	−
	TexasGB[a]	−	−	−	−	+	+	−	−	+	−
	CA 11914	−	−	−	−	−	+	−	−	+	−
	IN/Purdue 1250	−	−	−	−	+	+	−	−	+	−
III.	AUS Victoria[a]	−	+	−	+	−	+	−	−	−	+
	Miyadera[a]	+	+	−	+	−	+	−	−	+	−
	Hertfordshire	−	−	−	−	−	+	−	−	+	−
	Mukteswar	−	−	−	−	−	+	−	−	+	−
IV.	Herts 33[a]	−	+	−	−	−	+	−	−	+	−
	Italien[a]	−	+	−	−	−	+	−	−	+	−
	H/219/50	−	+	−	−	−	+	−	−	−	−
	H/221/54	−	+	−	−	−	+	−	−	+	−
	H/1/70	−	+	−	−	−	+	+	−	−	−
	H/5/70	−	+	+	−	−	+	+	−	−	−
	A/78/60	−	+	−	−	−	+	+	−	+	−
	A/82/60	−	+	−	−	−	+	+	−	+	−
	D/194/78	−	+	−	−	−	+	−	−	+	−
	D/195/79	−	+	−	−	−	+	−	−	+	−
	YU/246/83	−	+	−	−	−	+	−	−	+	−

Group	NDV strains	*Rsa*I 540	683	872	973	1055	1087	1160	1193	1593	1625
I.	Ulster 2C[a]	−	−	+	+	−	+	+	−	−	+
	V4 (Queensland)[a]	−	+	+	+	+	−	+	−	−	+
	D26/27[a]	−	+	+	+	+	+	−	+	−	+
	H/6/65	−	+	+	+	−	+	+	+	−	+
II.	LaSota[a]	−	+	−	−	−	+	+	−	−	+
	H/5L/70	−	+	−	−	−	+	+	−	−	+
	B1[a]	−	+	−	−	−	+	+	−	−	+
	F	−	+	+	−	−	+	+	−	−	+
	Roakin	−	+	+	−	−	+	+	−	−	+
	Komarov	−	+	+	−	−	+	+	−	−	+
	Lederle	−	−	+	−	−	+	+	−	−	−
	Beaudette C[a]	−	+	+	−	−	+	+	−	−	−
	TexasGB[a]	−	+	+	−	−	+	+	−	−	+
	CA 11914	−	+	+	−	−	+	+	−	−	+
	IN/Purdue 1250	−	−	+	−	−	+	+	−	−	−
III.	AUS Victoria[a]	+	+	+	−	−	+	−	−	−	+
	Miyadera[a]	+	+	+	−	−	+	−	−	−	−
	Hertfordshire	+	−	+	−	−	+	−	−	−	+
	Mukteswar	+	−	+	−	−	+	−	−	−	+
IV.	Herts 33[a]	−	+	+	−	−	+	−	−	+	+
	Italien[a]	−	+	+	−	−	+	−	−	+	+
	H/219/50	−	−	+	−	−	+	−	−	−	+
	H/221/54	−	−	+	−	−	+	−	−	+	+
	H/1/70	−	+	+	−	+	+	−	−	−	+
	H/5/70	+	+	+	−	+	+	−	−	−	+
	A/78/60	−	+	+	−	−	+	−	−	+	+
	A/82/60	−	+	+	−	−	+	−	−	+	+
	D/194/78	−	+	+	−	−	+	−	−	+	+
	D/195/79	−	+	+	−	−	+	−	−	+	+
	YU/246/83	−	+	+	−	−	+	−	−	+	+

