Comparison of Newcastle Disease Viruses Isolated from Cormorants in Canada and the USA in 1975, 1990 and 1992

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ABSTRACT

Seventeen Newcastle disease virus (NDV) isolates obtained from cormorants, turkeys, a pelican, and a gull in Canada and the USA collected in 1975, 1990 and 1992 were analyzed for relatedness by monoclonal antibody profiling. In addition, nucleotide sequence analysis was performed in two areas of the fusion (F) gene for 5 of the isolates. No difference in the antigenicity of these 17 viruses, as determined by monoclonal antibody binding patterns, was seen. The amino acid sequences obtained via nucleotide sequencing at the cleavage site of the F protein showed that all the isolates tested had two pairs of basic amino acids immediately upstream of the cleavage site, and a phenylalanine residue at the N-terminus of the F1 protein, which is consistent with velogenic NDV. The deduced amino acid sequence obtained at the cleavage site of the F protein from 6 of the isolates was virtually identical regardless of the species, year of isolation, or location. However, the 1975 cormorant isolate showed marked differences from the 1990–1992 isolates in the nucleotide and deduced amino acid sequence of the F gene signal region. These data indicate that the 1990 and 1992 outbreaks were caused by the same epizootic virus and further suggest that the population of NDV in these wild birds may be very stable. The belief that the velogenic NDV circulating in cormorants in 1992 was transmitted into the free-ranging turkey flocks located near the cormorants in North Dakota is supported by the present study in which no distinction could be made between the viruses isolated from turkeys or wild birds.

RÉSUMÉ

Le degré de similitude entre 17 isolats du virus de la maladie de Newcastle (VN) provenant du Canada et des États-Unis isolés de cormorans, de dindes, d'un pélican et d'un goéland lors des années 1975, 1990 et 1992 a été évalué par leur réactivité à des anticorps monoclonaux. Une analyse de la séquence nucléotidique dans deux régions du gène de fusion (F) a été effectuée sur 5 isolats. Aucune différence au niveau antigénique n'a été détectée entre les 17 isolats tel que déterminé par le profil de réaction avec les anticorps monoclonaux. Le séquençage nucléotidique a permis d'établir qu'au site de clivage de la protéine F, tous les isolats analysés avaient deux paires d'acides aminés basiques immédiatement en amont du site et un résidu phénylalanine du côté N-terminal de la protéine F1, ceci étant cohérent avec les caractéristiques des VN de type vélogénique. La séquence d'acides aminés au site de clivage de la protéine F, déduite à partir du séquençage nucléotidique, était pratiquement identique pour 6 isolats sans égard à l'espèce, année d'isolement ou provenance géographique. Toutefois, l'isolat de 1975 provenant d'un cormoran a montré des différences marquées avec un isolat de 1990–1992 quant à sa séquence nucléotidique et à la séquence d'acides aminés déduite dans la région signal du gène F. Ces résultats indiquent que les épidémies de 1990 et 1992 étaient dues à un même virus et que la population de VN était très stable chez les oiseaux sauvages. Les résultats de la présente étude viennent supporter l'hypothèse qu'en 1992, au Dakota du Nord, le VN vélogénique retrouvé chez les cormorans fut transmis aux élevages avoisinants de dinde.

(Traduit par docteur Serge Messier)

INTRODUCTION

Newcastle disease (ND) viruses form the avian paramyxovirus type 1 (PMV-1) serogroup in the genus *Paramyxovirus*, family *Paramyxoviridae* (1). Avian paramyxoviruses have been isolated from wild, captive, caged, and domestic birds; and the distribution of these viruses appears to be world-wide (2,3). Disease severity may vary from inapparent infection to death, depending mainly on the virus, but also on the host and environmental conditions. Because of the variable nature of ND viruses, a number of in vitro tests have been developed for strain characterization and identification. Panels of monoclonal antibodies (mAbs) raised to various strains of Newcastle disease virus (NDV) have been used for antigenic differentiation and strain characterization (4,5,6), which have proven useful in tracing epizootics of NDV. In addition, sequencing of viral genome regions is beginning to play a role in viral epidemiology and virulence determination (7,8).

The virulence of NDV is mainly related to the number of basic amino acids present at the cleavage site of the fusion (F) gene (9,10,11), although, for

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some viruses, this may also be modified by the requirement for cleavage of the hemagglutinin-neuraminidase precursor protein (12,13). In addition, other as yet undetermined factors may also play a role in determination of NDV virulence (9).

