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ACQUISITION OF PATHOGENICITY OF A NEWCASTLE DISEASE VIRUS ISOLATED FROM A JAPANESE QUAIL BY INTRACEREBRAL PASSAGE IN CHICKENS

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ABSTRACT

Newcastle disease virus (NDV) was isolated from a Japanese quail (*Cotornix cotornix japonica*). The effect of intracerebral and intranasal passages of the NDV in chickens on the pathogenicity was studied. Pathogenicity of the viruses of different passage levels was compared with that of the original isolate by the mean death time with the minimum lethal dose in chicken embryos, intracerebral pathogenicity index in day-old chicks, intravenous pathogenicity index with 6-week-old chickens and the mortality rates of chickens and quails inoculated intravenously or intranasally. The original isolate from the quail did not kill chickens but only embryos and some one-day-old chicks, exhibiting a mesogenic character. Pathogenicity of the virus of the 10th intranasal passage was not different from that of the original isolate. The viruses passaged intracerebrally, on the other hand, killed chickens of all ages by either route of inoculation, showing a velogenic property. Virus recovery from the blood and the brain was positive only in the chickens infected with brain-passaged viruses by any route of inoculation. Virus titers in the tissues of chickens infected with the brain-passaged viruses were higher than those with the original isolate and the virus of the 10th intranasal passage. These results indicate that the enhanced pathogenicity of the mesogenic NDV isolate from the quail for chickens was induced by acquiring the properties of neurotropism and pantropism through intracerebral passage in chickens.

Key Words: Pathogenicity, NDV, Intracerebral passage, Quail, Chicken

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INTRODUCTION

Newcastle disease is one of the most severe viral diseases of poultry because of its rapid spreading, high contagiousness and high lethality for chickens. NDV is an enveloped single-stranded RNA virus belonging to the Paramyxoviridae family[7,18]. Large numbers of NDVs have been isolated from different species of birds all over the world[3,4,8,13,18,19,21,22,23]. Strains of NDV are indistinguishable morphologically and serologically but different in their pathogenicity for chickens[22,23]. NDV strains are classified into velogenic, mesogenic and lentogenic types based on the mean death time (MDT) of chicken embryos, intracerebral pathogenicity index (ICPI) with day-old chicks and intravenous pathogenicity index (IVPI) with 6-week-old chickens[6]. Pathogenicity of NDV strains varies greatly with host species. Generally, the domestic chicken (*Gallus domisticus*) is a host bird that is highly susceptible to NDV. Other domestic and semidomestic species of birds, i. e., turkeys, quails, pigeons, ducks and geese are less susceptible to NDV infection, hence acting as asymptomatic carriers[11]. Although Japanese quails are rather resistant to NDV, they become infected under stress conditions[10,11]. However, little is known about the reason why the pathogenicity of NDV varies greatly with the host avian species. In the present study, to provide information on the possible factors involved in the variation of pathogenicity of NDV, an isolate from the quail was passaged in chickens through intracerebral or intranasal routes and the effects of passage through chickens on its pathogenicity were examined.

MATERIALS AND METHODS

Viruses: The original virus (Q0) was isolated from a dead Japanese quail (*Cotornix cotornix japonica*) of strain LWC and identified as NDV according to the method of Kida et al.[14] The CC10 and CN10 viruses were obtained after 10 serial intracerebral and intranasal passages of Q0, respectively, in six-week-old chickens. Intracerebral and intranasal serial passages in chickens were done with 10% brain and lung tissue extracts[13] of infected birds every three days according to the technique of Komarov et al.[17] Reference NDV strains used were Miyadera, Sato, Ulster, Italien, Ishii, Herts, Komarov, TCND and La Sota. Allantoic fluids of 10-day-old chicken embryos infected with each virus were used as the inoculum.

Cells and Media: Madin Darby bovine kidney (MDBK) cells were grown in Eagle's minimum essential medium (Nissui) supplemented with 10% calf serum and 200 U/ml penicillin, 200 mg/ml streptomycin and 40 mg/ml gentamycin.