V.	Essex 70	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–
	NY 70181	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–
	CA 1085/71	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–
	Northants 72	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–
	H/10/72	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–
	H/242/84	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–
	H/133/76	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	+	–	–	–	–	+	–	–	–	–
	H/143/79	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–
	Pa/WA/75	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–
	HR/271/84	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–
	D/372/86	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	+	–	–	–	+	–	–	–	–
VI.a	Warwick 66	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	Iraq AG68	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	Kuwait 256	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	Lebanon 70	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	Israel 70	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	Greece 68	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
VI.b	Pi/IRQ/78	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–	+
	Pi/M/82	–	–	+	+	–	+	–	–	+	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/I/82	–	–	+	+	–	+	–	–	+	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/GB/561/83	–	–	+	+	–	+	–	–	+	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/B/83	–	–	+	+	–	+	–	–	+	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/GB/454/84	–	–	+	+	–	+	–	–	+	–	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/H/83	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	PI/DK/83	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/HR/84	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/H/87	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/J/89	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/H/89	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	–	–	+
	Pi/ET/86	–	–	+	+	–	+	–	–	+	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	+	+	–	–	+
VI.c	HK/4/79	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	HK/109/78	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	H/310/82	–	–	+	+	–	+	–	–	+	–	–	+	+	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	H/386/85	–	–	+	+	–	+	–	–	+	–	–	+	+	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	H/345/86	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	+	+	–	–	–	–	–	–	+	–	–	–	+
	H/514/86	–	–	+	+	–	+	–	–	+	–	–	+	–	–	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	+

[a] Positions of restriction sites for these strains were taken from databank of published sequences

[b] Locations of restriction sites were determined by sequencing (to be published)

1160 which is characteristic of old North American strains (group II) and group I. Another such site seen already in groups I and III is cut by *Bst*OI at nt 953. VIc subgroup is composed of strains from Hong Kong and China isolated in the late 1970s and from Hungary from the early 1980s. The restriction site patterns of the F gene of VIa and VIc strains are very similar, in most cases identical. The distinction of these two subgroups is based on partial sequence comparison of the F genes (to be published) and on the restriction site patterns of the M gene. The M genes of subgroups VIa and VIb when compared by three restriction enzymes that generated 19 mapped cleavage sites were identical, while the cluster of the Hungarian strains (VIc) differed in 2 sites and HK-4 and KH-109 differed in one site from VIa strains (unpubl. results).

Discussion

Toyoda et al. [47] reported on a sequence diversity of 0.5–13% based on the coding region of the F genes of 11 NDV strains. In spite of this relatively high sequence homology substantial variation of the 30 restriction sites generated by three enzymes in the examined region could be observed when physical maps of individual strains were compared. After screening over 200 strains and comparing their physical maps it became evident that in addition to three major genetic groups (A, B and C) that could be constructed from strains isolated before the 1960s [47] at least three additional groups were recognised when more recent isolates were included in the study.

Some of the restriction sites appear to be quite strong markers and can allow identification of particular groups of viruses: e.g. *Rsa*I site at nt 973 is present only in group I strains, while two novel restriction sites, one by *Hinf*I at nt 1063, the other by *Bst*OI at position 1478 are present only in strains isolated after the mid-1960s although the latter may be lost in some recent isolates. However, the real identification power of restriction site analysis lies in the combination of the presence or absence of a set of cutting sites, as detailed in the Results (Table 2). The parallel loss of *Bst*OI site at nt 752 and the presence of *Rsa*I site at nt 1160 delineate North American isolates regardless of their virulence and this suggests a closer evolutionary relationship of these viruses. In strains isolated from psittacines or associated cases of chicken epizootics the loss of *Hinf*I cutting site at nt 883 and that of *Rsa*I site at nt 872 and at nt 1625 combined with the presence of two novel sites of the modern strains were characteristic of group V (Table 2). The chicken panzootic and pigeon PMV-1 viruses (group VI) could be distinguished easily from the above by the gain of *Rsa*I cutting site at nt 1625 and the loss of *Rsa*I site at nt 683, but retaining the characteristic two new restriction site markers of group V. Pigeon PMV-1 strains emerge as a distinct subgroup of group VI and are distinguishable by the loss of *Bst*OI site at nt 1601.

