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Viscerotropic velogenic Newcastle disease in six pet bird species: clinical response and virus-host interactions

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Viscerotropic velogenic Newcastle disease in six pet bird species: Clinical response and virus-host interactions

by

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INTRODUCTION

Psittacine and other pet bird species infected with viscerotropic velogenic Newcastle disease (VVND) virus have often been incriminated as the source of virus causing outbreaks of VVND in domestic poultry. Despite numerous isolations of the virus from various pet bird species, little was known about the relationship between pet birds and VVND virus. This investigation was therefore initiated in an attempt to determine the response of six pet bird species (Table 1) to a VVND viral isolate obtained in 1972 from yellow-headed Amazon parrots (Amazona ochrocephala oratrix) that had been illegally transported from Guadalajara, Mexico, to Lakewood, Colorado. The clinical and virologic responses of six pet bird species to this virus were examined, and the biological markers and pathogenicity of viral isolates obtained from the birds after exposure to VVND virus were evaluated.

Table 1. Species of birds exposed to viscerotropic velogenic Newcastle disease virus

Family	Subfamily	Genus	Species	Subspecies	Common name
Psittacidae					
	Psittacinae				
		Aratinga	canicularis	eburnirostrum	Halfmoon or Petz conure
		Amazona	ochrocephala	oratrix	Yellow-headed Amazon parrot
		Melopsittacus	undulatus		Budgerigar
Fringillidae					
		Serinus	canarius		Canary
Estrildidae					
		Lonchura	malacca		Black-headed nun
Sturnidae					
		Gracula	religiosa	indica	Lesser hill mynah

REVIEW OF LITERATURE

History

In a classic sense, four types of Newcastle disease (ND) have been described in the chicken. The peracute type, first recognized in 1926 by Doyle (19) and Kraneveld (31), is lethal to chickens. Following exposure to the virus the incubation period varies from two to four days when marked dyspnea, acute depression, and diarrhea are commonly observed. Conjunctivitis and edema of the head and wattles may occur. Paralysis may be present, but other signs of neural dysfunction are rare unless the course of the disease is protracted. Affected birds usually die within one to three days after clinical signs are noted.

At necropsy, petechial to ecchymotic hemorrhages with occasional necrosis are frequently found throughout the digestive tract, particularly in the proventriculus, and sometimes in the ventriculus and small intestine. Other lesions that may be present are tracheal hemorrhage and peritracheal edema. Newcastle disease viruses causing this type of disease have been termed viscerotropic pathotypes or viscerotropic velogenic (VV) ND viruses.

A second type of ND was called "avian pneumoencephalitis" by Beach in 1944 (6). Generally, marked dyspnea and signs of neural dysfunction such as tremors and torticollis are seen in affected chickens. Although mortality is often high, intestinal lesions are absent. Viruses producing this type of disease are referred to as neurotropic velogenic ND viruses.

The milder types of disease are primarily respiratory diseases. In 1946, Beaudette and Black (9) described a form of ND that produced an infection in adult chickens that was often inapparent but could cause respiratory and occasionally nervous signs in young chickens. Strains of the virus causing this type of disease are called mesogenic ND viruses.

The mildest form of ND was first described by Hitchner and Johnson (30) in 1948. It produced subclinical infections in adult chickens and very mild respiratory distress in young chicks. The viruses causing this syndrome are classified as lentogenic ND viruses (32). In vivo and in vitro characteristics of representative ND viruses are listed in Table 2.

Of the four types of ND, the viscerotropic pathotype with its demonstrated ability for rapid spread throughout the world is of greatest concern to the domestic poultry industry. Initially, the disease spread from the island of Java, Indonesia, to Asia, East Africa, and southern Europe over a period of 16 years (1926-1942). The second pandemic of VVND was recognized when the virus was isolated from a cockatoo in Indonesia in 1962. In 1966, a VVND outbreak occurred in Iran. From there the disease spread to the rest of the Middle East, Greece, and western Europe by 1970. While the source of spread to Europe may have been from the Middle East through southern Europe, Lüthgen and Wachendörfer (34) reported recovery of the virus from psittacine birds newly imported from Asia and South America. However, it was believed that diseased cage birds did not escape into poultry flocks.

The full impact of the pandemic was felt in the United States in 1970 and 1971. Viscerotropic velogenic ND virus first entered the country with

Table 2. Selected *in vitro* and *in vivo* characteristics for prototype Newcastle disease viruses (26,39,41,43)

Classification	Virus	%	Plaque assay clear/red	Diameter (mm)	MDT[a] (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)
Viscerotropic velogenic	Herts	1	Red	<1.0	56	48	30
		90	Clear	1.0			
		9	Red	2.8 (1.5-4.0)			
Neurotropic velogenic	Texas-GB	100	Clear	1.0-2.0	50	86	60
Mesogenic	Roakin	100	Clear	0.5-2.0	70	34	IS(5)[b]
Lentogenic	LaSota	100	Clear	<0.5-1.5	104	120	IS[c]
	B1	100	Clear	<0.5-1.5	128	3	IS(5)

[a]Chicken embryo mean death time.

[b]IS indicates that the hemagglutinin was not stable at ≥ 15 min. Estimated stability is listed in parentheses.

[c]Estimated stability was not available.

imported parrots that arrived in New York in March 1970. The 1971 VVND outbreak in California was also thought to have originated from infected parrots imported from South America (28,32). Table 3 depicts the world spread of VVND virus through movement of infected psittacine species from 1970 through April 1976.

Other pet bird species have also been implicated as potential sources for the spread of VVND. Mynah birds may be as efficient as parrots in transporting the virus. Of greater concern are the inapparent carriers of VVND virus. Viscerotropic virus has been isolated from apparently normal pittas and flowerpeckers (27). Pet bird species from which ND virus has been isolated are listed in Table 4.

Clinical manifestations in pet bird species

Cavill (13) has indicated that ND is severe in cage birds and commonly results in death. Initially birds are anorectic, markedly dyspneic, and have profuse ocular and nasal discharge. Diarrhea may be noted. When ND occurs in an aviary situation, the birds may huddle together in a corner with ruffled plumage. The course of the disease was stated to be short, and terminated in death within one to three days after clinical signs were observed. However, for some psittacine birds, sudden death is the first sign that ND is present. Surviving birds may show clinical signs due to central nervous system dysfunction. These include ataxia, muscular tremors, torticollis, and varying degrees of paralysis.

Scott and Winmill (40) reported similar observations for an outbreak of the disease in nine grey parrots (Psittacus erithacus). The pre-

Table 3. Known movement of psittacine species infected with viscerotropic velogenic Newcastle disease virus (VVNDV) from 1970 through April 1976 (21,23,24,28,32,46,)

Origin	Year	Destination[a]
Mexico	1972	Colorado, U.S.A. Georgia, U.S.A. Texas, U.S.A.
	1973	California, U.S.A.
	1974	California, U.S.A. Illinois, U.S.A. Michigan, U.S.A. Texas, U.S.A.
	1975	Texas, U.S.A.
Dominican Republic	1975	Puerto Rico
Honduras	1973	California, U.S.A.
	1975	Florida, U.S.A.
Nicaragua	1974	Florida, U.S.A.
Colombia	1970	Holland England Federal Republic of Germany
	1973	New York, U.S.A.
Paraguay	1970	Republic of South Africa via Holland England Federal Republic of Germany New York, U.S.A.
	1971	Connecticut, U.S.A. Florida, U.S.A.
	1975	Florida, U.S.A.

[a]Destination indicates the country or state and country that received VVNDV-infected psittacine species.

Table 3. (Continued)

Origin	Year	Destination
Argentina	1976	Illinois, U.S.A.
Belgium	1975	New York, U.S.A. Florida, U.S.A.
Holland	1972	Canada
	1976	Illinois, U.S.A.
Spain	1975	New York, U.S.A.
Ghana	1974	California, U.S.A. Florida, U.S.A.
Liberia	1973	New York, U.S.A.
Nigeria	1973	New York, U.S.A.
Senegal	1975	California, U.S.A.
	1976	Florida, U.S.A.
Zaire	1973	New York, U.S.A.
Hong Kong	1975	Hawaii, U.S.A.
Indonesia	1974	Illinois, U.S.A. California, U.S.A.
	1975	California, U.S.A.
Malaysia	1975	Florida, U.S.A.

Table 4. Newcastle disease infection in pet bird species (1,12,14,15,22,32,33,34,36,37,38,40,45, 46)

Family	Genus	Species	Subspecies	Common name	Probable pathotype[a]			
					VV	Meso-genic	Lento-genic	NK
Psittacidae	*Amazona*	*aestiva*		Blue-fronted Amazon	X			
	Amazona	*finschi*		Lilac-crowned Amazon	X			
	Amazona	*ochrocephala*	*oratrix*	Yellow-headed Amazon	X			
	Amazona	*vinacea*		Vinaceous Amazon	X			
	Amazona	*viridigenalis*		Green-checked Amazon	X			
	Pionus	*maximiliani*	*maximiliani*	Maximilian's Parrot	X			
	Poicephalus	*senegalus*		Senegal Parrot	X			
	Psittacus	*erithacus*		African Gray Parrot	X			
	Brotogeris	*versicolorus*	*chiriri*	Canary-winged parakeet	X			
	Psittacula	*alexandri*		Moustached parakeet	X			
	Psittacula	*columboides*		Malabar parakeet	X			
	Psittacula	*cyanocephala*		Plum-headed parakeet	X			

[a]Pathotypes of ND virus isolated from birds were not clear. Often chicken inoculation results and other pertinent laboratory data were lacking for definitive proof of the virus' pathotype. The indicated pathotypes were therefore classified as probable. VV=viscerotropic velogenic. NK=not known.

Table 4. (Continued)

Family	Genus	Species	Subspecies	Common name	Probable pathotype VV	Meso-genic	Lento-genic	NK
	Psittacula	eupatria		Alexandrine parakeet	X			
	Psittacula	krameri	borealis	Rose-ringed parakeet	X			
	Psittacula	krameri	krameri	Rose-ringed parakeet	X			
	Psittacula	krameri	manillensis	Rose ring necked parakeet	X			
	Cacatua	alba		White cockatoo	X			
	Cacatua	galerita		Sulphur-crested cockatoo	X			
	Cacatua	moluccensis		Salmon-crested cockatoo	X			
	Cacatua	sanguinea		Little corella	X			
	Cacatua	sulphurea	sulphurea	Lesser sulphur-crested cockatoo	X			
	Cacatua	sulphurea	parvula	Lesser sulphur-crested cockatoo	X			
	Eolophus	roseicapillus		Galah	X			
	Nymphicus	hollandicus		Cockatiel	X			
	Ara	macao		Scarlet macaw	X			
	Ara	severa		Chestnut-fronted macaw	X			
	Agapornis	pullaria		Red-faced lovebird	X			
	Lorius	rorytus		Lory	X			
Ramphastidae	Unidentified			Toucan	X			

Table 4. (Continued)

Family	Genus	Species	Subspecies	Common name	Probable pathotype			
					VV	Meso-genic	Lento-genic	NK
Trochilidae	Unidentified			Hummingbird	X			
Pittidae	*Pitta*	*guajana*		Banded Pitta	X			
	Pitta	*sordida*		Hooded pitta	X			
Dicaeidae	Unidentified			Flowerpecker	X			
Fringillidae	Unidentified			Finch	X			
	Serinus	*canarius*		Canary	X	X		
	Chloris	*chloris*		Greenfinch				X
	Fringilla	*coelebs*		Chaffinch				X
	Carduelis	*carduelis*		Goldfinch				X
Estrildidae	*Poephila*	*gouldiae*		Gouldian finch			X	
Ploceidae	*Coliuspasser*	*progne*		Weaverbird				X
	Padda	*oriziova*		Java Sparrow				X
Sturnidae	*Gracula*	*religiosa*	*religiosa*	Mynah	X			
Irenidae	*Irena*	*puella*	*cyanea*	Fairy bluebird			X	

dominant clinical signs were respiratory in nature. Nasal and ocular discharges were profuse. The birds had ruffled plumage and kept their eyes closed. Anorexia was followed by general debility and subsequent death. Eight of the birds succumbed to the disease.

Several outbreaks of VVND in newly imported psittacine birds were reported by Lüthgen (33), Lüthgen and Wachendörfer (34) and Wachendörfer and Lüthgen (45). In general, clinical signs were first noted within a few days after arrival at the quarantine station. Affected birds were depressed and had little interest in their new surroundings. Prominent clinical signs were torticollis, muscular tremors, and jerky or wobbly movements (chorea) of the head that occasionally included the entire body. The pupils were often dilated, and diseased birds were anorectic. Droppings were often watery and sometimes blood-tinged. Apparently normal birds at rest became quite excited at the sound of a human voice and clinical signs consisting of head tremors, and tremors of the extremities and body were observed. With progression of the disease, affected birds often became paralyzed and were not able to fly or sit on a perch. Shortly after paralysis was evidenced the birds died. Surviving birds were generally destroyed due to the persistence of central nervous symptoms. South American psittacines were more severely affected, with a predominance of central nervous symptoms, than were Asian psittacines. In Asian species the course of the disease was more protracted and enteric dysfunction dominated the clinical picture. Death losses were usually 80 to 100% for both South American and Asian psittacines. Peracute cases of the disease were also observed, in which birds that appeared normal on one day were found dead the next day.

Pohl (37) described an outbreak of VVND in yellow-headed Amazons (Amazona ochrocephala) that occurred in a Berlin pet shop. Mortality was lower than that in the outbreaks described by Wachendörfer and Lüthgen. Nevertheless, more than 30% of the diseased parrots died.

Cavill (14) reported two outbreaks of VVND in cockatoos (Cacatua sp.) imported into the United Kingdom from Singapore. The first outbreak involved 12 birds, one of which was dead on arrival. The remaining birds were dead within seven days. Twenty birds were received in a second shipment. Again, all birds succumbed to VVND within ten days of arrival. Clinical signs observed in the two outbreaks were anorexia and abnormally soft droppings for approximately 24 hours prior to death.

Post-mortem Lesions Observed in Pet Bird Species

The lesions vary in intensity, and in many birds, none are found (33,40,45,47). Lesions described are congestion of the trachea, lungs, and air sacs; blood-stained tracheal exudate; airsacculitis; hepatomegaly; splenomegaly; petechial hemorrhages on the pericardium; fibrinous inflammation of the abdominal membranes; diffuse petechial hemorrhages on the serous surface of the intestines; congestion of the proventriculus; hemorrhagic enteritis; and traumatic injuries of the skull with subdural hemorrhages (1,13,14,15,18,33,34,37,38,40,45,47).

In a differential diagnosis, two frequently described diseases of pet birds, psittacosis (ornithosis) and salmonellosis, must also be considered. Lesions commonly observed in pet birds infected with Chlamydia psittaci are airsacculitis; congestion of the lungs; serous, fibrinous, or purulent pericarditis; peritonitis; hepatomegaly; splenomegaly; and congestion of the intestinal tract. Also, mortality due to psittacosis or ornithosis can be very high in budgerigars (Melopsittacus undulatus)[1], lorikeets, rosellas (Platycercus cp.), South American parrots, finches, and canaries (Serinus canarius) (3).