Evidence of infection by NDV in wild birds has been well documented (2). Isolation of Newcastle disease virus in the great cormorant (*Phalacrocorax carbo*), the European shag (*Phalacrocorax aristotelis*), and the double-crested cormorant (*Phalacrocorax auritus*) has been reported several times in different parts of the world (14–19). In North America, there have been outbreaks of NDV in cormorants in Quebec 1975 (20), in Alberta, Manitoba, and Saskatchewan 1990 (19), and in several provinces of Canada and several states in the USA in 1992 (14,15,18). In many cases, high mortality was observed in young birds in rookeries. In addition to mortality, live birds were observed exhibiting unilateral wing and or leg paralysis and other abnormal neurological signs.

We report here an evaluation of the relatedness of some of these viruses collected over a 17-year period from a wide geographical area. The similarity of the viruses was compared antigenically by typing with a panel of mAbs and at the level of the deduced amino acid sequence through determination of the primary structure of portions of the F gene.

MATERIALS AND METHODS

VIRUSES AND CHARACTERIZATION

The viruses (n = 17) were collected from birds in several different locations in Canada and the USA from 1975 to 1992 (Table I). The isolates examined were obtained from the provinces of Saskatchewan, Ontario, and Quebec and from the states of Michigan, North Dakota, New York, and Minnesota. Most of the viruses were isolated from cormorants, but there were also isolates from a white pelican (*Pelecanus erythrorhynchos*) and a ring-billed gull (*Larus delawarensis*), which were located near the cormorants experiencing mortality in 1990. In addition, two isolates from turkeys suspected to be infected with the cormorant NDV were included (14). All of the viruses evaluated had been sent to the International Reference Laboratory for Newcastle Disease, Central Veterinary Laboratory, Weybridge, UK, where they were propagated in 9-to 10-day-old embryonating fowl eggs and confirmed to be NDV by hemagglutination inhibition tests with reference polyclonal chicken antisera. The isolates were inoculated into 6-week-old chickens to determine the intravenous pathogenicity indices (IVPI) and into 1-day-old chicks to determine the intracerebral pathogenicity indices (ICPI) (21). NDVs representative of both pathogenic (chicken/GB Texas/48) and nonpathogenic (Hitchner B1/45) biotypes were also included in this study as reference viruses.

TABLE I. Summary of the virus isolates, country of isolation, and pathogenicity

Sample #	PMV-1 isolates	ICPI[a]	IVPI[b]
1	cormorant/Quebec-Canada/457/75	1.72	2.20
2	cormorant/Saskatchewan-Canada/1288/90	1.51	2.19
3	cormorant/Saskatchewan-Canada/1479/90	1.55	2.08
4	cormorant/Saskatchewan-Canada/1480/90	1.55	2.37
5	gull/Saskatchewan-Canada/1477/90	1.51	1.93
6	pelican/Saskatchewan-Canada/1478/90	1.65	1.98
7	cormorant/Ontario-Canada/2175/92	1.22	1.77
8	cormorant/Ontario-Canada/2330/92	1.42	0.93
9	cormorant/Saskatchewan-Canada/2583/92	1.44	0.97
10	cormorant/Saskatchewan-Canada/2585/92	1.39	1.66
11	cormorant/Ontario-Canada/2603/92	1.55	1.67
12	cormorant/Saskatchewan-Canada/2652/92	1.40	1.92
13	cormorant/Michigan-USA/40068/92	1.51	1.83
14	cormorant/Minnesota-USA/40140/92	1.35	1.66
15	cormorant/New York-USA/51234-1/92	1.46	1.80
16	turkey/North Dakota-USA/43084/92	1.55	1.57
17	turkey/North Dakota-USA/48032-2/92	1.07	0.36
18	chicken/GB Texas/48	1.75	2.66
19	Hitchner B1/45	0.25	0.00

[a] ICPI: intracerebral pathogenicity index in 1-day-old chicks; indices represent the mean score of 10 birds per observation; maximum index 2.00 = all birds dead within 24 h, minimum index 0.00 = no clinical signs in any bird over 8 d

[b] IVPI: intravenous pathogenicity index in 6-week-old birds; indices represent the mean score of 10 birds per observation; maximum index 3.00 = all birds dead in 24 h, minimum index 0.00 = no clinical signs in any bird over 10 d

MONOCLONAL ANTIBODY TYPING

All of the viruses were propagated in VERO cells (African green monkey kidney cell line) and assessed for their ability to bind a panel of 28 mAbs in an indirect immunoperoxidase assay as previously described (5). The assay was conducted twice, and in some cases three or more times, where there was discrepancy between the first reading and the second. Binding of the monoclonal antibodies to the infected cells was scored as either positive, negative, or indeterminate if binding was seen but the staining pattern was not consistent with that mAb.