Previously, NDV strains were classified into 10 antigenic groups based on mAb binding patterns [6, 7, 41]. The relationship of groupings based on mAb binding pattern and restriction site fingerprints is summarised in Table 3.

Table 3. Groupings of NDV strains by different methods

Origin of strains	Virulence in chickens [25]	Antigenic groups by mAbs [6, 7, 41]	Lineages HN and F gene sequences [42, 47]	Genetic groups Restriction site (this study)
North America				
prior to the 1960s(?)	L	E	B	II
	M, V	D	B	II
1st panzootic				
(until the 1960s?)				
a) Far East	V	B	C	III
b) Europe	V	B	C	IV
2nd panzootic				
(from the early 1960s)				
a) "Psittacine"	V	A	–	V
b) Chicken	V	B, C1	–	VIa/c
3rd panzootic				
(from the late 1970s)				
Pigeon PMV-1	L, M, (V)	P	–	VIb
Others				
a) Chicken				
F vaccine	L	F	–	II
H vaccine	M	B	–	III
V4, Ulster	L	G	A	I
b) Waterfowl	L	G	A	I
	L	H	–	–
	L	L	–	–
	L	C2	–	–

L Lentogenic; *M* mesogenic; *V* velogenic; – not done

In group I strains from mAb binding group G are represented. Lentogenic strains of waterfowl origin isolated in Hong Kong and China, although displaying some restriction site variability, all fall into group I (to be published). However, lentogenic strains from waterfowl of other geographical area will have to be examined to see if all these belong to the same genetic cluster.

Group II unifies antigenic mAb binding group D (American velogenic and mesogenic strains), E and F (lentogenic vaccines). These antigenic groups, with the exception of strain F and Komarov, contained North American strains. It was reported, that strain Komarov had been obtained by attenuation of a local virulent strain in 1945 in Palestine [31]. Its restriction site fingerprint is identical with that of strain Roakin that is a mesogenic field isolate from 1946 in the United States [11]. Strain F was isolated in England [9]. It is distinct from the vaccinal lentogenic strains (LaSota and B1). H/5L/70, a lentogenic strain that was isolated from post-vaccinal respiratory conditions was identical with strain LaSota. The regrouping of different mAb binding groups into a single category,

however, does not mean that restriction site analysis has less resolving power, because lentogenic vaccine strains LaSota and B_1 can be easily distinguished from the remainder of the group by *Hinf*I cutting site at nt position 1603 and the two vaccine strains can be separated from each other by *Cfo*I (unpubl.).

The mesogenic strain H is closest to group III and differs considerably from strain Herts 33. If any relationship of strain H is excepted it is to strain Herts 33, because it was reported that strain H had been attenuated by passaging Herts 33 in eggs [27]. However both the restriction site pattern and F gene sequences (to be published) of strain H and Herts 33 (F gene sequences from [47]) are quite distinct, making progenitor-offspring relationship unlikely. It is to be seen if the true parent candidate for strain H will be found. It is interesting that our strain H is identical with vaccine strain Mukteswar that is also claimed to be derived by egg passage, but in this case from an Indian Ranikhet disease isolate [24]. Strain H was brought to Hungary in 1948 as a vaccine under the designation Hertfordshire (in short H) and it has been maintained in Phylaxia (Budapest) since then.

Groups III and IV include strains that were formerly classified into mAb binding group B [41]. No group specific restriction site pattern could be established for strains that were classified into group IV on the basis of sequence comparison. Viruses of group IV may represent a composite cluster of fairly different strains belonging to the first panzootics. It is to be noted that strains with identical restiction site fingerprint with Herts 33 were circulating as late as in the mid-1980s. Endo et al. [17] also reported on the high stability of H3 haemagglutinin gene of equine influenza viruses and suggested that it was an example of frozen replication.