Salmonellosis is widespread in caged birds and aviaries. Lesions in birds dying of a septicemic infection are congestion of the lungs, hepatomegaly and splenomegaly with congestion, and pericarditis (42,44). Thus, a rapid presumptive diagnosis of VVND in pet birds on the basis of post-mortem lesions is not possible. More definitive, controlled studies were needed to determine the type and extent of lesions that might be expected in pet birds exposed to VVNDV.

Information about the duration of virus shedding, mortality from uncomplicated VVNDV infections and virus-host adaptation was clearly lacking. For these reasons, six species of birds were selected for exposure to VVNDV. Representatives of the family Psittacidae were needed to assess the significance of the numerous gross lesions described in the literature. The mynah bird (Gracula sp.) has also been implicated in the spread of VVND (27,32,46). Sporadic isolations have also been reported from finches. Importation data were therefore reviewed to determine which species were most frequently imported into the United States.

For the four year period of 1968 through 1971, 52,000 Amazon parrots, 157,000 conures, 101,000 mynahs, 522,000 canaries, and 187,000 nuns were

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[1]Personal communication with Dr. L. A. Page, National Animal Disease Center, Ames, Iowa, June, 1976.

brought into this country. The total number of birds in the families represented by those species imported over the same period were Psittacidae - 533,000, Sturnidae (mynahs) - 103,000, Fringillidae (canaries) - 595,000, and Estrildidae (nuns) - 1,606,000 (4,5,16,17). As listed in Table 1, representative species chosen for exposure to VVNDV were the yellow-headed Amazon parrot, halfmoon conure, and the budgerigar for the Psittacidae; the hill mynah for the Sturnidae; the common canary for the Fringillidae; and the black-headed nun for the Estrildidae.

MATERIALS AND METHODS

Viruses

The Colorado Psittacine Isolate of VVNDV (COPI-VVNDV) was isolated from two yellow-headed parrots illegally transported from Guadalajara, Mexico, to Lakewood, a suburb of Denver, Colorado, in 1972. Prototype NDV strains LaSota and Roakin were obtained from the respository of the Veterinary Science Department of the University of Wisconsin at Madison. A third prototype strain, B1, was obtained from Dr. P. D. Beard, Veterinary Services Laboratories, Ames, Iowa.

Working stocks of the viruses were prepared by inoculation of specific pathogen free (SPF) eight- to eleven-day-old embryonated chicken eggs[1] by the allantoic route. Inoculated eggs were incubated at 37C and candled daily for embryo deaths. Amnionic and allantoic fluids were harvested from eggs dying after 48 hours of incubation and were tested for hemagglutinating activity (HA). Fluids positive by the HA test were evaluated by the NDV hemagglutination-inhibition (HI) test to confirm the presence of NDV in the HA positive fluids. The bulk virus stocks were then divided into 1.0 ml aliquots and maintained at $\leq$-70C.

Experimental Birds

Six species of pet birds and domestic chickens were used in the experiments. Four of the pet bird species were obtained from commercial

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[1]SPAFAS, Inc., Norwich, Connecticut, or Larson Laboratory Eggs, Inc., Fort Dodge, Iowa, or National Animal Disease Center, Ames, Iowa.

bird importers and were directly imported to the laboratory under U.S. Department of Agriculture (USDA) permits. Domestic budgerigars were purchased through a local pet shop. The yellow-headed Amazon parrots were collected in Mexico and quarantined in USDA facilities, where they received chlortetracycline treatment to clear them of Chlamydia psittaci infection, if present. After treatment the parrots were likewise directly imported under USDA permit.

Forty-two yellow-headed Amazon parrots, 52 halfmoon conures, 105 budgerigars, 48 mynahs, 132 canaries, and 71 black-headed nuns were used for exposure to VVNDV. All birds were divided into groups of three or four individuals and placed in isolettes (Figures 1 through 6). Each isolette had a filtered air intake and was under negative air pressure to effect maximal biological security for the experiments.

Eight-week-old, NDV-susceptible White Leghorn chickens were used for characterization of NDV isolates obtained from exposed pet bird species.

Evaluation of Clinical and Virologic Responses of Experimental Birds Exposed to VVNDV

Exposure of birds

Five of the six pet bird species were exposed in the following manner: Three birds were randomly assigned to each isolette upon arrival at the laboratory. After a minimum adjustment period of two weeks one bird was removed from each isolette and placed in separate isolation facilities. The two remaining birds were then exposed to nebulized COPI-

Figures 1-6. Representative individuals of the six pet bird species exposed to viscerotropic velogenic Newcastle disease virus

Figure 1. Budgerigar (upper left)

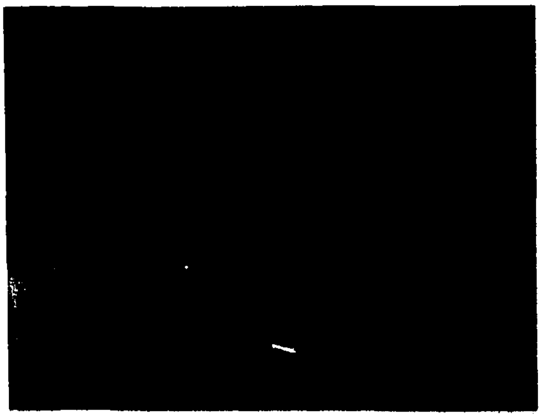
Figure 2. Conure (upper right)

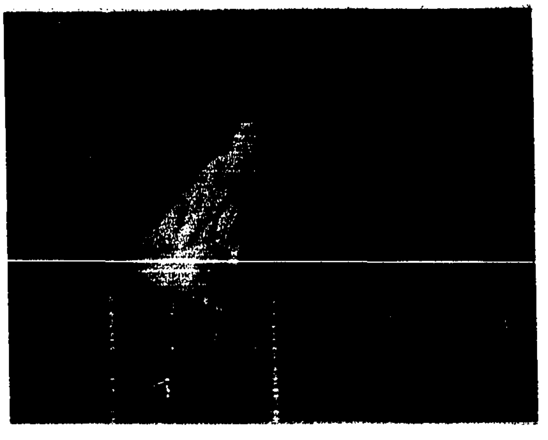
Figure 3. Canary (center left)

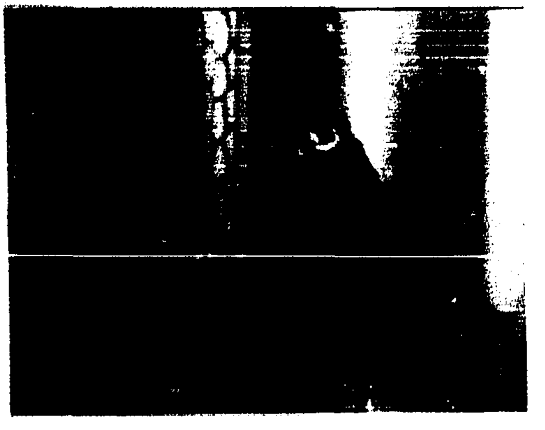
Figure 4. Mynah (center right)

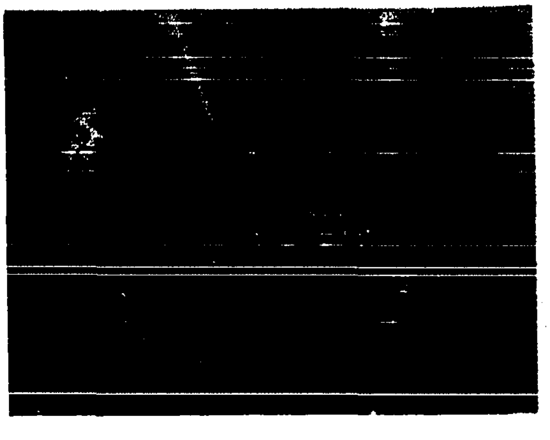
Figure 5. Parrot (lower left)

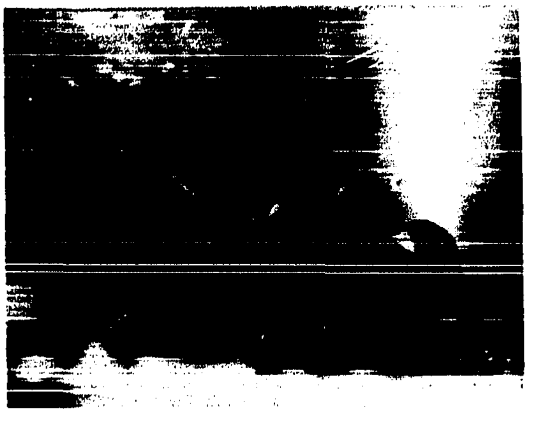
Figure 6. Nun (lower right)











VVNDV. At three days postexposure (DPE) the third bird that had been removed was returned to serve as a contact exposure bird.

The sixth species, the black-headed nun, was exposed in a different fashion due to limited isolette space. Upon arrival four nuns were randomly assigned to each isolette. After adjustment one bird was removed from each isolette and placed in separate isolation facilities. The three remaining birds were then exposed to nebulized COPI-VVNDV. At three DPE the fourth bird that had been removed was returned to function as a contact exposure bird.

Venous blood samples were obtained from all birds at zero DPE and from 20% of the birds that had been randomly selected at 12 or 14 DPE, 21 DPE, and at weekly intervals thereafter.

Oral and cloacal swab specimens were collected in sterile brain-heart-infusion broth from all birds at 0, 3, 5, 8, 12, and 21 DPE, and at weekly intervals thereafter. Lung, trachea, spleen, and brain were collected from birds that died during the studies.

Each species was held until all birds had apparently ceased to shed NDV for a minimum period of two weeks. At that time, the remaining birds were killed, and lung, trachea, spleen, and brain were collected for attempted NDV isolation.

Virus isolation

Swab and tissue specimens were stored at -70C immediately following collection. Swab specimens were usually processed in the following fashion within one week after collection. The specimens were thawed at room temperature (23C) and centrifuged at 1500 x g for clarification. A

1.2 ml aliquot of supernatant fluid from each specimen was then aspirated for inoculation of six embryonated chicken eggs, 0.2 ml per egg, as previously described.

Inoculated eggs were incubated at 37C for seven days and were candled daily. Embryonal deaths occurring at the second and subsequent DPE were considered to be due to virus infection of inoculated eggs. Amnionic and allantoic fluids (AAF) were harvested from dead eggs and examined by the HA and HI tests for the presence of NDV as previously described. Fluids positive for NDV were divided into 2.0 ml aliquots and stored at -70C.

Ten percent tissue suspensions were prepared from necropsy specimens for inoculation of four embryonated chicken eggs, 0.25 ml per egg, as described above. Generally, the lung and trachea, and spleen and brain were pooled to provide sufficient quantities of tissue suspension for egg inoculation.

Occasionally, HA negative fluids were obtained from dead embryonated eggs that were also negative for bacterial contaminants. Such fluids were subpassaged into four embryonated chicken eggs, 0.25 ml per egg, and incubated and candled as described above. Amnionic and allantoic fluids were harvested from embryonated eggs dying at the second and subsequent DPE. Fluids positive for NDV were divided into 2.0 ml aliquots and stored at -70C.

Zero DPE swab specimens from parrots, budgerigars, and conures were also examined for the presence of _Chlamydia psittaci_. Clarified broth material was inoculated into 6 six- to eight-day-old SPF, embryonated chicken eggs, 0.2 ml per egg, by the yolk sac route. Inoculated eggs were incubated at 37C and candled daily for embryo deaths. At the end of the ten-day passage period, AAF were harvested from the eggs and pooled for each specimen. Each pool of AAF was subpassaged into four embryonated chicken eggs by the yolk sac route as described above. Yolk sac membranes were harvested from dead eggs and all remaining eggs at the end of the seven-day subpassage period for preparation of yolk sac chlamydiosis complement fixation test antigens. The resulting antigens were then tested for the presence of *C. psittaci* by the microtitration chlamydiosis complement fixation test (25).

Necropsy tissue specimens negative for NDV isolation were subsequently examined for bacterial pathogens according to the methods described in Merchant and Packer (35).

Serology

Blood samples were examined for antibodies against NDV by two HI tests. The HI tests were performed according to a standard method (2) using the inactivated LaSota NDV HA antigen described, and an HA antigen prepared from a 133 DPE budgerigar NDV isolate.

Budgerigar blood samples were also examined for NDV-neutralizing antibodies by the egg-bit technique of C. W. Beard (7). The 133 DPE budgerigar NDV isolate was again used for this test.

Organ culture studies

Organ explants were cultured in an attempt to determine a nidus for chronic NDV infection. Cultures were prepared from the trachea, lung, cloaca, brain, and a segment of the intestinal tract that correlated to the ileocecocolic junction in the chicken. One sampling was made for five of the pet bird species examined in these studies. Two samplings were obtained from the yellow-headed Amazon parrots. The number of birds sampled for each species and DPE for sampling are listed in Table 5.

Table 5. Birds sampled for organ explant

Pet bird sampled	Number sampled	DPE[a] for sampling
Parrot	6	104-105
	8	402
Conure	10	181
Budgerigar	33	123-133
Mynah	10	134
Canary	30	89-93
Nun	10	136

[a]Day postexposure.

Organ explants were prepared according to the method of Heuschele and Easterday (29), and were maintained in Eagle's basal medium with 50 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer and 5% fetal bovine serum as described by Blaskovic et al. (11). Antibiotics employed in the culture medium were 200 IU penicillin, 250 μg streptomycin, 50 μg kanamycin, and 2.5 μg amphotericin B per milliliter. All organ cultures were maintained in a moist 5% carbon dioxide atmosphere at 37C for a minimum observation period of three weeks. The culture medium was aspirated from all flasks and replenished with fresh medium at seven-day intervals. Media harvested from the cultures were then inoculated into six embryonated chicken eggs, 0.2 ml per egg, as previously described, to determine if NDV had been propagated from the organ explants.

Characterization of Selected NDV Isolates

Two hundred and seventy-nine of the NDV isolates obtained after exposure of the six pet bird species to VVNDV were screened for pathogenicity by both the plaque assay technique of P. D. Beard et al. (8), and the chicken embryo mean death time (MDT) technique (43).

Fifty-seven isolates were subsequently selected for additional characterization by the hemagglutination-elution and hemagglutinin thermostability (56C) techniques (41,43). Nineteen of the 57 isolates were then chosen for chicken backpassage studies.

Chicken backpassage studies

Initially, one NDV-susceptible eight-week-old chicken was placed in each of 19 isolettes. Each chicken was then inoculated with one of the 19 isolates by swabbing its cloaca with a 1:100 dilution of the virus.

At four days postinoculation (DPI) a second susceptible chicken of the same age was placed with each inoculated chicken. Another susceptible chicken was placed in each cage at eight DPI. After eight DPI all cages remained closed except for the removal of dead chickens. Dead chickens were necropsied, trachea, lung, spleen, intestine, and brain were collected for virus isolation attempts, and the isolates were pathotyped according to the gross lesions observed at necropsy.

The backpassage studies were terminated when a minimum observation period of 22 days had elapsed. At that time oral and cloacal swab, and blood specimens were obtained from the chickens for NDV isolation attempts and the HI test.

Newcastle disease viral isolates obtained from the chickens were characterized by the NDV plaque assay, mean death time, hemagglutination-elution, and hemagglutinin thermostability (56C) techniques to determine if viral mutation had occurred during the course of these studies.

RESULTS

Clinical Response of Six Pet Bird Species

No clinical signs were observed in the canaries and mynahs, but progressive death losses occurred.