Eggs and birds: Quails and fertile eggs of white leghorn chickens were purchased from commercial farms. Chickens were hatched and raised exclusively in our laboratory.

Pathogenicity tests: The mean death time with the minimum lethal dose (MDT/MLD) was determined with 9-day-old chicken embryos as described by Hanson et al.[9]

For this test, 0.1 ml of each of the 3 highest virus dilutions (10^{-6}–10^{-8}) was inoculated into the allantoic cavities of eight 9-day-old chicken embryonated eggs, four of the embryos receiving the inoculum in the morning and the other 4 in the afternoon. The mortality of the embryo was observed by candling the eggs at 8- and 16-hour intervals up to 72 hours. The MDT value was calculated based on the data at the highest dilution in which all of the embryos had died. The intracerebral pathogenicity index (ICPI) with day-old chicks and intravenous pathogenicity index (IVPI) with six-week-old chickens were determined according to Allan et al.[6] For the ICPI test, 0.1 ml of undiluted virus with approximately 10^9EID_{50} was inoculated into eight one-day-old chicks intracerebrally. The chicks were observed daily until death or for 8 days. The ICPI index was calculated by scoring each chick daily (0, normal; 1, diseased; 2, dead), and the resulting sum was divided by the number of birds observed. In the IVPI test, 0.1 ml of undiluted virus was inoculated into each of 8 chickens six weeks old. The birds were observed daily until death or for 10 days. Each bird was scored daily (0, normal; 1, sick; 2, paralysed; 3, dead). The sum of the score was divided by the number of birds observed.

Experimental infection of chickens and quails: To compare the virulence of the passaged viruses with that of Q0, three sets of experiments were performed using six-week-old chickens and quails using intravenous, intranasal and intracerebral routes of inoculation. For intranasal inoculation, 0.2 ml of virus was dropped into both nostrils of each bird. For intravenous and intracerebral inoculation, 0.1 ml of the infectious allantoic fluid of 10^9EID_{50} was given to each bird.

Virus recovery from the tissues of birds: From each of the experimental groups, two chickens were sacrificed by cardiac puncture at 12- and 24-hour intervals up to day 6 post inoculation (p. i.) and tissue samples were collected aseptically. Titration of the virus from the tissue was done by plaque assay in MDBK cells as by Raming[20].

Serological tests: Enzyme-linked immunosorbent assay (ELISA) was done according to Kida et al.[12] using monoclonal antibodies against the HN and F proteins of NDV, which had been established in our laboratory[1,2]. Identification of Q0 was done by immunodiffusion test according to Kida et al.[14]

Results

Isolation and characterization of a Newcastle disease virus from the quail: Four groups of the LWC strain of SPF Japanese quail (*Cotornix cotornix japonica*) (Nippon Institute for Biological Science, Yamanashi, Japan) consisting of 12–59 birds were raised in the laboratory of the Department of Comparative Pathology, School of Veterinary Medicine, Hokkaido University, Japan. Groups of 50–100 Japanese quails were purchased every 3 weeks from a commercial farm and kept for use in experimental studies as control birds in the same room. On December 5th 1991, one of the SPF quails showed respiratory sings, loss of appetite and depression, and other

birds were affected one by one in the group. The number of diseased birds also increased gradually in the other groups. Diseased birds started dying 2 weeks after the first onset. On the other hand, the control quails, which were considered to play a role in the introduction of NDV into SPF quails, were apparently healthy. At the beginning of February, approximately half of the birds of one group died. On February 21, 1992 the remaining birds were sacrificed. A paramyxovirus was isolated from the tracheal sample of one of the dead quails. Antigenicity of the isolate was compared with that of the reference strains of NDV. The isolate was identified as NDV but was antigenically different from the reference strains tested (Table 1). The results of pathogenicity tests indicated that the isolate was mesogenic type of NDV (Table 2). The isolate was also inoculated into chickens through the intranasal or intravenous route (Table 2). The results showed that the isolate was not virulent for chickens.