The 2nd panzootic of VVND (viscerotrop velogenic ND) is thought to have started in the Far East in the early 1960s followed by a series of outbreaks between 1966 and 1970 in Iran, the Middle East, Greece and in Europe [32]. While the origin of the 1970–71 VVND epizootic in the United States was clearly traced to imported exotic birds [49], that of the European epizootics were thought to be the continuation of outbreaks at the Middle East [32]. Russell and Alexander [41] showed, however, that viruses isolated from the 1970–72 epizootic in Great Britain fell into mAb-binding group A just as the American VVNDV strains isolated at the same time from chickens (CA 1085/71) and from imported parrots (New York 70181). Our results have confirmed that Essex 70 (the first isolate of the epizootics in England), New York 70181 and CA 1085/71 are identical, but differ from any of the Middle East isolates. The similarity of American and European isolates is not surprising in the light of the fact that imports of exotic birds from the South America to Europe were taking place at the time [20] and ND outbreaks at quarantine stations were also reported [35]. Restriction site analysis classified Northants 72 (a late isolate of the England epizootics) together with Essex 70 and New York 70181, in spite of the fact that on the basis of mAb-binding Northants 72 was placed into group B rather than A [41]. Based on restriction site patterns, a number of other isolates could be assigned into group V as well: e.g. a West African psittacine virus and strains from Central Europe. Newcastle disease outbreaks were sporadic in Hungary

before 1971 when a dramatic increase of VVND incidence occurred in 1973 and the pre-existing group IV strains were replaced by group V (Table 2). The epizootic was followed by sporadic outbreaks for about ten years and still yielded similar strains (e.g. H/242/84, Table 2). A related strain (HR/247/84) was present in Croatia as well, as late as 1984. Some of the Hungarian isolates of group V were confirmed to belong to mAb-binding group A (D. J. Alexander, pers. comm.) that support the notion that group A binding mAbs recognise genetic group V strains.

Group VI appears to be a heterogeneous group, and based on both partial sequence data (to be published) and restriction site patterns, we have assigned the strains in this group into three subgroups (a, b and c) which reflect epizootiological differences. Subgroups VIa to VIc comprise strains that have been classified into mAb-binding groups B, C1 and P [6]. Subgroup VIa includes an early panzootic strain from England (Warwick 66), Middle East and Greece isolates of 1968–70, including Kuwait 256 that has C1 binding properties in contrast to the above strains that are characterised by group B binding [41]. Pigeon PMV-1 viruses, mAb binding group P [7], have been placed in subgroup VIb. All Middle East viruses have identical restriction site fingerprint and they appear to be the direct progenitor of the pigeon PMV-1 strains, while the VIc subgroup composed of Hungarian isolates from the early 1980s may not be so closely related. It is interesting that this group was not found earlier in Hungary.

The pigeon PMV-1 subgroup (VIb) is also heterogeneous to some extent. It remains to be seen if the genetic variation of the pigeon PMV-1 strains represent a very fast evolution in this different host, or if the recurrence of restriction sites seen in old isolates indicates multiple transition events of more than one strain from the chicken host to the pigeon.

The presence of a group VIa strain (Warwick 66) in England in the sixties shows the association of the epizootics of the Middle East and those that prevailed in England in the mid-1960s. The difference of Warwick 66 (mAb group B binding) from the 1970–1972 strains (group A binding) was also shown by Russell and Alexander [41]. Our studies clearly show that epizootics during the 2nd panzootic were caused by at least two distinct genotypes. More strains have to be examined to see to what extent the Far East epizootics (group VIa) spread in Europe and to find out that what other countries were victims of the "psittacine derived" (group V) strains.

Wherever the introduction of group V strains took place in Europe, the epizootic that broke out in Hungary in the early 1970s must have belonged to the secondary wave of the primary epidemics. The origin and more than ten years delay of the emergence of VIc group in Hungary may reflect both circulation and further evolution of group VIa in the 1970s. The existence of VIc patterns, however, cannot be excluded in other regions: this is supported by the presence of similar strains in Hong Kong and China in 1978–79 that have VIc pattern of the F gene, while their M genes show an intermediate pattern between those of VIa and VIc strains (unpubl. res.). Examination of strains from other pools might reveal the whereabouts of VIc group before the 1980s. It is possible that velogenic

strains isolated in some European countries in the early 1980s and showed group C1 binding [3, 6] may be related to VIc strains in Hungary.