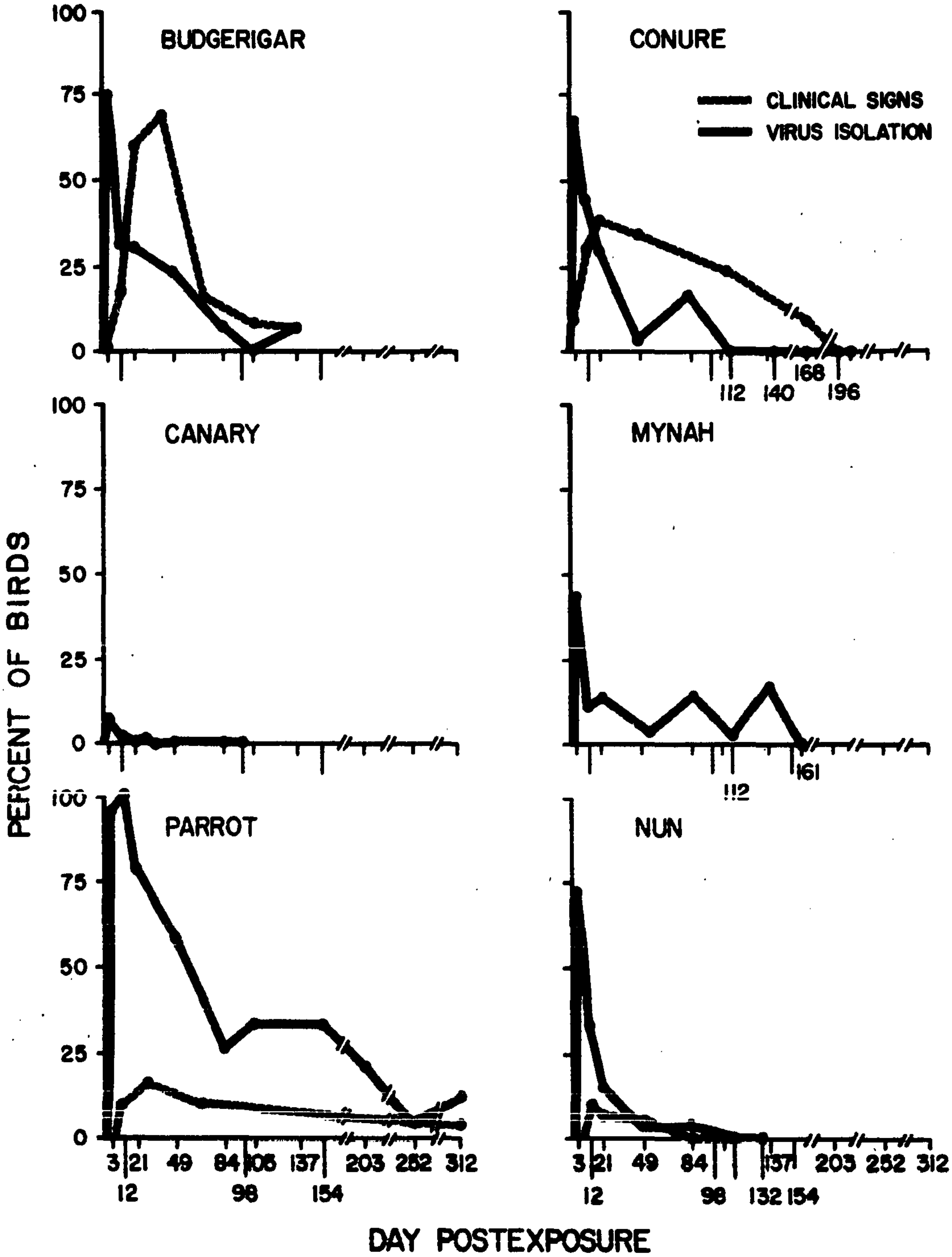
In conures, parrots, budgerigars, and nuns depression, inappetance, and ruffled plumage were initially observed within three days to two weeks after the birds were exposed to VVNDV. With the exception of the nuns, a progression of clinical signs was seen in the birds. These included conjunctivitis, serous nasal discharge, diarrhea, and various manifestations suggestive of a viral encephalomyelitis. Central nervous system involvement was first evidenced as fine tremors of the neck and head during the third and subsequent weeks postexposure. When affected birds were excited by sudden movements or loud noises they would often exhibit a more exaggerated jerking or bobbing movement of the head and neck. As the birds moved away from such a stimulus, many of them were markedly ataxic as they walked, ran, or flew. With progression of the disease, varying degrees of paralysis were observed after three weeks postexposure.

Paralysis was manifested as unilateral or bilateral wing droop and/or leg paralysis. Budgerigars and parrots with bilateral leg paralysis were observed pulling themselves across the isolettes with their beaks to reach food and water. Birds with bilateral leg paralysis usually succumbed within one to three weeks after onset of paralysis. However, conures, parrots, and budgerigars with one leg paralyzed often adapted to their affliction and lived a fairly normal existence for the duration of the studies. Similarly, wing droop often persisted for the duration of the studies, and both types of clinical disease were reflected (as illustrated in Figure 7) by a chronic low incidence of clinical signs for those three species. Many of the birds that were depressed, anorectic, or ataxic as well as those that had minor tremors of the neck and head eventually recovered. Clinical signs were most marked in the budgerigars, less severe in the parrots, and least severe in the conures. The predominant clinical signs observed for the budgerigars were tremors of the neck and head, wing droop, leg paralysis, and ataxia. In the parrots tremors of the neck and head, ruffled plumage, and wing droop predominated. The principal clinical signs in conures were ruffled plumage and ataxia. Clinical signs observed in affected budgerigars and conures are depicted in Figures 8 through 13.

Fourteen of the 71 nuns exposed to VVNDV subsequently had clinical signs during the studies. Clinical signs observed were tremors of the neck and head, ataxia, conjunctivitis, and paralysis. Only one bird had conjunctivitis and a second bird had a paralyzed foot. Three of the 14 affected birds died at 13, 14, and 19 DPE. The remaining birds were asymptomatic by 84 DPE (Figure 7).

Mortality was most severe for the conures (55%). Parrots and canaries had lower cumulative mortalities of 29 and 25%, respectively. Budgerigars, nuns, and mynahs had similar respective cumulative mortalities of 22, 21, and 21% (Figure 14). When dead budgerigars, parrots, canaries, mynahs, and nuns were necropsied, gross lesions similar to those seen in chickens succumbing to VVNDV were not observed. Lesions compatible with a presumptive diagnosis of salmonellosis or psittacosis were also lacking. However, parrots that died during the first two weeks after

Figure 7. Chronology of clinical signs and Newcastle disease virus isolation


BUDGERIGAR
CONURE
CLINICAL SIGNS
VIRUS ISOLATION
CANARY
MYNAH
PARROT
NUN
PERCENT OF BIRDS
100
75
50
25
0
112 140 168 196
112 161
3 21 49 84 105 137 203 252 312
12 98 154
3 21 49 84 137 203 252 312
12 98 132 154
DAY POSTEXPOSURE

Figures 8-13. Clinical signs observed for affected budgerigars (8-10) and conures (11-13)

Figure 8. Marked incoordination; severely impaired righting reflex (upper left)

Figure 9. Depression (upper right)

Figure 10. Chorea of the upper trunk and head with wing droop (center left)

Figure 11. Ruffled plumage (center right)

Figure 12. Disorientation and lack of coordination (lower left)

Figure 13. Paralysis (lower right)

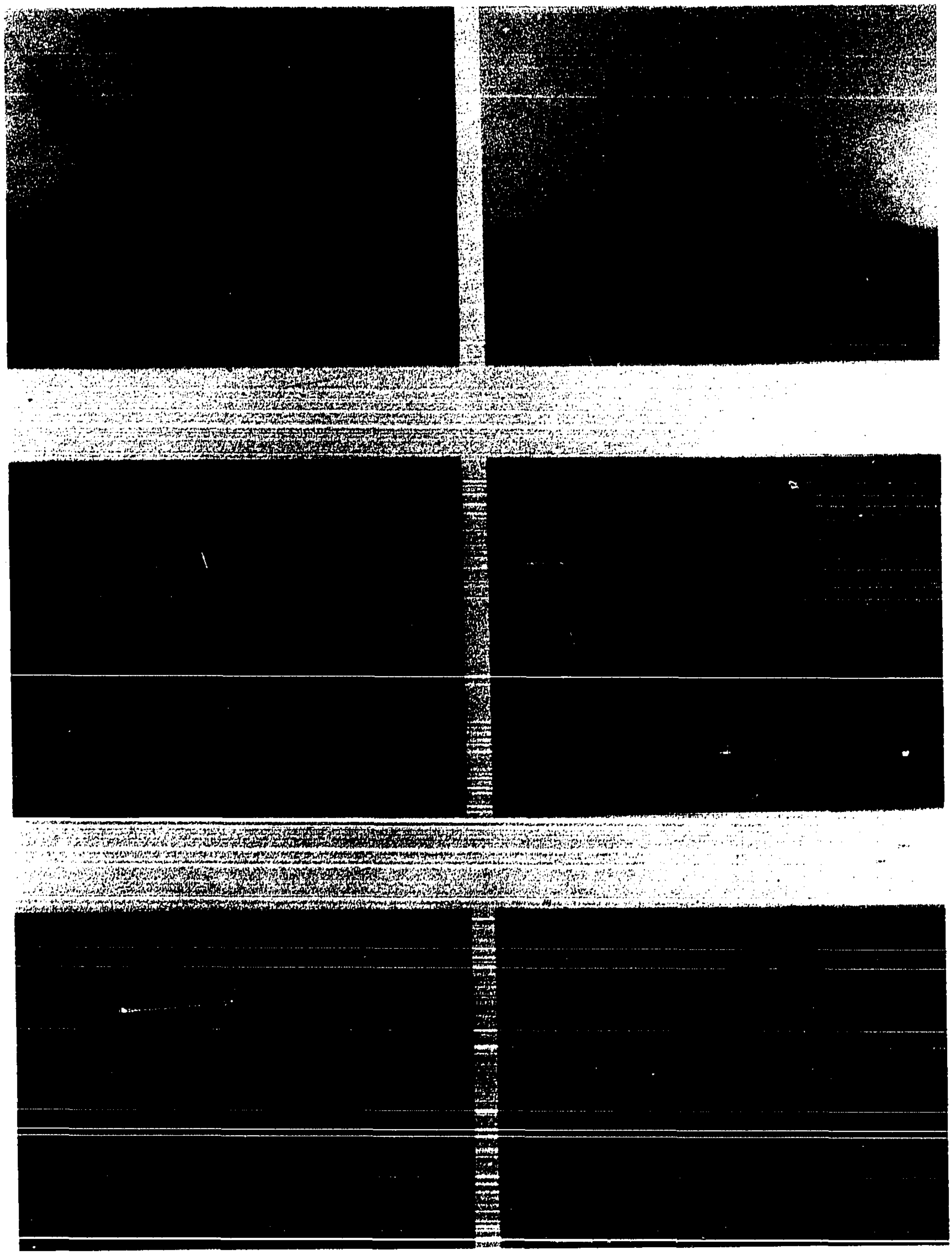
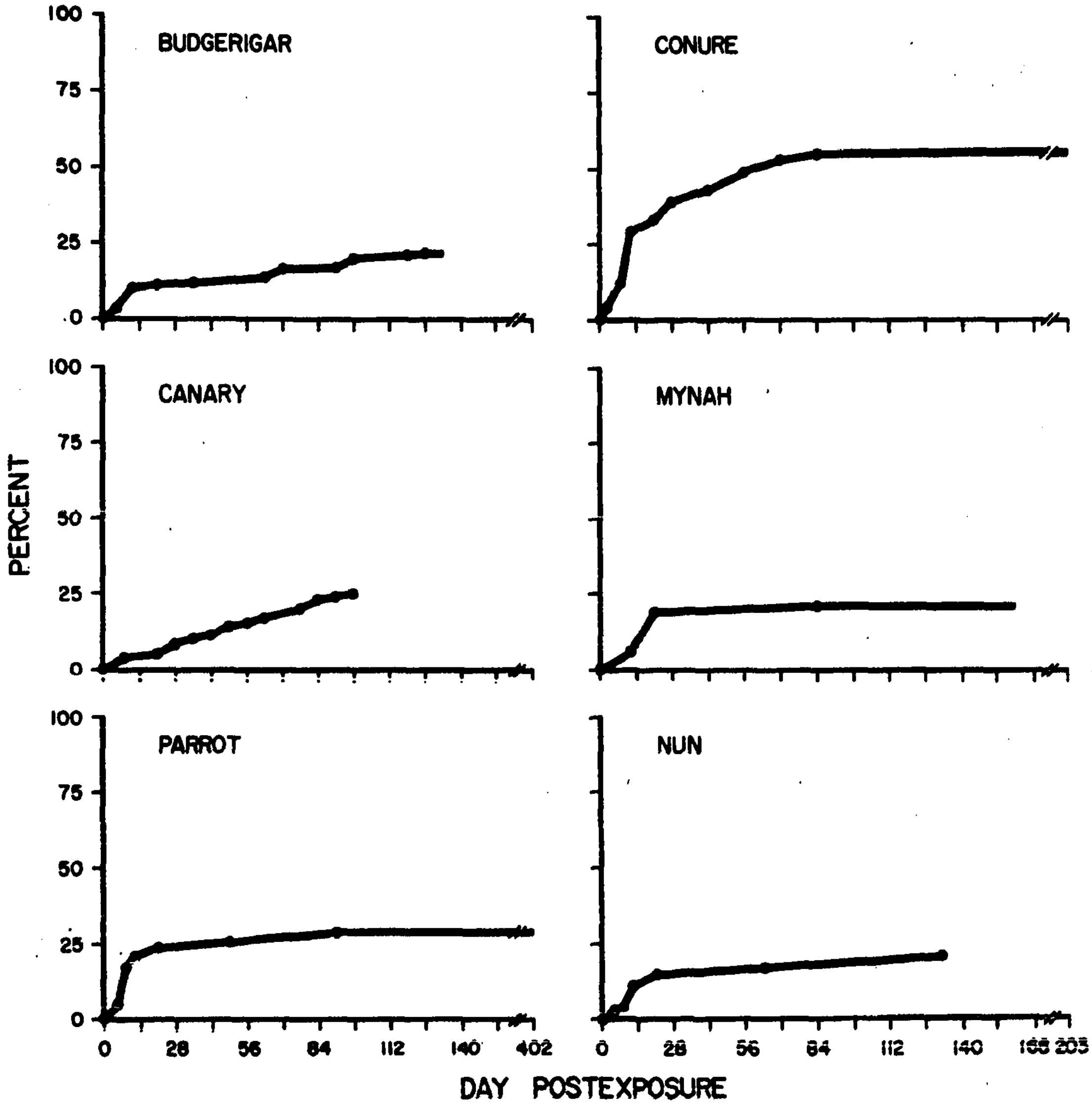


Figure 14. Cumulative mortality for six species of birds exposed to similar doses of viscerotropic velogenic Newcastle disease virus


BUDGERIGAR
CONURE
CANARY
MYNAH
PARROT
NUN
PERCENT
100
75
50
25
0
0
28
56
84
112
140
402
140
DAY POSTEXPOSURE

exposure to VVNDV had mycotic airsacculitis and pneumonitis at necropsy. The remaining parrots were treated with Amphotericin B in an attempt to eliminate mycotic pathogens.[1] Parrot mortality abruptly subsided following treatment of the birds.