NUCLEIC ACID SEQUENCE ANALYSES

Nucleic acid sequence analyses was done on six of the viruses using procedures similar to those described by Collins et al (7,9). For the reverse transcription step, the forward primer (5' TTAGAAAAAACACGGGTAGAA 3') was used for all the viruses. For the polymerase chain reaction (PCR), the reverse primer (5' AGTCGGAGGATGTTGGCAGC 3') was also included. A PCR product of approximately 500 bases was obtained in each case, which included the areas corresponding to both the signal sequence and the F0 cleavage site. Sequencing of the PCR product was done using the ΔTaq cycle sequencing kit (United States Biochemical, Cleveland, Ohio). Two primers were used: one of negative sense (5' GCTTTATCTCCTGTTACCACAAT 3') for sequencing across the signal sequence region; the other (5' CAGAACACTGACCACTTTACTCAC 3') for sequencing across the F0 cleavage site of the gene.

RESULTS

VIRUS PATHOGENICITY

The values obtained in IVPI and ICPI for the isolates in this study are presented in Table I. As shown, considerable variation could be seen

TABLE II. Binding profiles of 28 monoclonal antibodies with various Newcastle disease viruses isolated from wild water birds and domestic turkeys

	Monoclonal antibodies																										
Isolate #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26[b]	27	28
1–17	+[a]	+	−	+	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	−?	+	−
18	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	N	−	+	N
19	+	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	N	−	+	N

[a] Binding was scored as positive (+), negative (−), or indeterminate (?). N = Not determined. The mAbs used were: 1–6 are 14, 424, 445, 479, 481, and 688 prepared against NDV Ulster 2C (Russell, 1983); 7–20 are U11, U23, U32, U45, U48, U49, U55, U57, U67, U68, U69, U70, U79, and U85 prepared against NDV Ulster 2C (Collins and Alexander, unpublished observations); 21–27 are 3/617, 38/617, 43/617, 54/617, 83/617, 161/617, and 165/617 prepared against isolated PMV-1/pigeon/England/617/83 (Collins, 1989); 28 is 9A raised against NDV Hertz 33 (Collins and Alexander, unpublished observations)

[b] Four isolates (#1, 13, 14, 16) gave indeterminate results with monoclonal antibody #26

TABLE III. Comparison of the deduced amino acid sequences at the cleavage site and at the signal region of the fusion gene for selected isolates

Isolate	Fusion gene cleavage site amino acid sequence	Fusion gene signal region amino acid sequence
cormorant/Quebec-Canada/457/75	SGGRRQKR*FVGA	MGPKPSTRISAPLMLITRTMLILSCICL
gull/Saskatchewan-Canada/1477/90	R *	P GTP V A A V G V P
cormorant/Ontario-Canada/2330/92	R *	P GTP V A A V G V P
cormorant/Minnesota-USA/40140/92	R *	P GTP V A A V G V P
turkey/North Dakota-USA/43084/92	R *	P GTP V A A V G V P
turkey/North Dakota-USA/48032-2/92	R *	P GTP V A A V G V P
chicken/GB Texas/48	* I	R NPT M TV VA V P
Hitchner B1/45	G G *LI	SR P M TI VA V P

* = cleavage site of the fusion protein
Only differences from the 457/75 isolate are shown

among the isolates, with a range in ICPI values of 1.07 to 1.72 and a range in IVPI values of 0.36 to 2.37.

MONOCLONAL ANTIBODY BINDING PROFILES

The results of the mAb binding patterns of the different virus isolates with the 28 mAbs are shown in Table II. This panel of mAbs was used because previous work demonstrated this panel to be very useful in differentiating a wide variety of NDV isolates (Alexander, personal communication). In this study, there were no differences seen among the isolates as determined by any of the reactions with the mAbs, except for 161/617 (#26) which showed some uncharacteristic binding with some isolates. However, the patterns obtained were quite different from those seen with GB Texas and Hitchner B1 NDV strains, which were included as typical examples of a neurotropic velogenic strain and a live vaccine strain, respectively.

SEQUENCE ANALYSES

The results of the sequence analyses of six of the viruses are shown in Table III. The deduced amino acid sequence obtained for the 12 residues at the cleavage site of the F protein was identical for the five viruses that were isolated in 1990 and 1992. The 1975 cormorant isolate showed variation in that there was a glycine substituted for an arginine at residue −7 from the cleavage site. The published sequences for Texas GB and Hitchner B1 show that these viruses also have glycine in this position and isoleucine for valine at residue +2 from the cleavage site (22). The amino acid sequence of the lentogenic Hitchner B1 virus showed further differences related to the requirement of basic amino acids at −2 and −5 and phenylalanine at +1, characteristic for virulent viruses.