Results presented here confirm the observations [42, 47] on the existence of geographical restriction of the North American strains isolated before the 1960s. The strains of North America that exist in geographical isolation and are showing a relatively moderate degree of sequence diversity have evolved into very different pathotypes including avirulent, intermediate and fully virulent viruses. Further studies are required to elucidate the circumstances or selection forces that allowed this course of evolution to happen under natural conditions. Other cases in which pathogenically diverse viruses emerged from the same genetic group under natural conditions is associated with the evolution of PMV-1 viruses in the pigeon host [5, 16] and with possible mutation of low virulent strains of waterfowls to highly pathogenic one [15].

Strain comparison by restriction site analysis has proved to be a useful technique for grouping NDV strains into distinct categories in which strains share epizootiological relationships or possibly common descent. It is also a valuable tool for the unambiguous identification of individual strains (e.g. vaccines). For the study of phylogenetic relationships, however, sequencing is to be used. A 378 nt long stretch of the F gene appeared to be adequate to unravel genetic relatedness of NDV strains and phylogenetic tree constructed by using the sequences of 24 representative strains reflected a strikingly similar genetic relationship to those proposed on the basis of restriction site distributions (to be published).

Note added in proof

Since submission of this paper, genetic relationships of 33 NDV isolates, based on sequence analysis of portions of F and M genes, were reported [Seal et al. (1995) J Clin Microbiol 33: 2624–2630].

Acknowledgements

Parts of this work was supported by a grant from the National Science Fund, Hungary (OTKA, No. T016345). We thank those who provided NDV strains.

References

1. Albiston HE, Gorrie BV (1942) Newcastle disease in Victoria. Aust Vet J 18: 75–79
2. Alexander DJ (1991) Newcastle disease and other paramyxovirus infections. In: Calnek BW, Barnes HJ, Beard CW, Reid WM, Yoder HW Jr (eds) Diseases of poultry. Iowa State University Press, Ames, pp 496–519
3. Alexander DJ (1995) Newcastle disease in countries of the European Union. Avian Pathol 24: 3–10
4. Alexander DJ, Allan WH (1973) Newcastle disease. The nature of the virus strains. Bull Off Int Epiz 79: 15–26
5. Alexander DJ, Parsons G (1984) Avian paramyxovirus type 1 infections of racing pigeons: 2 pathogenicity experiments in pigeons and chickens. Vet Rec 114: 466–469
6. Alexander DJ, Manvell RJ, Kemp PA, Parsons G, Collins MS, Brockman S, Russell PH, Lister SA (1987) Use of monoclonal antibodies in the characterisation of avian para-

myxovirus type 1 (Newcastle disease virus) isolates submitted to an international reference laboratory. Avian Pathol 16: 553–565