Two types of lesions were most prevalent in 15 conures that died during the first two weeks of the study. The first type was characterized by hyperemia, hemorrhage and occasionally necrosis. Ten conures had hyperemia, petechial or ecchymotic hemorrhage, and occasionally necrosis of the gastrointestinal mucosa. Skull cap hemorrhage or hemorrhage around the orbit of the skull was frequently seen. Hyperemia, hemorrhage and necrosis of the gastrointestinal tract was usually seen in the proventriculus, at the junction of the proventriculus and ventriculus, and sometimes in the small intestine.

The second lesion type was characterized by inflammation of the membranous lining of the body cavity. A serous to fibrinous peritonitis was observed in six conures. Occasionally, excessive peritoneal fluid was also noted. Other lesions observed in the 15 conures were hepatomegaly or splenomegaly (four birds), focal hepatic necrosis (one bird), airsacculitis (one bird), and hemorrhagic tracheitis (one bird).

A variety of lesions without any apparent pattern was seen in birds necropsied after 14 DPE. Lesions present were mycotic ingluvitis, occluded vent with megacolon, skull cap hemorrhage (five birds), petechial hemorrhages at the junction of the proventriculus and ventriculus, and

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[1]Each parrot was given 0.75 mg of Amphotericin B per kg intravenously every other day for three treatments and three days after the third treatment. Antibiotic therapy was initiated at 11 DPE.

focal hepatic and renal necrosis. Cultural results for bacterial pathogens from necropsy tissues negative for NDV isolation are listed in Appendix B.

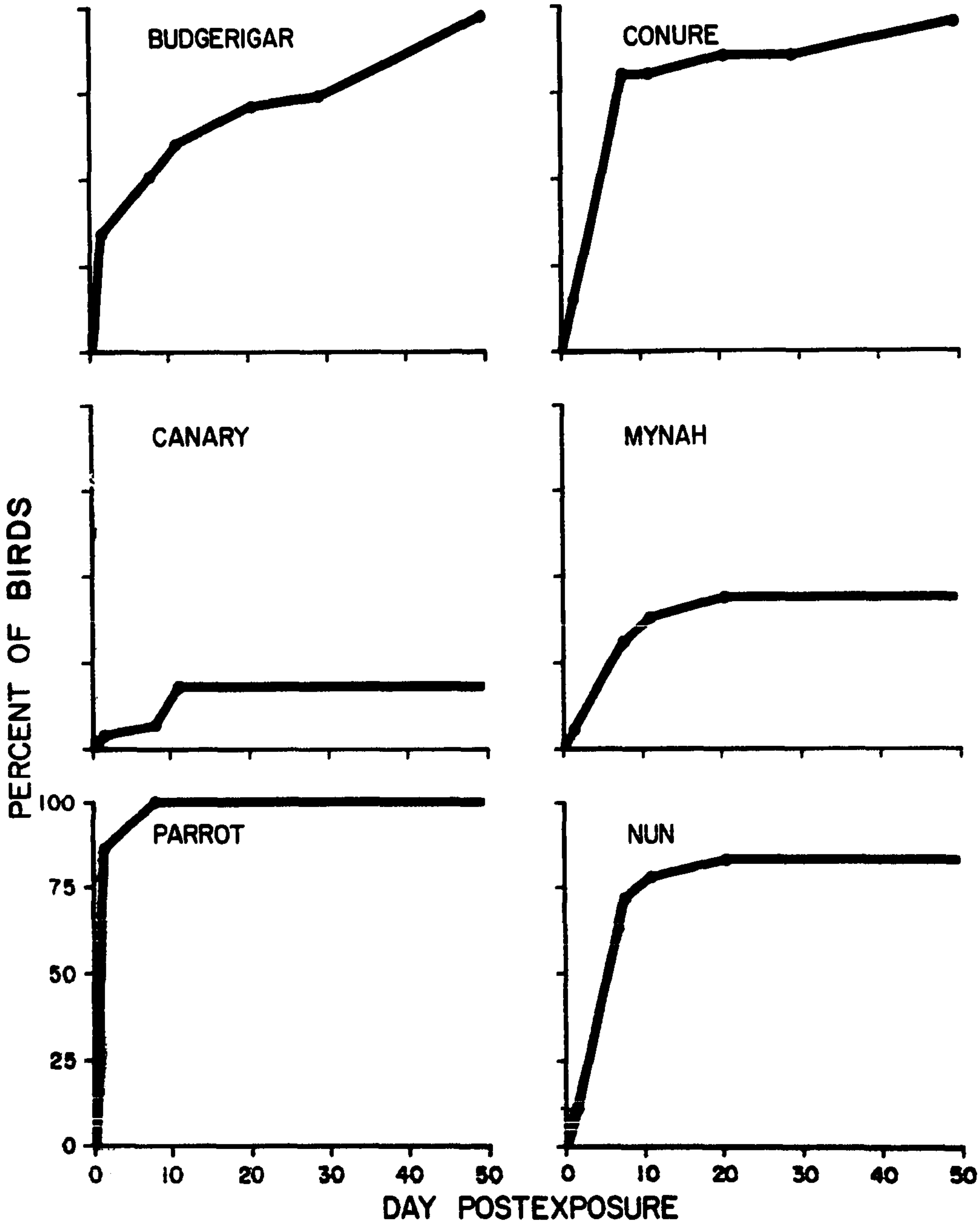
Virologic and Serologic Responses of Six Pet Bird Species to VVNDV

Parrots, conures, budgerigars, mynahs, canaries, and nuns were monitored for virus excretion and circulating antibodies against NDV for 402, 203, 137, 161, 98, and 132 days after exposure to COPI-VVNDV, respectively. As shown in Figure 7, by 12 days after exposure to VVNDV, all parrots were positive for NDV isolation from oral and/or cloacal swab specimens. The budgerigars, conures, and nuns were also highly susceptible to infection with NDV. Mynahs were somewhat more resistant to NDV, while canaries were almost refractory to the virus.

Contact exposed birds of the same species became infected with NDV almost as rapidly as the directly exposed birds (Figure 15).

Chronic NDV infections were apparently established in three of the six species examined. The budgerigars were killed at 137 days after exposure to VVNDV because all birds were negative for NDV isolation at 126 DPE. Mynahs were positive for NDV isolation through 119 DPE. Newcastle disease virus was isolated from parrots in excess of one calendar year after exposure to VVNDV. To determine if the chronic excretion of NDV was due to the infection circulating among parrots within each isolette, 50% of the remaining birds (12) were placed in individual isolettes at 268 DPE. One of the 12 single parrots was positive for NDV isolation at 283 DPE, two at 312 DPE, and two at 376 DPE. One of the three isolettes

Figure 15. Percentage of birds infected with Newcastle disease virus following contact with infected birds of the same species


BUDGERIGAR
CONURE
CANARY
MYNAH
PARROT
NUN
PERCENT OF BIRDS
100
75
50
25
0
0
10
20
30
40
50
0
10
20
30
40
50
DAY POSTEXPOSURE

containing four parrots had one parrot positive for NDV isolation at 312, 340, and 383 DPE. A second isolette had a positive parrot at 376 DPE.

Newcastle disease virus was isolated from conures and nuns up to 112 and 84 DPE, respectively. Sporadic NDV isolates were obtained from canaries for up to 35 DPE.

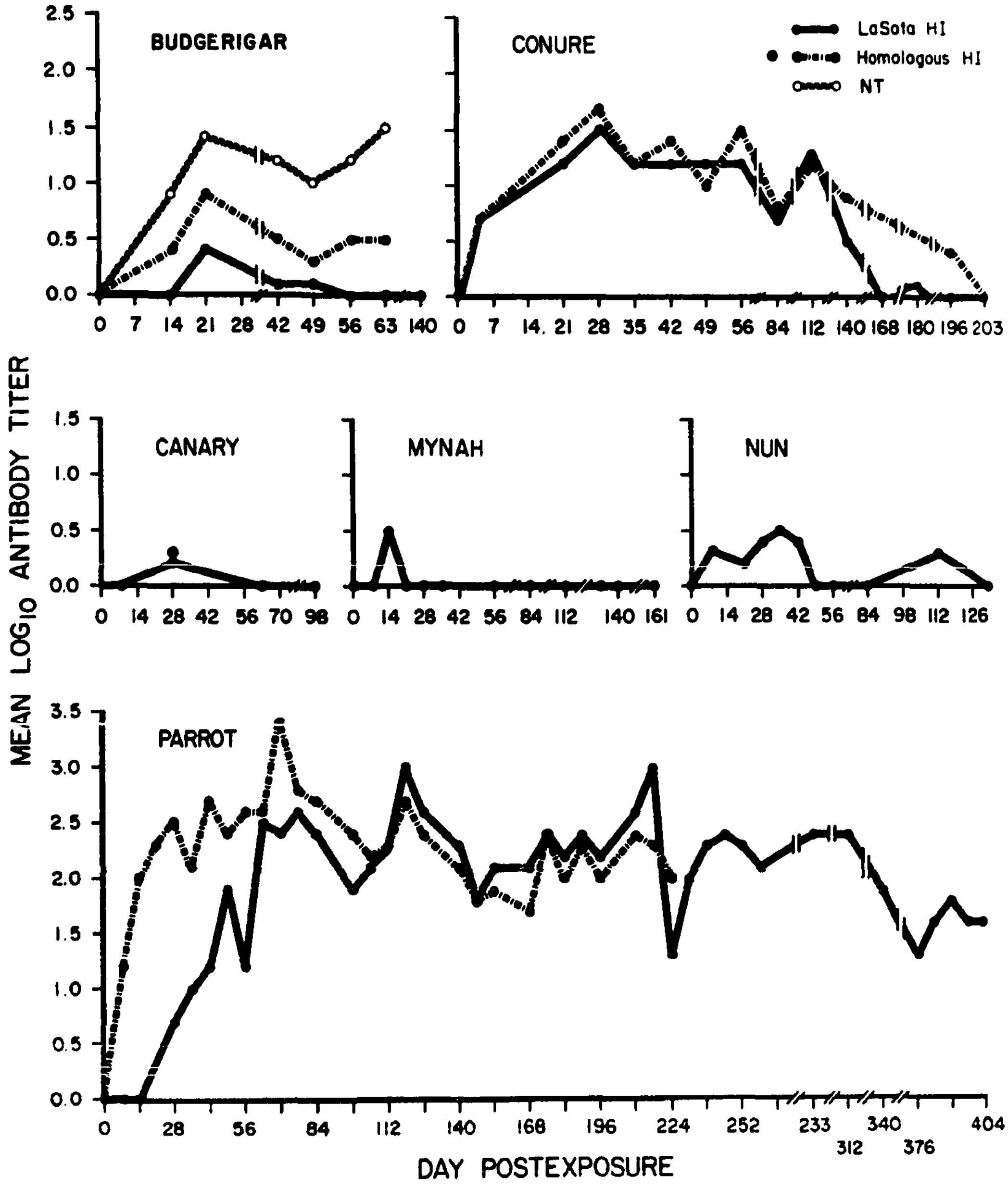
The frequency of detection of NDV in oral and cloacal swab specimens from all six species was also compared. In parrots 205 isolates were obtained between 63 and 402 DPE. One hundred and fifty-seven of these isolates were obtained from cloacal swab specimens and 48 from oral swabs, a ratio of 3:1. In the other five species a significant difference in isolation rate from the two orifices was not observed.

With the exception of the mynahs, tissue specimens collected from birds prior to 18 DPE were positive for NDV isolation. Virus was not recovered from two dead mynahs at 12 (one bird) and 15 DPE, despite positive NDV isolations obtained for tissues from four mynahs at 12 (two birds) and 13 DPE. Overall NDV isolation rates from tissue specimens were from parrots, 83%; budgerigars, 67%; nuns, 67%; conures, 64%; mynahs, 33%; and canaries, 11%.

Serologic response

Using the LaSota antigen for the NDV HI test a minimal serologic response was observed for budgerigars as compared to the parrots and conures (Figure 16). Two other assays were subsequently conducted on budgerigar blood specimens to more completely evaluate the birds' serologic response to VVNDV. The second assay used was a homologous HI test. For that test, a 133 DPE budgerigar NDV isolate was used for preparation of the HA antigen. To assess the sensitivity of the homologous HI test,

Figure 16. Mean serologic response of six species of birds as determined by the hemagglutination-inhibition (HI) test using LaSota and homologous (A21-C/133) Newcastle disease virus (NDV), and by the egg bit neutralization test (NT) using homologous NDV

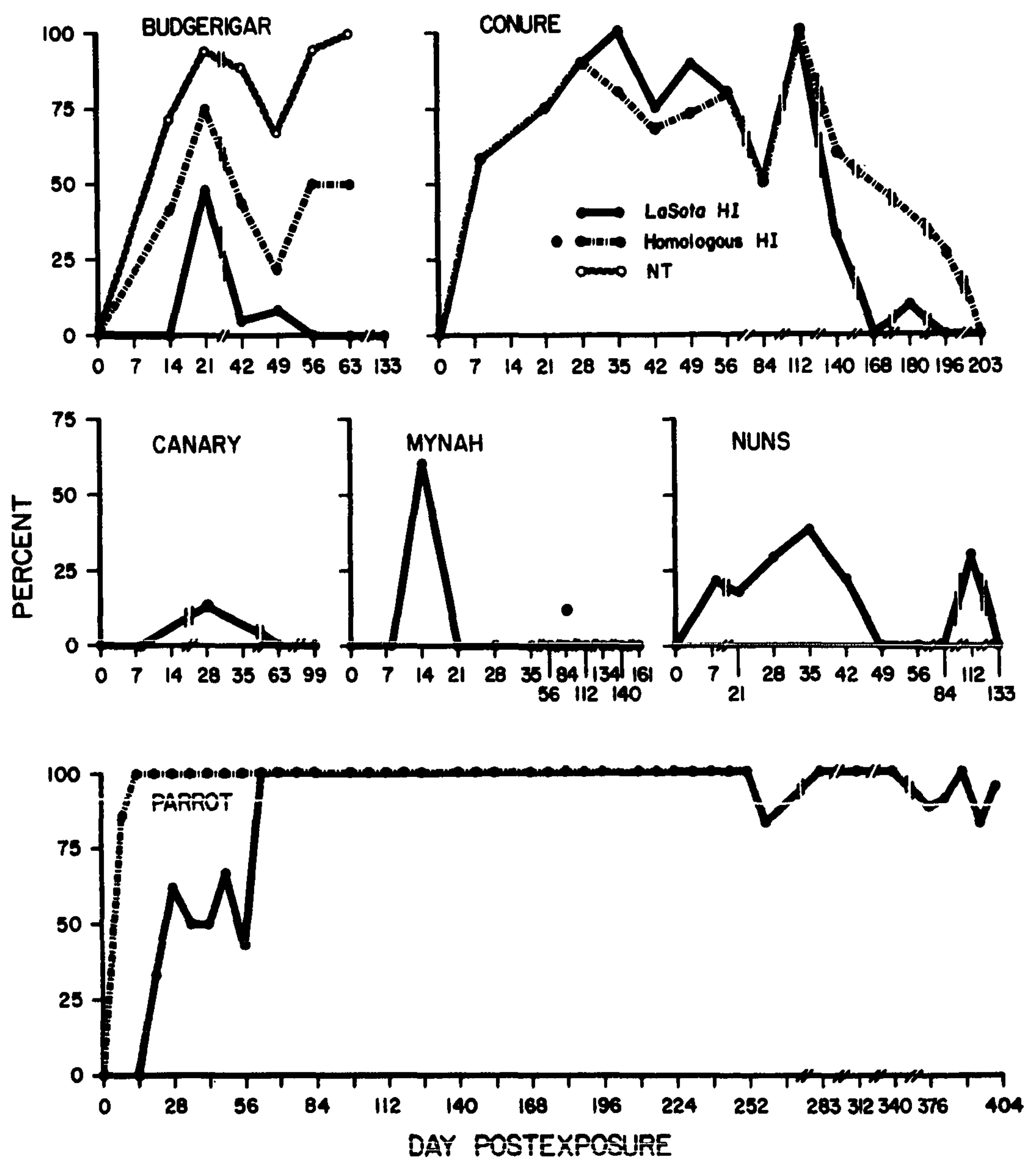

BUDGERIGAR
CONURE
LaSota HI
Homologous HI
NT
CANARY
MYNAH
NUN
PARROT
MEAN LOG10 ANTIBODY TITER
DAY POSTEXPOSURE

blood specimens from the parrots, conures, mynahs, and canaries were also evaluated by the technique. Regrettably, most of the canary and mynah, and all of the nun blood specimens had been discarded by the time this test was conducted (one year after completion of the animal phase of the experiments). Budgerigar mean $\log_{10}$ antibody titers were consistently higher by this technique. Specimens negative by the LaSota HI test at 14, 56, and 63 DPE had mean $\log_{10}$ antibody titers of 0.4, 0.5, and 0.5, respectively. For the other four species, homologous mean $\log_{10}$ antibody titers were usually greater than those obtained by the LaSota HI test (Figure 16).