Comparison of pathogenicity of viruses passaged through chickens with the original isolate from the quail: The original isolate from the quail (Q0) killed chicken embryos and day-old chicks, but did not kill chickens older than 6 weeks (Table 2). To investigate the effect of passaging through chickens on the pathogenicity of the mesogenic strain of NDV, Q0 was passaged in chickens intracerebrally or intranasally. Pathogenicity of the viruses passaged intracerebrally in chickens (CC1–10) and the

Table 1. Antigenic analysis of NDV strains with monoclonal antibodies to the glycoproteins

Virus	Antigenic site on HN													Antigenic site on F			
	I	II												I		II	III
	388/2	301/1	397/1	500/1	705/1	815/1	410/1	333/1	289/1	38/1	98/1	343/1	284/1	320/1	743/1	70/1	59/1
Q0	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	−
CC10	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	−
Miyadera	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+
Sato	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Herts	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
Ulster	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Italien	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
Ishii	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Komarov	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
TCND	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
La Sota	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+

− indicates no binding of the monoclonal antibodies at the dilution of 1 : 400 with the antigens in ELISA.

+ indicates binding to the viral antigens at more than 1 : 6400 dilution of the antibodies.

Table 2. Comparison of NDV-strain pathogenicity for chicken embryos, chickens and quails

Virus[a]	Pathogenicity				Mortality rates (%) of 6-week-old chickens by route of inoculation		Mortality rates (%) of 6-week-old quails by route of inoculation	
	MDT/MLD (Hours)	ICPI	IVPI/C[b]	IVPI/Q[c]	I.V.[d]	I.N[e]	I.V	I.N
Q0	60	0.85	0.00	0.40	0(0/6)	0(0/6)	20(2/10)	10(1/10)
CC1	55	0.87	0.00	NT[f]	NT	NT	NT	NT
CC3	53	1.41	0.58	NT	50(4/8)	0(0/6)	NT	NT
CC5	51	1.52	0.89	NT	82(5/8)	17(1/6)	NT	NT
CC7	50	1.50	1.24	NT	88(7/8)	50(3/6)	NT	NT
CC10	52	1.44	1.24	1.14	88(7/8)	84(5/6)	60(6/10)	20(2/10)
CN10	50	1.24	0.00	NT	0(0/8)	0(0/8)	NT	NT

a) Q0=original isolate from quail, CC1=virus recovered from a chicken inoculated with the isolate intracerebrally, CN10=virus of 10th intranasal passage in chicken, b) indicates intravenous pathogenicity index with chickens, c) indicates intravenous pathogenicity index with quails, d) indicates intravenous route, e) indicates intranasal route, (%) indicates number of dead birds/number of birds treated in each experimental group, f) indicates not tested, MDT/MLD=mean death time of minimum lethal dose, ICPI=intracerebral pathogenicity index ; MDT/MLD, ICPI & IVPI values are shown as the mean of the results of 3 tests ; standard deviation values ranged from 1.0−1.5 for MDT/MLD, 0.01−0.05 for ICPI & 0.01−0.03 for IVPI.

virus of the 10th intranasal passage (CN10) for chicken embryos was examined by measuring MDT/MLD of viruses (Table 2). The results showed that passage of Q0 through chickens enhanced the virulence of the virus for chicken embryos.