7. Alexander DJ, Russell PH, Parsons G, Abu Elzein EME, Ballouh A, Cernik K, Engstrom B, Fevereiro M, Fleury HJA, Guittet M, Kaleta EF, Kihm U, Kosters J, Lomniczi B, Meister J, Meulemans G, Nerome K, Petek M, Pokomunski S, Polten B, Prip M, Richter R, Saghy E, Samberg Y, Spanoghe L, Tumova B (1985) Antigenic and biological characterisation of avian paramyxovirus type 1 isolates from pigeons – an international collaborative study. Avian Pathol 14: 365–376
8. Alexander DJ, Wilson GWC, Russell PH, Lister SA, Parsons G (1985) Newcastle disease outbreaks in fowl in Great Britain during 1984. Vet Rec 117: 429–434
9. Asplin FD (1952) Immunization against Newcastle disease with a virus of low virulence (Strain F) and the observations on subclinical infection in partially resistant fowls. Vet Rec 64: 245–249
10. Beach JR, Bankowski RA, Quantrup ER (1948) A preliminary report on the modification of avian pneumoencephalitis (Newcastle disease) virus by cultural methods. Cornell Vet 38: 341–357
11. Beaudette JA, Bivins JA, Miller BR (1949) Newcastle disease immunization with live virus. Cornell Vet 39: 302–334
12. Biancifiori F, Fioroni A (1983) An occurrence of Newcastle disease in pigeons: virological and serological studies on the isolates. Comp Immunol Microbiol Infect Dis 6: 247–252
13. Boney WA (1951) The isolation of a neurotropic strain (GB) of Newcastle disease virus. SW Vet 5: 19–21
14. Chambers P, Millar NS, Emmerson PT (1986) Nucleotide sequence of the gene encoding the fusion glycoprotein of Newcastle disease virus. J Gen Virol 67: 2685–2694
15. Collins MS, Bashiruddin JB, Alexander DJ (1993) Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. Arch Virol 128: 363–370
16. Collins MS, Strong I, Alexander DJ (1994) Evaluation of the molecular basis of pathogenicity of the variant Newcastle disease viruses termed "pigeon PMV-1 viruses". Arch Virol 134: 403–411
17. Endo ///A, Pecoraro R, Sugita S, Nerome K (1992) Evolutionary pattern of the H3 haemagglutinin of equine influenza virus: multiple evolutionary lineages and frozen replication. Arch Virol 123: 73–87
18. Espion D, De Henau S, Letellier C, Wemers CD, Brasseur R, Young JF, Gross M, Rosenberg M, Meulemans G, Burny A (1987) Expression at the cell surface of native fusion protein of the Newcastle disease virus (NDV) strain Italien from cloned cDNA. Arch Virol 95: 79–95
19. Fenner F (1976) Classification and nomenclature of viruses. Intervirology 6: 1–12
20. Francis DV (1974) South American parrots and VVND. Proc 8th California Poult Hlth Symp Davis CA
21. Goldhaft TM (1980) Historical note on the origin of the LaSota strain of Newcastle disease virus. Avian Dis 24: 297–301
22. Granoff A (1959) Studies on mixed infection with Newcastle disease virus. I. Isolation of Newcastle disease virus mutants and tests for genetic recombination between them. Virology 9: 636–648
23. Gustafson DP, Moses HE (1951) Isolation of Newcastle disease virus from the eye of a human being. JAMA 118: 1–2
24. Haddow JR, Idnani JA (1946) Vaccination against Newcastle (Ranikhet) disease. Ind J Vet Sci 16: 45–53