The third assay used for budgerigar blood specimens was the egg bit neutralization test (NT). By that test, mean $\log_{10}$ antibody titers were consistently 0.9 log or more higher than LaSota HI titers, with a peak mean $\log_{10}$ NT antibody titer of 1.5 at 63 DPE (Figure 16).

The percent seroconversion of randomly sampled birds by those serologic assays was also considered. For the budgerigars, peak seroconversion occurred at 21 DPE by the two HI techniques (LaSota 68%; homologous 75%) and at 63 DPE by the NT (100%). Respective peak seroconversion for the parrots and conures occurred at 63 and 35 DPE by the LaSota HI test, and at 14 and 112 DPE by the homologous HI test. Lower respective peak seroconversion rates of 60, 38, and 13% were observed for the mynahs, nuns, and canaries (Figure 17).

Figure 17. Serologic response of six species of birds as determined by the hemagglutination-inhibition (HI) test using LaSota and homologous (A21-C/133) Newcastle disease virus (NDV) and by the egg bit neutralization test (NT) using homologous NDV: Percent seroconversion of randomly sampled birds


BUDGERIGAR
CONURE
LaSota HI
Homologous HI
NT
CANARY
MYNAH
NUNS
PARROT
PERCENT
DAY POSTEXPOSURE

Organ Culture Studies

Newcastle disease virus was not propagated from organ explant cultures prepared for these studies. All organ explants were examined for evidence of viable cells by light and electron microscopy.[1] Either metaplastic epithelial and fibroblastic, or only fibroblastic cells were found in four of the five types of organ explants prepared from the six avian species examined in these studies. No viable cells were found in brain explant cultures.

Characterization of Selected NDV Isolates

Pathogenicity screening

Mean death times for the NDV isolates assayed varied from 42 to 94 hours. The average MDT for the swab specimen isolates obtained from the six species through 12 DPE was 61 hours. Average budgerigar MDT for 43 swab specimen isolates obtained from 56 through 84 DPE, and 12 swab specimen isolates over a four-week period prior to termination of the study, were 64 and 67 hours respectively.

Many of the 279 isolates had large (≥1.5 mm) red plaques by the NDV plaque assay technique, which indicated that those viruses were probably VVNDV. According to that test, 100% of the canary, 90% of the conure, 81% of the budgerigar, 75% of the mynah, 74% of the nun, and 41% of the parrot isolates were VVNDV. See Appendix A for listing of the mean death time and plaque assay results.

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[1]Through the courtesy of Dr. S. J. Proctor, National Animal Disease Center, Ames, Iowa 50010.

Hemagglutination elution and hemagglutinin thermostability studies

Five sequential isolates were chosen for each of three budgerigars and two parrots to determine if viral mutation had occurred within individual birds. In addition, 32 isolates were chosen for further evaluation on the basis of plaque assay results. An isolate was selected if it had produced predominantly clear plaques when tested.

Characterization results for sequential isolates from the five individual birds have been depicted in graphic fashion in Figures 18, 19, 20, and 21. If a biological variation of ±5 hours for mean death times is considered insignificant, only the 56 and 141 DPE isolates from parrot 8 were significantly different (Figure 18). Conversely, a uniform trend was seen for the five birds according to the NDV plaque assay. One hundred percent clear-plaquing isolates were respectively obtained from budgerigars 73, 89, and 99, and parrots 8 and 22 at 56, 126, 112, 56, and 112 DPE (Figure 19).

The 25 sequential isolates were all slow-eluting viruses (>24 hours for complete elution). A marked increase in the time required for complete elution was noted for later isolates from budgerigars 73, 89, and 99, and parrot 8. Peak respective elution times were 120, 78, 120, and 120 hours (Figure 20).

The hemagglutinin of three of the sequential isolates was thermostable at 56C for 15 minutes. Twenty-two of the 25 isolates had thermolabile hemagglutinins (Figure 21). See Appendix A for listing of hemagglutination elution and hemagglutinin thermostability results for the remaining 32 selected NDV isolates.

Figure 18. Mean death time for sequential Newcastle disease viral (NDV) isolates from three budgerigars and two parrots

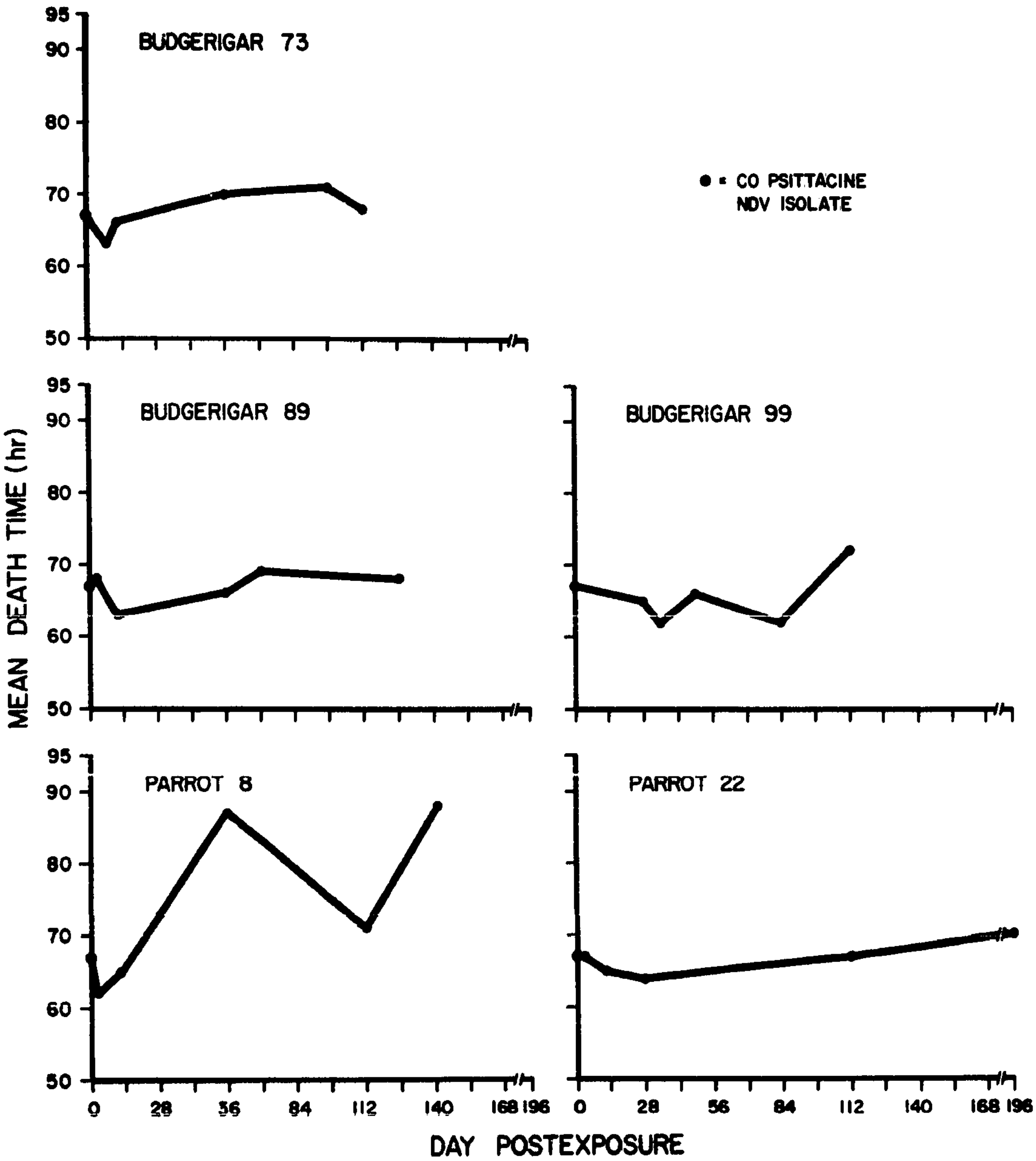
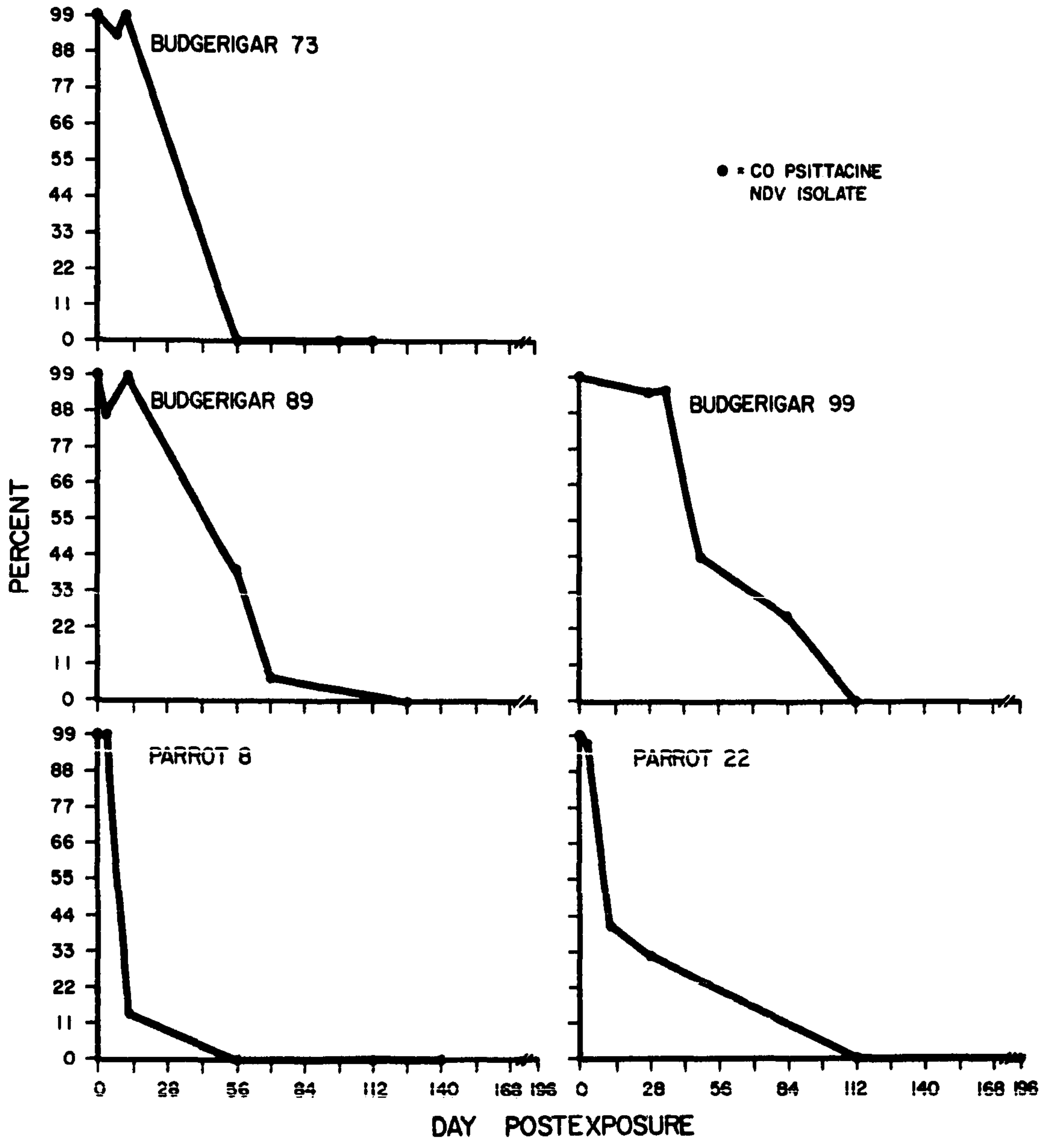

BUDGERIGAR 73
● = CO PSITTACINE
NDV ISOLATE
BUDGERIGAR 89
BUDGERIGAR 99
PARROT 8
PARROT 22
MEAN DEATH TIME (hr)
DAY POSTEXPOSURE
95
90
80
70
60
50
0
28
56
84
112
140
168
196

Figure 19. Plaque assay of sequential Newcastle disease viral (NDV) isolates from three budgerigars and two parrots: Percentage of red plaques



BUDGERIGAR 73
● = CO PSITTACINE
NDV ISOLATE
BUDGERIGAR 89
BUDGERIGAR 99
PARROT 8
PARROT 22
PERCENT
99
88
77
66
55
44
33
22
11
0
0
28
56
84
112
140
168
196
DAY POSTEXPOSURE

Figure 20. Hemagglutination elution times at 4C for sequential Newcastle disease viral (NDV) isolates from three budgerigars and two parrots