To examine *in vivo* pathogenicity of these viruses, each virus was inoculated into chickens intracerebrally or intranasally. The intracerebral pathogenicity index (ICPI), intravenous pathogenicity index (IVPI) and mortality rate for chickens inoculated by intravenous and intranasal routes with each virus are shown in Table 2. With the level of passage, ICPI, IVPI and the mortality rate of chickens with viruses passaged intracerebrally (CC3–10) increased. The pathogenicities of CC7 and CC10 were highly virulent and indistinguishable from each other. ICPI of CN10 was significantly higher than that of Q0 but the IVPI and mortality rates were similar, suggesting that CN10 is not virulent for chickens older than 6 weeks. These results indicate that the virus acquired virulence for chickens by intracerebral passage in the chicken, but that intranasal passage did not affect its pathogenicity for chickens older than 6 weeks. The viruses passaged through chickens were also examined for their pathogenicity for quails (Table 2). The results showed that the IVPI and mortality rate of quails

receiving intravenous inoculation with CC10 were higher than that of Q0. This indicated that intracerebral passage of the mesogenic NDV in chickens also enhanced the pathogenicity for quails.

Comparison of virus recovery from the tissues of chickens infected with Q0 and viruses passaged in chickens : To examine the mechanism of acquisition of pathogenicity of the virus by passaging through chickens, distribution of the virus in the tissues of chickens after intranasal, intravenous or intracerebral inoculation with each virus was examined. The titers of the virus recovered from chicken tissues on days 1–4 p.i. are shown in Tables 3, 4 and 5. In the case of intranasal and intravenous inoculation, on days 3 and 4 p. i., a high titer of the virus was recovered from each of the tissues tested, including the brain and the blood of the chickens infected with CC10. Failure to detect the virus in the brains of chickens inoculated with Q0 by intravenous or intranasal routes indicated that the original isolate from the quail was not neurotropic for adult chickens. Virus recovery from the brains of chickens infected with CC10 by intranasal, intravenous and intracerebral routes indicated that the virus acquired neurotropism. The mortality rate of the chickens was correlated to the titer of the viruses recovered from the tissues of the chickens. The high titers of the virus recovered from most of the tissues of chickens infected with CC10 indicated that it acquired the property of pantropism, too. The virus was not detected in the brain or the blood of the chickens infected with CN10, indicating that 10 intranasal passages of

Table 3. Virus recovery from the tissues of chickens after intranasal inoculation with NDV strains

Virus	day p.i.	Tissue virus titers									
		Sinus	Blood	Brain	Trachea	Lung	Spleen	Colon	Thymus	Bursa	Kidney
Q0	1	4.2	—	—	—	—	—	—	3.0	—	—
	2	4.2	—	—	—	3.5	—	3.4	4.1	5.0	—
	3	3.8	—	—	4.2	5.1	4.0	3.7	3.6	5.1	4.9
	4	3.5	—	—	4.0	5.0	3.7	3.8	3.8	5.1	3.9
CC10	1	4.3	—	—	—	—	—	—	3.1	—	—
	2	4.3	—	—	3.6	3.4	3.7	3.8	5.0	5.0	—
	3	5.3	2.6	—	4.2	4.2	4.2	3.6	5.1	5.1	5.0
	4	5.3	3.4	3.2	5.1	5.1	3.9	4.0	4.1	5.1	5.0
CN10	1	4.3	—	—	3.9	4.1	—	—	3.0	3.0	—
	2	4.3	—	—	4.0	4.1	—	—	4.1	3.0	—
	3	4.1	—	—	4.1	4.1	3.3	3.1	5.1	3.4	3.5
	4	4.0	—	—	4.1	4.0	4.1	3.1	5.2	3.7	3.7

Titers are expressed as $\log_{10}$p.f.u./g tissues ; — indicates < 2.30 ; virus titers are the mean values of two chickens.

Table 4. Virus recovery from the tissues of chickens after intravenous inoculation with NDV strains

Virus	day p.i.	Tissue virus titers								
		Blood	Brain	Trachea	Lung	Spleen	Colon	Thymus	Bursa	Kidney
Q0	1	—	—	3.1	3.4	3.7	3.2	4.0	5.0	—
	2	—	—	3.7	3.4	3.5	4.6	4.5	5.2	3.3
	3	—	—	4.0	3.6	3.0	3.6	4.6	5.0	4.8
CC10	1	—	—	3.4	4.6	5.0	4.9	5.0	6.1	—
	2	—	3.1	4.0	4.6	6.1	4.2	5.0	6.2	—
	3	3.8	4.0	6.2	5.6	3.3	4.2	6.2	6.2	5.2
CN10	1	—	—	3.0	4.0	4.0	3.0	4.1	5.2	—
	2	—	—	4.0	4.0	5.0	3.0	5.1	5.1	3.0
	3	—	—	4.0	4.0	4.1	3.1	5.1	5.1	3.8