A deduced sequence of 28 amino acids at the highly variable F protein signal region was obtained for each of the six viruses examined. The five isolates obtained during 1990–1992 were identical in this region regardless of the original host (1 × gull, 2 × turkey, 2 × cormorant), year (1 × 1990, 4 × 1992), or country (2 × Canada, 3 × USA). In marked contrast, the 1975 cormorant isolate showed considerable differences from this sequence with 11/28 different amino acids. The 1990 and 1992 isolates differed from the published signal sequences for GB Texas and Hitchner B1 in 12/28 amino acid residues, and these two viruses differed by 11/28 and 10/28 amino acids, respectively, when compared with the 1975 isolate. In contrast, the signal sequences of Texas GB and Hitchner B1 viruses differed by only 4/28 amino acid residues.

DISCUSSION

Analyses of several NDV isolates from Canada and the USA collected in 1975, 1990, and 1992 were done by mAb profiling and by nucleotide sequence analyses of two areas of the fusion gene. No differences in the antigenicity of these viruses, as determined by monoclonal antibody binding patterns, were seen (Table II). The mAb binding pattern observed for the isolates in this study was different from that of a neurotropic velogenic NDV (GB Texas) and from that of a typical vaccine virus (Hitchner B1).

The finding that there was little difference upon sequence analysis, and no difference in mAb binding patterns, among the isolates obtained in 1990 and 1992 and from different geographical locations, indicates that

the outbreaks were caused by related viruses. It further suggests that the population of NDV in these wild birds may be very stable. With no vaccination pressure and a new susceptible chick population each year, this finding is not unexpected. Similarly, it is not surprising that there were no differences found among the isolates obtained from the different geographical areas. The Mississippi flyway branches out over the states and provinces involved, and it is likely that the migratory birds shared common overwintering sites in the south and would therefore have been infected with a similar virus population.

Despite an identical monoclonal antibody binding profile and only one amino acid difference in the 12 deduced amino acids at the cleavage site, the 1975 cormorant isolate showed marked differences from the 1990–1992 isolates in the deduced amino acid sequence of the F gene signal region. From the available data (22), the F gene signal sequence appears to be a highly variable region. This region was therefore chosen for this study, since analysis of this region may show small phylogenetic differences, whereas analysis of the entire fusion gene sequence may show no or little sequence difference for different NDV strains (Collins, unpublished observations). The differences between the 1990/1992 cormorant isolates and the 1975 isolate may be due to: a) an accumulation of random point mutations while the virus was maintained in the cormorant population; b) introductions of two separate viruses from an unknown source 15 years apart; c) the existence of more than one NDV species circulating in the cormorant population; or d) the viruses coming from two different populations or subspecies of cormorants (those that use the Mississippi flyway and those that use the east coast flyway).

The amino acid sequences obtained at the cleavage site of the F protein showed that all isolates had two pairs of basic amino acids upstream of the cleavage site and a phenylalanine residue at the N-terminus of the F1 protein. This is consistent with viruses that are velogenic for chickens (7). However, the pathogenicity indices obtained, particularly the IVPI values, varied between the isolates and were often much lower than would be expected for fully velogenic viruses (21). This was particularly evident with the turkey isolate 48032-2/92, which was identical in sequence and mAb binding pattern to the other turkey isolate, but had markedly lower ICPI and IVPI values. This phenomenon has been recorded before, both for isolates of the panzootic NDV in racing pigeons (9) and for isolates of the velogenic virus obtained from falcons (23). It has been demonstrated for the pigeon panzootic virus that passage through chickens increases the pathogenicity indices obtained (24). Collins et al (9) have suggested that viruses isolated from nonpoultry hosts may require some host adaptation to chickens before they express their full virulence. However, it is worth recording that all of these isolates would be considered velogenic under the regulations for the control of ND in European Union countries and Canada, which define ND, for which the control measures will be enforced, as infection with a virus which has an ICPI of greater than 0.7 (25). Therefore, by all definitions, the isolates from these wild birds were pathogenic and constituted a threat to domestic poultry populations.

The possibility that the velogenic NDV circulating in cormorants in 1992 was transmitted into the free-ranging turkey flocks located near the cormorants in North Dakota is supported by the present study in which no distinction could be made between the viruses isolated from turkeys or wild birds. This emphasises the danger of rearing commercial birds in conditions that allow wild birds to mix freely with domestic poultry.

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