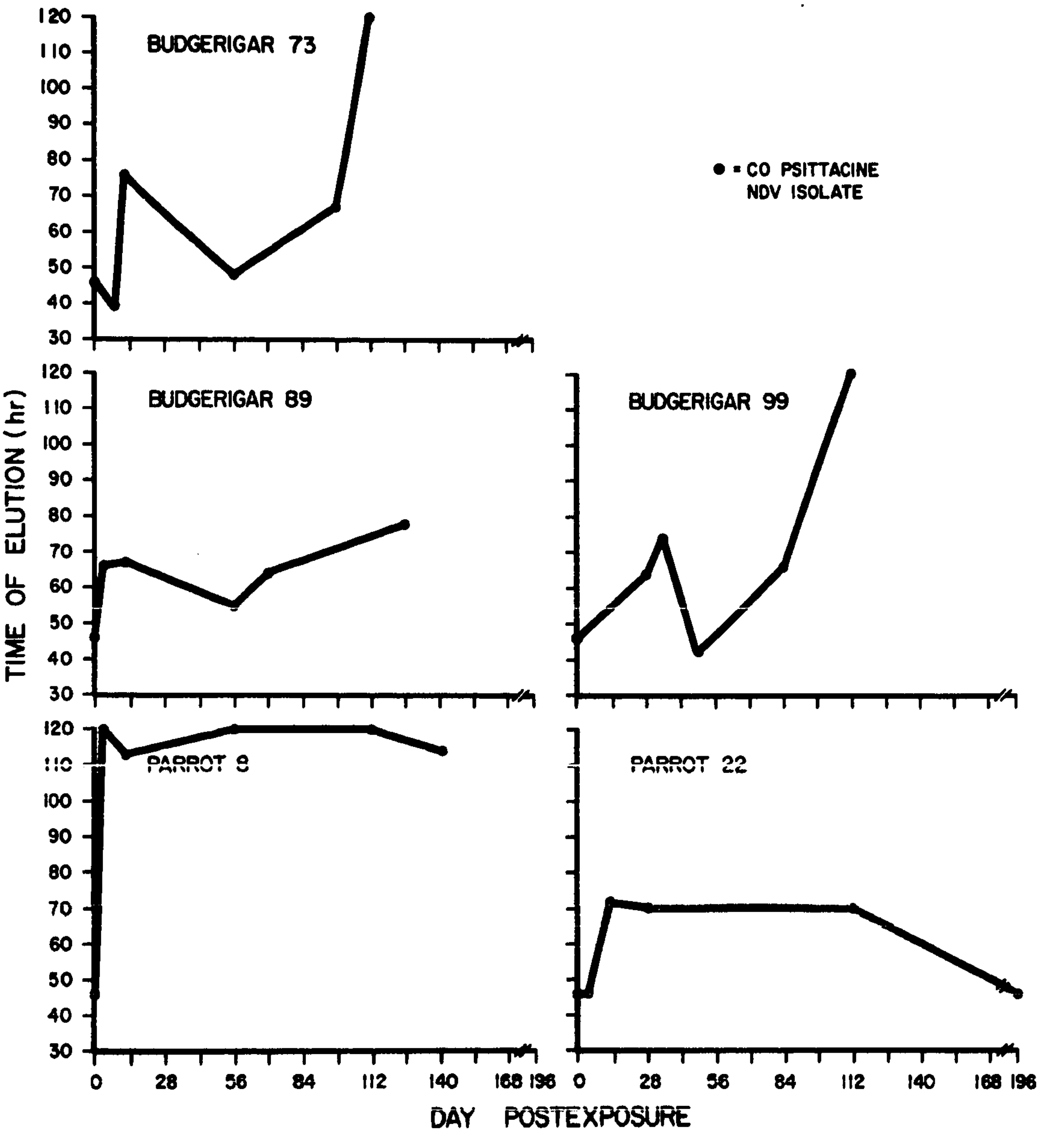
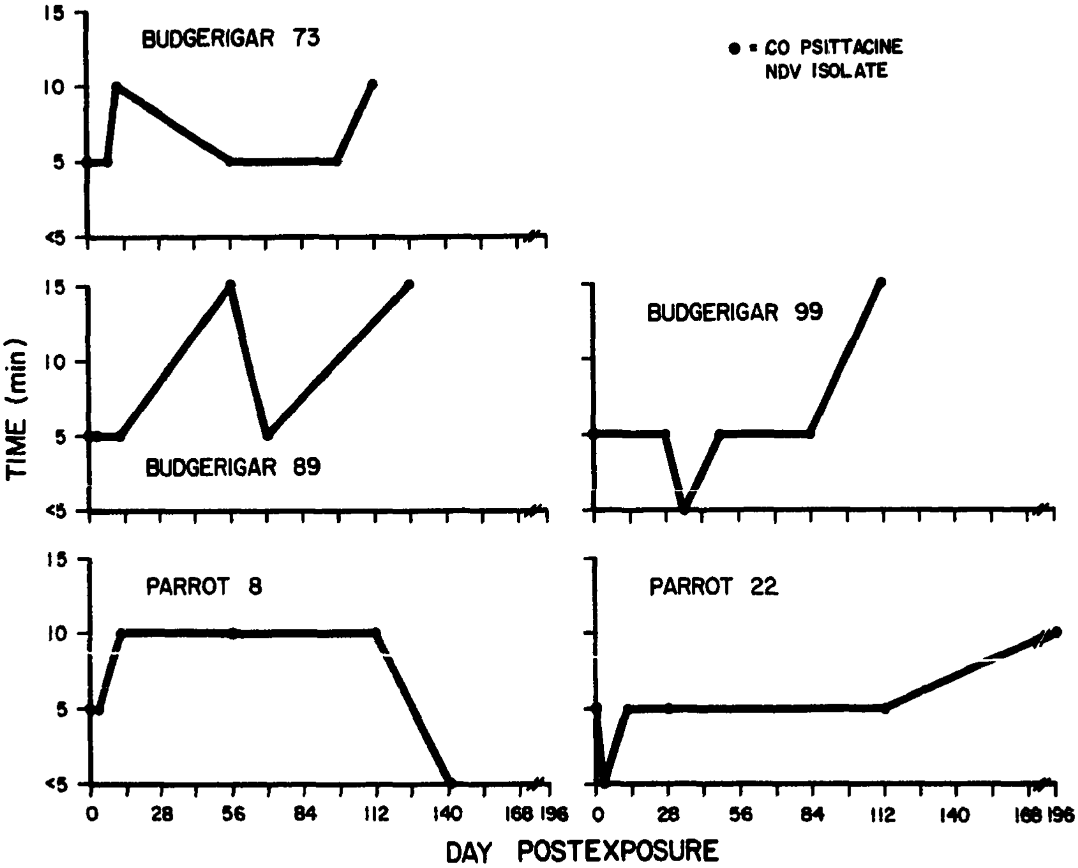

BUDGERIGAR 73
● = CO PSITTACINE NDV ISOLATE
BUDGERIGAR 89
BUDGERIGAR 99
PARROT 8
PARROT 22
TIME OF ELUTION (hr)
120
110
100
90
80
70
60
50
40
30
0
28
56
84
112
140
168
196
DAY POSTEXPOSURE

Figure 21. Hemagglutinin thermostability at 56C for sequential Newcastle disease viral (NDV) isolates from three budgerigars and two parrots



● = CO PSITTACINE NDV ISOLATE
BUDGERIGAR 73
BUDGERIGAR 89
BUDGERIGAR 99
PARROT 8
PARROT 22
TIME (min)
15
10
5
<5
0
28
56
84
112
140
168
196
DAY POSTEXPOSURE

Chicken backpassage studies

Fifteen of the 19 isolates used in the backpassage studies were selected for their potential to be relatively avirulent for eight-week-old domestic chickens. The isolates produced predominantly small (0.5 to 1.5 mm) clear plaques when assayed and had mean death times similar to those previously described for Roakin, a mesogenic strain of NDV. Those isolates were A99-0/112, A73-0/112, A89-0/126, A21-0/133, B8-C/56, B8-C/112, B8-C/141, B22-C/112, B18-C/224, B1-C/312, B17-C/312, Co8-0/84, M33-C/28, N36-0/28, and N56-C/56.

The remaining isolates had varying percentages of large red plaques when assayed and were used as "virulent" NDV controls likely to be pathotyped as VVNDV. Table 6 lists the *in vivo* and *in vitro* characterization and a summary of the chicken backpassage results for the nineteen isolates, and *in vivo* and *in vitro* characteristics of COPI-VVNDV. Eleven of the 15 isolates selected for potential avirulence established asymptomatic infections in backpassage chickens. Eight of the 11 chickens inoculated with the avirulent isolates and one contact exposure chicken had NDV HI antibody titers ranging from 1:4 to 1:256 (Table 7). Serologic evidence of NDV infection was lacking for the remaining 24 chickens used in evaluation of the 11 isolates.

A more extensive disease process was observed for chickens inoculated and subsequently exposed to isolates A73-0/112, A89-0/126, B1-C/312, and N36-0/28. Affected birds underwent a mild respiratory disease that was not readily observed. One of the chickens exposed to B1-C/312 NDV, No. 4145, later developed neurologic disease manifested as torticollis. The

Table 6. In vivo and in vitro characterization of 20 selected Newcastle disease viral isolates

Isolate[a]	Plaque diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)	Response of chickens exposed to isolate[b]
CO Psittacine[c]	0.5			67	46	IS(5)[d]	By 4 to 6 days postinoculation (DPI) acute clinical disease evidenced. Symptoms included anorexia, dyspnea, and depression. Isolate pathotyped as viscerotropic velogenic NDV at necropsy.
	1.0		3				
	1.5	1	9				
	2.0		41				
	2.5		23				
	3.0		17				
	3.5		4				
	4.0		2				
A99-C/84	0.5	10		62	66	IS(5)	Inoculated chicken - acute depression and anorexia by 4 to 6 DPI with eventual recovery.
	1.0	19					
	1.5	6	11				
	2.0	2	32				

[a]Viral isolate code indicates the bird species and the individual's number - oral (O) or cloacal (C) swab specimen/day postexposure of isolation: A=budgerigar; B=parrot; Co=conure; M=mynah; N=nun.

[b]Chicken exposure results for project virus were obtained from direct exposure chickens. Results for experimental bird species' isolates were from three chickens - one exposed by cloacal swabbing and two by contact exposure to that chicken.

[c]CO Psittacine isolate was obtained in 1972 from yellow-headed Amazon parrots and was used for exposure of experimental birds.

[d]IS indicates that the hemagglutinin was not stable at ≥15 min. Estimated stability is listed in parentheses.

Table 6. (Continued)

Isolate	Plaque diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)	Response of chickens exposed to isolate
	2.5 3.0		10 10				First contact exposure chicken (CEC) severe dyspnea prior to death at 7 days postexposure (DPE). Pneumotropic lesions at necropsy. Second CEC-anorexia and dyspnea evident at 6 DPE. When trial terminated at 24 DPE conjunctivitis and torticollis were noted. Pneumoencephalitic lesions at necropsy.
A99-0/112	0.5 1.0	97 3		72	120	15	Frank disease absent through 29 DPI.[e]
A73-0/112	0.5 1.0 1.5	28 59 13		68	120	IS(10)	Inoculated chicken-extensive airsacculitis, pericarditis, and multiple lung and visceral adhesions noted on necropsy at 29 DPI.

[e]The DPI given was calculated from the time the first of three chickens was exposed.

Table 6. (Continued)

Isolate	Plaque diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)	Response of chickens exposed to isolate
							First and second CEC-frank disease absent through 29 DPI.[e] No gross lesions were observed on necropsy at 29 DPI.[e]
A89-0/126	0.5	45		68	78	15	Inoculated chicken-extensive airsacculitis noted on necropsy at 29 DPI. First and second CEC-frank disease absent through 29 DPI.[e] No gross lesions were observed on necropsy at 29 DPI.[e]
	1.0	44					
	1.5	10					
	2.0	1					
A21-0/133	0.5	42		82	120	20	Frank disease absent through 29 DPI.[e]
	1.0	58					
B8-C/12	0.5	3		65	113	IS(10)	Inoculated, and first and second contact exposure chickens-acute depression and anorexia prior to death at 8, 9, and 9 DPE respectively. Isolate pathotyped as viscerotropic velogenic NDV at necropsy.
	1.0	23					
	1.5	57					
	2.0	3	14				

Table 6. (Continued)

Isolate	Plaque diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemaggluti-nation elu-tion (hr)	Stability of hemagglutinin at 56C (min)	Response of chickens exposed to isolate
B8-C/56	0.5 1.0	88 12		87	120	IS(10)	Frank disease absent through 22 DPI.[e]
B8-C/112	0.5 1.0 1.5	58 39 2		71	120	IS(10)	Frank disease absent through 22 DPI.[e]
B8-C/141	0.5	100		88	114	IS(<5)	Frank disease absent through 22 DPI.[e]
B22-C/112	0.5 1.0	72 28		67	70	IS(5)	Frank disease absent through 22 DPI.[e]
B18-C/224	0.5 1.0	89 11		72	113	15	Frank disease absent through 32 DPI.[e]
B1-C/312	0.5 1.0	38 62		67	4	20	Inoculated chicken-severe depression with torticollis at 8 DPI when killed. No gross lesions noted at ne-cropsy. First and second CEC-mild respira-tory disease; no gross lesions noted when ne-cropsied at 32 DPI.[e]

Table 6. (Continued)

Isolate	Plaque diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)	Response of chickens exposed to isolate
B17-C/312	0.5	100		91	120	IS(5)	Frank disease absent through 29 DPI.[e]
B9-0/376	0.5	4		73	43	30	By 4 to 6 DPI acute clinical disease evidenced. Symptoms included anorexia, dyspnea and depression. Isolate pathotyped as viscerotropic velogenic NDV at necropsy.
	1.0	10					
	1.5	7	11				
	2.0		36				
	2.5		25				
	3.0		7				
Co8-0/84	0.5	91		70	94	15	Frank disease absent through 22 DPI.[e]
	1.0	9					
Co8-0/181	0.5	14		67	94	IS(10)	By 4 to 6 DPI acute clinical disease evidenced. Symptoms included anorexia, dyspnea, and depression. Isolate pathotyped as viscerotropic velogenic NDV at necropsy.
	1.0	23					
	1.5	20	7				
	2.0		19				
	2.5		12				
	3.0		5				
M33-C/28	0.5	100		71	55	IS(10)	Frank clinical disease absent through 22 DPI.[e]

Table 6. (Continued)

Isolate	Plaque diameter (mm)	Plaque assay		MDT (hr)	Hemaggluti-nation elu-tion (hr)	Stability of hemagglutinin at 56C (min)	Response of chickens exposed to isolate
		% clear	% red				
N36-0/28	0.5 1.0 1.5	16 82 2		76	75	IS(<5)	Inoculated chicken and first CEC-frank clinical disease absent through 29 DPI.[e] Second CEC airsacculitis noted on necropsy at 29 DPI.[e]
N56-C/56	0.5	100		94	120	IS(10)	Frank clinical disease absent through 22 DPI.[e]

Table 7. Serologic response of backpassage chickens

Isolate[a]	Backpassage chicken identification[b]		Newcastle disease virus hemagglutination-inhibition test antibody titer
Asymptomatic response; absence of gross lesions at necropsy:			
A99-O/112	I	3952	1:32
	CEC-1	3698	Negative (Neg)
	CEC-2	2702	Neg
A21-O/133	I	3977	1:256
	CEC-1	3949	Neg
	CEC-2	2612	Neg
B8-C/56	I	3732	1:32
	CEC-1	2739	Neg
	CEC-2	3865	Neg
B8-C/112	I	2738	Neg
	CEC-1	3729	Neg
	CEC-2	3707	Neg
B8-C/141	I	3836	1:4
	CEC-1	3940	Neg
	CEC-2	3935	1:16
B22-C/112	I	3641	Neg
	CEC-1	3767	Neg
	CEC-2	2758	Neg

[a]Viral isolate code indicates the bird species and the individual's number - oral (O) or cloacal (C) swab specimen/day postexposure of isolation: A=budgerigar; B=parrot; Co=conure; M=mynah; N=nun.

[b]Chicken code designates the inoculated chicken (I), first contact exposure chicken (CEC-1), and second contact exposure chicken (CEC-2).