Titers are expressed as $\log_{10}$p.f.u./g tissues ; — indicates < 2.30 ; virus titers are the mean values of two chickens.

Table 5. Virus recovery from the tissues of chickens after intracerebral inoculation with NDV strains

Virus	day p.i.	Tissue virus titers								
		Blood	Brain	Trachea	Lung	Spleen	Colon	Thymus	Bursa	Kidney
Q0	1	—	3.0	—	—	—	—	—	—	—
	2	—	3.2	4.2	—	3.0	3.5	3.3	4.4	—
	3	3.0	3.3	4.1	—	—	—	—	3.3	—
	4	—	3.0	—	—	—	—	—	—	—
CC10	1	—	3.7	—	—	4.7	4.0	4.3	4.2	—
	2	3.3	4.2	4.2	4.4	4.2	3.9	4.3	4.7	4.4
	3	3.0	5.7	4.6	4.3	3.7	3.9	4.7	5.7	4.0
	4	—	5.6	4.3	4.3	—	3.8	4.3	4.6	3.6

Titers are expressed as $\log_{10}$p.f.u./g tissues ; — indicates < 2.30 ; virus titers are the mean values of two chickens.

NDV in chickens did not confer these properties.

Antigenic comparison between Q0 and CC10 using monoclonal antibodies: Antigenicity of Q0 and CC10 viruses was examined using monoclonal antibodies against the HN and F proteins in ELISA (Table 1). Both viruses showed identical reactivity patterns with monoclonal antibodies, indicating that the acquisition of pathogenicity did not accompany antigenic variation on the surface glycoproteins of the viruses.

DISCUSSION

The present results showed that the NDVs obtained after serial intracerebral passages in chickens (CC7–10) were highly pathogenic both for chickens and quails as compared to the original isolate, Q0. Acquisition of virulence of the virus was detected at the third intracerebral passage and reached its peak at the 7th passage, whereas the pathogenicity of the virus at the 10th intranasal passage in chickens (CN10) remained unchanged. Q0 killed day-old chicks and six-week-old chickens after intracerebral inoculation, suggesting that the original isolate from the quail possessed some degree of affinity for nerve cells. Virus recovery from the brain and the other tissues of chickens infected with Q0 by intravenous or intranasal routes showed that the isolate was not neurotropic for chickens but pantropic. CC10 acquired higher neurotropic and pantropic properties after adaptation to the chicken brain. These findings suggest that the virulence of NDV could be altered by several passages of the virus in chickens through unnatural routes. This study supports the findings of Alexander et al.[5] that an NDV strain isolated from pigeons, which was not initially virulent for chickens, acquired virulence after several intramuscular passages in chickens. The pathogenicity of measles virus increased tremendously after several intracerebral passages of the virus in rats.[15] On the other hand, Komarov et al.[16] reported that a virulent strain of NDV from chickens lost its pathogenicity for them after several intracerebral passages of the virus in ducklings. The mechanism of alteration of pathogenicity of NDV by serial passages in different bird species through various routes of inoculation is not yet clear.

It is known that the quail is rather resistant to NDV. In the case of the present isolation of NDV, it appears that the virus was transmitted to the SPF quail population of strain LWC from quails introduced from a commercial farm that had been infected with NDV but were apparently healthy. The rate of mortality of quails indicates that the LWC strain of quails is more susceptible to NDV than normal domestic quails. To clarify the mechanism of acquisition of pathogenicity of NDV, genetic analyses of the viruses are now under way.

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