25. Hanson RP (1980) Newcastle disease. In: Hitchner SB, Domermuth CH, Purchase HG, Williams JE (eds) Isolation and identification of avian pathogens. American Association of Avian Pathology, Kennett Square, pp 63–66a
26. Hitchner SB, Johnson EP (1948) A virus of low virulence for immunizing fowls against Newcastle disease (Avian pneumoencephalitis). Vet Med 79: 525–530
27. Iyer SG, Dobson N (1940) A successful method of immunization against Newcastle disease of fowls. Vet Rec 52: 889–894
28. Kaleta EF, Alexander DJ, Russell PH (1985) The first isolation of the PMV-1 virus responsible for the current panzootic in pigeons? Avian Pathol 14: 553–557
29. Kawashima H, Sato T, Hanaki T (1953) The latest outbreak of ND in Japan. Bull Natl Inst Anim Health 27: 151–167
30. Kissi Bachir, Erdei Judit, Erdei J, Palya V, Sághy E, Meder M, Tanyi J, Lomniczi B (1988) Virulence and identification by monoclonal antibodies of pigeon paramyxovirus-1 strains isolated in Hungary. Magyar Áo Lapja 43: 357–360
31. Komarov A, Goldsmit L (1946) Preliminary observation on the modification of a strain of Newcastle disease virus by intracerebral passage through ducklings. Vet J 102: 212–218
32. Lancaster JE (1976) A history of Newcastle Disease with comments on its economic effects. Wlds Poultry Sci 32: 167–175
33. Lomniczi B (1973) Studies on interferon production and interferon sensitivity of different strains of Newcastle disease. J Gen Virol 21: 305–313
34. Lomniczi B (1975) Thermostability of Newcastle disease virus strains of different virulence. Arch Virol 47: 249–255
35. Lüthgen W, Wachendöfer G (1970) Newcastle-disease bei frisch importierten grosspapageien (vorläufige mitteilung). Dtsch Tieraerztl Wochenschr 77: 407–408
36. Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New York
37. McFerran JB, Nelson R (1971) Some properties of an avirulent Newcastle disease virus. Arch Ges Virusforsch 34: 64–74
38. McGinnes LW, Morrison TG (1986) Nucleotide sequence of the gene encoding the Newcastle disease virus fusion protein and comparisons of paramyxovirus fusion protein sequences. Virus Res 5: 343–356
39. Millar NS, Chambers P, Emmerson PT (1988) Nucleotide sequence of the fusion and haemagglutinin-neuraminidase glycoprotein genes of Newcastle disease virus strain Ulster: molecular basis for variations in pathogenicity between strains. J Gen Virol 69: 613–620
40. Nagai Y, Yoshida T, Hamaguchi M, Naruse H, Inuma M, Maeno K (1980) The pathogenicity of Newcastle disease virus isolated from migrating and domestic ducks and the susceptibility of the viral glycoproteins to proteolytic cleavage. Microbiol Immunol 24: 173–177
41. Russell PH, Alexander DJ (1983) Antigenic variation of Newcastle disease virus strains detected by monoclonal antibodies. Arch Virol 75: 243–253
42. Sakaguchi T, Toyoda T, Gotoh B, Inocenico NM, Kuma K, Miyata T, Nagai Y (1989) Newcastle disease virus evolution. I. Multiple lineages defined by sequence variability of the hemagglutinin-neuraminidase gene. Virology 169: 260–272
43. Sato H, Oh-Hira M, Ishida N, Inamura Y, Hattori S, Kawakita M (1987) Molecular cloning and sequence of P, M and F genes of Newcastle disease virus avirulent strain D26. Virus Res 7: 241–255
44. Schaper UM, Fuller FJ, Ward MDW, Mehrotra Y, Stone HO, Stripp BR, De Buysscher E (1988) Nucleotide sequence of the envelope protein genes of a highly virulent, neurotropic strain of Newcastle disease virus. Virology 165: 291–295

45. Simmons GC (1967) The isolation of Newcastle disease virus in Queensland. Aust Vet J 43: 29–30
46. Toyoda T, Sakaguchi T, Imai K, Inocenco NM, Gotoh B, Hamaguchi M, Nagai Y (1987) Structural comparison of the cleavage-activation site of the fusion glycoprotein between virulent and avirulent strains of Newcastle disease virus. Virology 158: 242–247
47. Toyoda T, Sakaguchi T, Hirota H, Gotoh B, Kuma K, Miyata T, Nagai Y (1989) Newcastle disease evolution. II. Lack of gene recombination in generating virulent and avirulent strains. Virology 169: 273–282
48. Vilček S, Elvander M, Ballagi-Pordány A, Belák S (1994) Development of nested PCR assays for detection of bovine respiratory syncytial virus in clinical samples. J Clin Microbiol 32: 2225–2231
49. Walker JW, Heron BR, Mixton MA (1973) Exotic Newcastle disease eradication program in the United States. Avian Dis 17: 486–503

Authors' address: Dr. B. Lomniczi, Veterinary Medical Research Institute, P.O. Box 18, H-1581 Budapest, Hungary.

Received June 20, 1995