Table 7. (Continued)

Isolate	Backpassage chicken identification	Newcastle disease virus hemagglutination-inhibition test antibody titer
B18-C/224	I 614	1:4
	CEC-1 1992	Neg
	CEC-2 4355	Neg
B17-C/312	I 2701	Neg
	CEC-1 2694	Neg
	CEC-2 3779	Neg
Co8-0/84	I 3734	1:64
	CEC-1 3839	Neg
	CEC-2 3702	Neg
M33-C/28	I 556	1:256
	CEC-1 4195	Neg
	CEC-2 588	Neg
N56-C/56	I 3948	1:32
	CEC-1 3926	Neg
	CEC-2 3750	Neg
Mild disease with or without gross lesions at necropsy:		
A73-0/112	I 2755	1:512
	CEC-1 3692	Neg
	CEC-2 3722	Neg
A89-0/126	I 2675	Neg
	CEC-1 3601	1:128
	CEC-2 3618	Neg
B1-C/312	I 4145	-
	CEC-1 4067	1:128
	CEC-2 1919	Neg

Table 7. (Continued)

Isolate	Backpassage chicken identification	Newcastle disease virus hemagglutination-inhibition test antibody titer
N36-0/28	I 0	1:16
	CEC-1 3697	1:32
	CEC-2 3781	1:128
Severe clinical disease with loss of one or more chickens:		
A99-C/84	I 1972	1:1024
	CEC-1 653	-
	CEC-2 4033	1:128
B8-C/12	I 2618	-
	CEC-1 2630	-
	CEC-2 3789	-
B9-0/376	I 2635	-
	CEC-1 3776	-
	CEC-2 3983	-
Co8-0/181	I 1979	-
	CEC-1 1926	-
	CEC-2 4011	-

contact exposure chickens (CEC) placed with that bird remained clinically normal for the duration of the study. As indicated in Table 7, the CEC placed at four DPE subsequently seroconverted. It is not known if the second CEC developed an NDV infection since no antibodies could be demonstrated.

The two chickens inoculated with budgerigar isolates A73-0/112 and A89-0/126 had extensive airsacculitis at necropsy. Isolate A73-0/112 was apparently more transmissible as indicated by seroconversion of the first CEC, but no gross lesions were seen in that bird at necropsy. Similarly, no gross lesions were observed at necropsy of the second CEC and the two CEC placed with the chicken inoculated with A89-0/126.

Nun isolate N36-0/28 was highly transmissible as evidenced by seroconversion of the three chickens used for evaluation of the virus. The second CEC had an extensive airsacculitis at necropsy.

Three of the four isolates used as "virulent" ND control viruses, B8-C/12, B9-0/376, and Co8-0/181, were readily transmitted from inoculated chickens to CEC's. The three isolates were pathotyped as VVNDV at necropsy. Gross lesions observed in the chickens used for evaluation of isolate B9-0/376 are depicted in Figures 22 through 27.

Infection with the fourth control isolate, A99-C/84, resulted in a very severe upper respiratory disease that was characterized by extreme depression and a marked dyspnea in all three birds. Eventually the inoculated bird recovered. The first CEC rapidly developed an acute infection characterized by depression and dyspnea that terminated in death at seven DPE. Pneumonic lesions were observed at necropsy. The second CEC

Figures 22-27. Visceral lesions observed in chickens exposed to velogenic Newcastle disease virus isolate B9-0/376

Figure 22. Edematous hemorrhagic conjunctivitis (upper left)

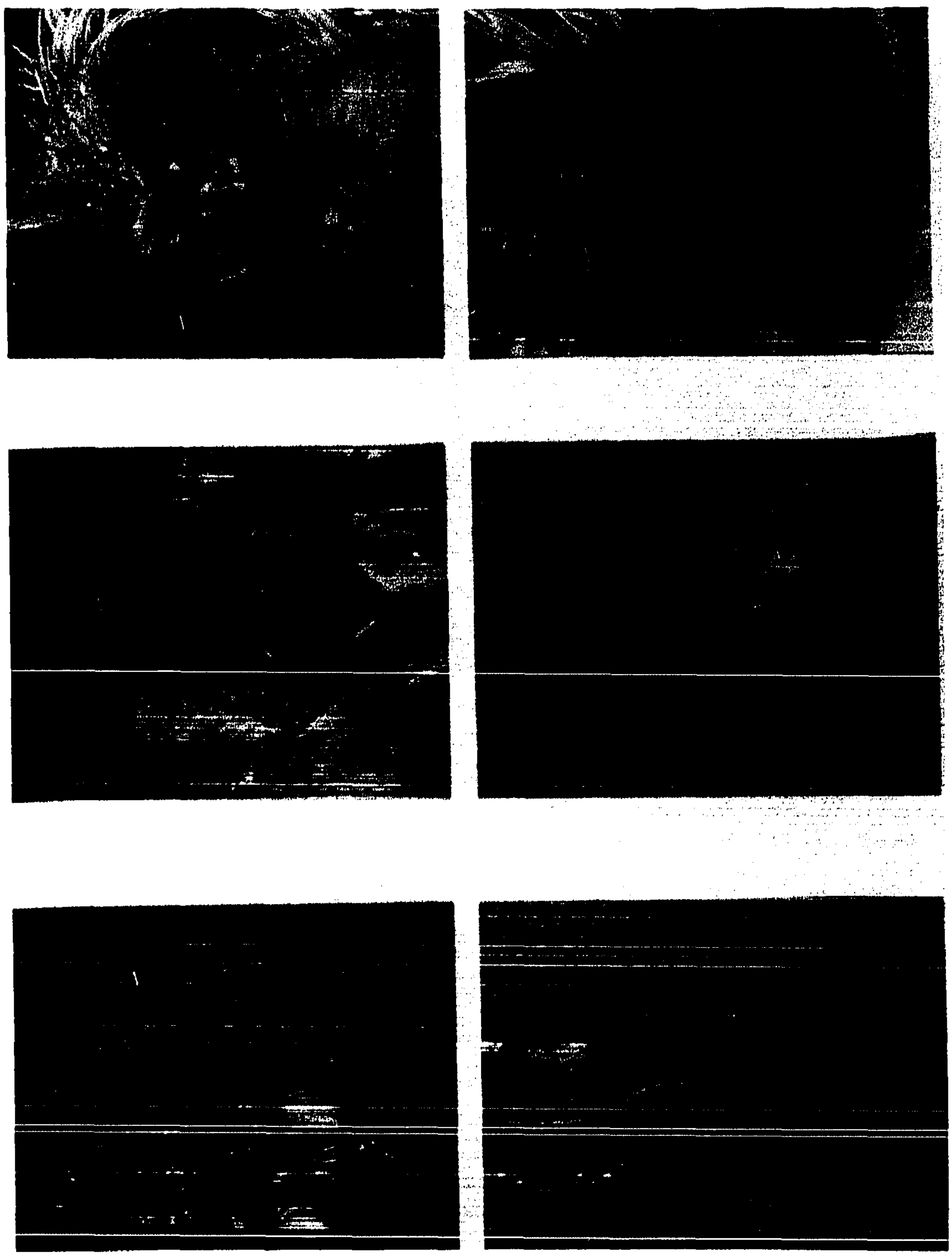
Figure 23. Edema in the subcutis of the anterior cervical region (upper right)

Figure 24. Hemorrhagic tracheitis (center left)

Figure 25. Petechial hemorrhage in the coronary fat band (center right)

Figure 26. Petechial and ecchymotic hemorrhages and necrosis in the proventriculus, ventriculus, ceca, colon, and cloaca (lower left)

Figure 27. Hemorrhagiconecrotic foci of the ileum and ceca (lower right)



also developed an acute infection and had a marked dyspnea by seven DPE. With progression of the disease the chicken developed torticollis and had pneumonic and encephalitic lesions when the study was terminated at 24 DPE.

Newcastle disease virus was recovered from the tissues of all chickens used for evaluation of isolates B8-C/12, B9-0/376, and Co8-0/181; the first CEC used for isolates A99-C/84 and B22-C/112; and the chicken inoculated with isolate B1-C/312. Nine of the 13 NDV isolates obtained from backpassage chickens were then selected for in vivo and in vitro characterization. The four viruses not characterized were isolated from the inoculated and second contact exposure chickens used for evaluation of isolates B9-0/376 and Co8-0/181. The results of those studies are listed in Table 8.

Table 8. *In vivo* and *in vitro* characterization of nine Newcastle disease viral isolates obtained from backpassage chickens

Isolate[a]	Backpassage chicken No.[b]	Diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)
A99-C/84	CEC 1-653A[c]	0.5			69	32	IS(5)[d]
		1.0					
		1.5	3				
		2.0	74				
		2.5	23				
	CEC 1-653B[c]	0.5			62	30	IS(<5)
		1.0	5				
		1.5	14				
		2.0	56				
		2.5	20	1			
		3.0	4				

[a]Viral isolate code indicates the bird species and the individual's number - oral (O) or cloacal (C) swab specimen/day postexposure of isolation: A=budgerigar; B=parrot; Co=conure.

[b]Backpassage chicken No. code is as follows: IC=inoculated chicken; CEC 1 and CEC 2=first and second contact exposure chicken, respectively.

[c]Isolate 653A was obtained from the brain and 653B was obtained from a composite of the lung, trachea, and spleen.

[d]IS indicates that the hemagglutinin was not stable at ≥15 min. Estimated stability is listed in parentheses.

Table 8. (Continued)

Isolate	Backpassage chicken No.	Diameter (mm)	Plaque assay % clear	Plaque assay % red	MDT (hr)	Hemagglutination elution (hr)	Stability of hemagglutinin at 56C (min)
B8-C/12	IC-2618	0.5			66	49	IS(10)
		1.0					
		1.5	83	17			
	CEC 1-2630	0.5			66	98	IS(10)
		1.0		13			
		1.5		33			
		2.0		47			
		2.5		7			
	CEC 2-3789	0.5	17		64	98	IS(10)
		1.0	83				
B22-C/112	CEC 1-3767	0.5	4		62	120	IS(10)
		1.0	46				
		1.5	50				
B1-C/312	IC-4145	0.5	16		62	49	IS(5)
		1.0	68				
		1.5	11				
		2.0	5				
B9-0/376	CEC 1-3776	0.5	5		70	114	15
		1.0	24				
		1.5	6	59			
		2.0		6			
Co8-0/181	CEC 1-1926	0.5			66	60	IS(5)
		1.0		8			
		1.5		79			
		2.0		13			

DISCUSSION

The clinical responses of the three psittacine species evaluated in these studies were consistent with those previously described by Lüthgen (33), Lüthgen and Wachendörfer (34), Pohl (37), and Wachendörfer and Lüthgen (45). Fourteen of 71 nuns exposed to the COPI-VVNDV developed similar symptoms. Canaries and mynahs exposed to VVNDV remained relatively asymptomatic.

The clinical disease observed in pet birds resembled the neurologic sequelae seen in domestic chickens surviving an epidemic of VVND. However, death losses often exceed 90% for susceptible chickens. This is in sharp contrast with the maximum mortality rate of 55% observed in these studies for halfmoon conures.

Gross lesions comparable to those found in chickens succumbing to VVNDV were not found in budgerigars, parrots, canaries, mynahs, and nuns at necropsy. Also, the distribution of hemorrhagic visceral lesions in conures differed from that seen in domestic fowl. In chickens hemorrhages are usually present in the proventriculus and ventriculus, and in the upper and lower intestinal tract associated with lymphoid aggregates. Hemorrhagic lesions in the conures were restricted to the proventriculus, proventriculus-ventriculus junction, and the small intestine.[1] Since extrapolation of data between species of pet birds is not possible, the

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[1]No bacterial pathogens were consistently isolated from necropsy tissue specimens negative for NDV isolation for five of the six species evaluated. *Escherichia coli* was isolated from the two parrots that succumbed to COPI-VVNDV infection, but its significance is unknown. See Appendix B.

responses of six pet bird species were evaluated to determine the epidemiologic impact of VVND.

Parameters considered were cause specific morbidity rate, point prevalence, crude and cause specific death rates, and absolute risk. For calculation of the cause specific morbidity rate and point prevalence, a case was defined as any bird that had seroconverted to NDV, had clinical signs of ND, or had NDV isolated from oral or cloacal swab specimens following direct or contact exposure to the COPI-VVNDV. Death was considered to have been due to NDV if NDV had previously been isolated from oral or cloacal swab specimens, or from tissue specimens collected at necropsy for calculation of the cause specific death rate. All the criteria listed above were used to determine the absolute risk of budgerigars, parrots, canaries, conures, mynahs or nuns becoming infected with NDV following direct or contact exposure to COPI-VVNDV.

In an epidemiologic sense, the communicability of a disease tends to vary inversely with the length of the period of shedding. Therefore, for the most accurate assessment of the cause specific morbidity rate, point prevalence, and crude and cause specific death rates, an evaluation period of 0 through 28 DPE was used. However, on an individual basis, the absolute risk of becoming infected with NDV if chronic shedding of the virus occurred would be greatest over the longest period of time. For that reason, the absolute risk was calculated from all data collected for each species during the studies.

It is important in the epidemiologic evaluation of these data whether free-flying birds of the six species would normally be found in groups of three to four birds as used in these studies. According to Forshaw and Cooper (22), the three psittacine species examined are commonly observed feeding in groups ranging from two birds up to flocks of 100 or more birds. Specifically, one to two pairs and as many as 20 Amazon parrots have been found roosting in trees. When conures are not breeding, the birds are often seen in groups of four to eight birds. During the breeding season both the parrots and conures are found in pairs.

Budgerigars are limited to Australia in their natural distribution and have also become established in Florida, U.S.A. They ordinarily congregate in small parties of several birds, as well as in large flocks. Again, during the breeding season, the birds are found in pairs.

The three remaining species evaluated in these studies are members of very gregarious families; the Fringillidae (finches), Estrildidae (finch-like birds), and Sturnidae (starlings). Thus, the communal habits of the six species examined would offer ample opportunity for exchange of NDV from bird to bird in groups comprised of three or more birds.

The results of the epidemiologic analyses are listed in Tables 9, 10, 11, and 12. According to the results obtained for the cause specific morbidity rates, it would be reasonable to expect that all budgerigars, parrots and conures would develop NDV infection after direct or contact exposure to COPI-VVNDV over a 28-day observation period. Almost all directly exposed nuns and 83% of the nuns exposed by contact would become infected with NDV. Similarly, a large percentage (84%) of aerosol-exposed mynahs and 50% of the mynahs exposed by contact would subsequently develop NDV infections. As indicated in Table 9, canaries were more refractory to infection by direct or contact exposure to VVNDV.

Table 9. Cause specific morbidity rate[a] for six species of birds after aerosol and contact exposure to viscerotropic velogenic Newcastle disease virus

Bird	Aerosol exposure	Contact exposure
Budgerigar	100/100	100/100
Parrot	100/100	100/100
Canary	19/100	11/100
Conure	100/100	100/100
Mynah	84/100	50/100
Nun	98/100	83/100

[a]Cause specific morbidity rate = $\frac{\text{Number of new cases over a time period}}{\text{Population at beginning of period of study}} \times K$; K = 100.

The weekly morbidity rates (point prevalence) for the six species evaluated in these studies are listed in Table 10. Again, the three psittacine species were most susceptible to NDV as indicated by their point prevalence values on eight and twelve DPE. However, a marked difference in the susceptibility of nuns and mynahs was demonstrated from that determined by the cause specific morbidity rates. Mynahs were markedly less susceptible than nuns to infection by NDV during the first two weeks postexposure. Canaries were almost refractory to infection by NDV.

Table 10. Point prevalence[a] for six species of birds after aerosol and contact exposure to viscerotropic velogenic Newcastle disease virus

	Day postexposure			
Bird	8	12	21	28
Budgerigar	63/100	31/100	30/100	15/100
Parrot	100/100	100/100	78/100	84/100
Canary	2/100	2/100	0/100	1/100
Conure	76/100	44/100	29/100	3/100
Mynah	15/100	11/100	13/100	8/100
Nun	54/100	33/100	15/100	14/100

[a]Point prevalence = $\frac{\text{Total cases present at a given time}}{\text{Population at that time}}$ x K; K = 100.

By 28 DPE, death losses were most severe for the conures. As indicated in Table 11, all conure, parrot, and budgerigar deaths could be directly related to previous infection with NDV. Parrot mortality rates probably would have been lower if the initial disease situation had not been complicated by mycotic airsacculitis and pneumonitis. As reflected by final respective cumulative mortality rates of 43% and 0%, very little parrot mortality occurred after treatment of the birds with amphotericin B. A more remarkable fact was that all contact-exposed parrots survived infection with COPI-VVNDV.

Cause specific death rates for aerosol-exposed mynahs and nuns were similar to those observed for aerosol-exposed budgerigars. Canary crude and cause specific death rates were minimal.

Table 11. Crude and cause specific death rates[a] for six species of birds after aerosol and contact exposure to viscerotropic velogenic Newcastle disease virus

	Crude death rate		Cause specific death rate	
Bird	Aerosol exposure	Contact exposure	Aerosol exposure	Contact exposure
Budgerigar	16/100	6/100	16/100	6/100
Parrot	30/100	0/100	30/100	0/100
Canary	10/100	5/100	6/100	2/100
Conure	41/100	35/100	41/100	35/100
Mynah	22/100	12/100	22/100	6/100
Nun	19/100	11/100	17/100	11/100

[a]Crude death rate = $\frac{\text{Deaths occurring in a selected time period}}{\text{Population at beginning of period of study}} \times K$;

cause specific death rate = $\frac{\text{Deaths from a given cause over a time period}}{\text{Population at beginning of period of study}}$ x K; K = 100.

Death rates observed for contact-exposed birds would likely reflect the mortality pattern that might occur following the introduction of VVNDV into a free-flying bird population. Such an introduction would best be effected by one to several birds of a given species being directly exposed to VVNDV on infected domestic poultry premises where large quantities of highly infectious aerosols would be generated by diseased chickens.

The absolute risk of becoming infected after exposure to VVNDV was greatest for the budgerigar, parrot, conure, and nun (100/100). Mynahs were slightly less susceptible to NDV. Canaries were most refractory to infection with COPI-VVNDV. Thus, in a highly restricted area, the risk that free-flying birds would become infected with NDV by contact exposure was great for five of the six species evaluated in these studies (Table 12).

After 28 DPE three of the six species, budgerigars, mynahs, and parrots, developed chronic NDV infections. Similar infections of long duration were observed for conures and nuns. Infected canaries rapidly eliminated the virus and the birds did not develop chronic NDV infection as determined by virus isolation results for oral and cloacal swab specimens. Therefore, only recently exposed canaries would be likely to serve as a source for infection of other susceptible avian species. Conversely, from three through 84 DPE two or more budgerigars, parrots, conures, mynahs, or nuns were shedding NDV in their oral and/or cloacal secretions and could have served as a source for exposure of domestic fowl to VVNDV.

Peak seroconversion rates for four of the six species were similar to their 28 DPE cause specific morbidity rates. The maximum seroconversion rates observed for mynahs and nuns were lower than their morbidity rates but may have been similar if the blood samples had been retained for later evaluation by the egg bit NT, as was done for budgerigar blood specimens.

Mean $\log_{10}$ LaSota HI antibody titers were greatest for parrots (3.0) and conures (1.5). Maximal serologic responses were $\leq$0.5 for budgerigars, canaries, mynahs, and nuns. However, budgerigar blood specimens further tested by the homologous HI and egg bit NT revealed a definite serologic response coinciding with a marked clinical response. This supported an emerging hypothesis that COPI-VVNDV might have mutated by passage through

Table 12. Absolute risk[a] of becoming infected following aerosol or contact exposure to viscerotropic velogenic Newcastle disease virus

Bird	Aerosol exposure	Contact exposure
Budgerigar	100/100	100/100
Parrot	100/100	100/100
Canary	19/100	11/100
Conure	100/100	100/100
Mynah	90/100	62/100
Nun	100/100	100/100

[a]Absolute risk = $\frac{\text{Number of cases}}{\text{Population at risk}}$ x K; K = 100.

the budgerigar. A 100% seroconversion rate by egg bit NT at 63 DPE for birds that were negative by the LaSota HI test supported that hypothesis.

Ongoing characterization studies for 167 budgerigar NDV isolates also demonstrated that later isolates were markedly different than COPI-VVNDV. Thirteen of 17 isolates obtained after 84 DPE no longer produced large red plaques when assayed. Plaques produced by the 13 viruses were clear and similar in size to those produced by Roakin NDV. However, the four remaining isolates did produce large red plaques when assayed, and as indicated by the chicken backpassage studies, were probably VVND viruses. Therefore, chronically infected budgerigars previously exposed to VVNDV could serve as a source of VVNDV for other susceptible avian species. Similarly, 25 of 30 selected parrot isolates obtained after 84 DPE were clear-plaquing NDV mutants. The five remaining isolates were VVND strains which demonstrates that the parrot can serve as a source for dissemination of VVNDV after 84 DPE (Appendix A). Despite the sporadic isolation of VVNDV from budgerigars and parrots as late as 137 and 376 DPE, respectively, attempts to identify a nidus for chronic NDV infection by the culture of organ explants were unsuccessful.

Concurrent embryonated chicken egg mean death time assays did not characterize any of the isolates as lentogenic (≥100 hours) NDV. All isolates had mean chicken embryo death times that were characteristic of velogenic and/or mesogenic ND viruses (42 to 94 hours).

To further evaluate the apparent mutation of COPI-VVNDV to mesogenic NDV, five sequential isolates from three budgerigars and two parrots were examined by the hemagglutination-elution and hemagglutinin-thermostability assays. Thirty-two other isolates from budgerigars, parrots, canaries, conures, mynahs, and nuns were also examined for comparison with findings for the sequential isolates.

As depicted in Figure 20, the rate of elution generally increased with the progression of time for sequential isolates from four of the five birds evaluated. The same trend was observed for the other 32 isolates evaluated by this technique. Fifty-six of the 57 isolates as well as COPI-VVNDV were slow eluting viruses (>24 hours). One isolate, B 1-C/312, was a rapidly eluting virus with an elution time of four hours that was similar to the time range reported for B 1 virus (one to eight hours; average time of 3.2 hours). However, isolate B 1-C/312 produced 0.5 mm to 1.0 mm plaques without $MgSO_4$ and DEAE dextran, whereas

B 1 virus produces pinpoint plaques only with $MgSO_4$ and DEAE dextran when assayed (8,41). This indicates that B 1-C/312 is a mild mesogenic and not a lentogenic strain.

Newcastle disease viral strains with virus populations containing two or more genotypes as determined by plaque type have an elution pattern that is determined by one of the genotypes. This relationship was clearly illustrated in studies conducted by Spalatin et al. (41). From those studies, it was determined that only a marked change in the genotype composition of a heterogenous culture, such as COPI-VVNDV, would change the elution pattern. Therefore, it is reasonable to assume that isolate B 1-C/312 had undergone marked mutation in passage through parrots. Conversely, the results of the hemagglutination elution assays also indicate that the 56 remaining isolates had not changed markedly. Rather, it appears that the viral plaque populations present when the viruses were isolated were similar in their genetic makeup to plaque populations of COPI-VVNDV. Thus, according to the results of these assays a mutation of COPI-VVNDV probably did not occur by passage through pet birds. Instead, the small (0.5 to 1.5 mm) clear-plaquing viral subpopulation of that virus was apparently favored in passage through the pet birds to the disadvantage of the large red-plaquing viral subpopulation. Repeated sporadic isolations of NDV from the pet birds after four to eight months postexposure that produced large red plaques when assayed supports the following hypothesis.

After the acute VVNDV infection had subsided by three weeks to two months postexposure depending on the species under evaluation, the small clear-plaquing viral subpopulation of COPI-VVNDV was by an unknown mechanism favored in propagation within the birds to the disadvantage of the large red-plaquing viral subpopulation. The large red-plaquing viral subpopulation may have barely been maintained in the birds or replicated in very small quantities. By plaque assay only $10^{1.5}$ to $10^{2.0}$ tissue culture 50% infective doses ($TCID_{50}$) of a viral isolate can be assayed. Therefore, with an average NDV isolate titer of 10^7 to 10^8 $TCID_{50}$ as much as $10^{5.5}$ to $10^{6.5}$ $TCID_{50}$ of virus would not be detected by a given test. Accordingly, it is likely that a large red-plaquing viral subpopulation with a titer of $10^{0.5}$ to $10^{2.0}$ $TCID_{50}$ would not be found when plaque assays were conducted. More likely, however, is that if the large red-plaquing viral subpopulation was not being propagated in large quantities within pet birds having chronic NDV infections, that viral subpopulation may not have been collected with the moistened swab when virus isolation specimens were obtained.

According to the second assay used to assess the characteristics of many predominantly small clear-plaquing NDV isolates obtained from the pet birds, similar results were obtained with respect to possible mutation of COPI-VVNDV by passage through the six bird species. For the sequential viral isolates (Figure 21) the hemagglutinin of three isolates was stable at 56C for ≥15 minutes whereas the 22 remaining isolates as well as COPI-VVNDV were not stable at 56C for 15 minutes. Likewise, a majority (66%) of the hemagglutinins of the 32 other isolates evaluated (Appendix A) were not stable at 56C for 15 minutes. Therefore, the results of the hemagglutinin-thermostability assays also lend support to the previously stated hypothesis that marked changes of COPI-VVNDV genotype composition did not occur by passage through the six pet bird species. Rather, apparently, the replication of the clear-plaquing subpopulation was somehow favored in passage through pet bird species.

To determine if predominantly small clear-plaquing isolates had a large red-plaquing viral subpopulation in low titer ($10^{0.5}$ to 10^2 $TCID_{50}$) or if the genetic potential existed for reversion of a small clear-plaquing isolate to a large red-plaquing VVNDV, susceptible chickens were inoculated with 15 clear-plaquing isolates for backpassage studies (Table 6). The clinical response observed for the three chickens used for each of the 15 isolates was consistent with the response seen in fully susceptible chickens vaccinated with mesogenic strains of NDV. The absence of seroconversion by the NDV HI test for one or more of the chickens in three of the 15 groups does not necessarily indicate that a NDV infection was not established in those chickens. That would be possible, but a more likely occurrence would be that those three isolates established a limited, local infection in the intestinal tract of the inoculated chickens, and consequently failed to produce a circulating antibody response. Similar responses have been observed in studies using NDV vaccines. Benson et al. (10) reported a mean HI antibody titer of 1:2.6 for 29 ten-week-old chickens vaccinated with LaSota NDV by the intraocular route at 16 days of age, and the spray technique at five weeks of age.

The four NDV isolates, A 99-C/84, B 8-C/12, B 9-0/376, and Co 8-0/181, were used as "virulent" control viruses on the basis of production of large red plaques when the viruses were assayed. Infection with isolates B 8-C/12, B 9-0/376, and Co 8-0/181 resulted in an acute fulminating disease typical of VVND, and at necropsy the three isolates were pathotyped as VVNDV. Similarly, infection with isolate A 99-C/84 resulted in an acute clinical response for both inoculated and contact-exposed chickens. The inoculated chicken eventually recovered while both contact-exposed chickens succumbed to the disease by six and seven DPE. Pneumonic and pneumoencephalitic lesions were observed at necropsy. The neurologic clinical sign, torticollis, as observed for the second contact-exposed chicken, is commonly seen in chickens that have survived an outbreak of VVND. If, for pathotyping purposes, ten susceptible chickens were inoculated via the vent with a 1:10 or 1:100 dilution of that virus (A 99-C/84), it is highly probable that at least one of the chickens succumbing to the subsequent disease would have hemorrhagic visceral lesions at necropsy and the virus would therefore be pathotyped as VVNDV.[1]

Nine of the 13 viral isolates obtained from backpassage chickens were subsequently analyzed by the plaque-assay, embryonated-chicken-egg-mean-death time, hemagglutination-elution, and hemagglutinin-thermostability techniques to determine if any dramatic shifts had occurred for these parameters during the backpassage process.

Plaque-assay results for backpassage chicken isolates CEC 1-653A and CEC 1-653B indicated the following. The viral subpopulation (A) present in the brain was a large clear-plaquing mutant derived from the 1.5 and 2.0 mm clear-plaque subpopulation of isolate A 99-C/84. A different viral

[1]Confirmed in personal communication with Dr. R. P. Hanson, Department of Veterinary Science, University of Wisconsin at Madison, June, 1976.

subpopulation mixture was present in isolate B that was obtained from a composite tissue pool of lung, trachea, and spleen. For that isolate apparently 99% of the viral population was derived from the clear-plaquing components of A 99-C/84, and 1% of the viral population was derived from the red-plaquing components of A 99-C/84. Values obtained for mean death time, hemagglutination elution, and hemagglutinin thermostability were comparable to those of A 99-C/84.

Viral subpopulations detected by plaque assay for three chickens exposed to B 8-C/12 were again variations from the parent virus stock as indicated by comparison of Table 8 to Table 6. Most of the remaining in vitro parameters for the three backpassage chicken isolates were similar to those of B 8-C/12. Only the hemagglutination-elution time of 49 hours for isolate IC-2618 was appreciably different from the value obtained for B 8-C/12. However, virus IC-2618 would still be classified as a slow-eluting strain of NDV since its elution time was in excess of 24 hours.

The virus isolated from backpassage chicken CEC 1-3767 was quite similar to parrot isolate B 22-C/112 used for that study. An increase in plaque diameter was demonstrated for the backpassage chicken isolate, as well as an increased elution time. Again, the changes detected were minor and of little significance.

Likewise, an increase in plaque diameters was also observed for backpassage chicken isolate IC-4145. Of greater importance was the isolate's reversion to slow elution and hemagglutinin instability at 56C as compared to the parrot isolate (B 1-C/312) used for the study. The marked changes in those three _in vitro_ parameters indicate that one of two things may have occurred. In view of the minor changes observed for A 99-C/84, B 8-C/12, and B 22-C/112, the 1.5- and 2.0 mm-plaquing viral subpopulations were probably present at very low concentrations in parrot isolate B 1-C/312. By backpassage in susceptible chickens those two subpopulations may have been replicated at a greater multiplicity rate than in the embryonated chicken eggs used for propagation of isolate B 1-C/312 from the parrot cloacal swab specimen. With such a marked change in viral subpopulation concentrations it would not be unreasonable to expect other changes in the _in vitro_ assays used for evaluation of NDV isolates.

Alternatively, depending on the conditions for each assay of a given NDV isolate, a minor shift in plaque diameters was not uncommon. By inadvertent application of the second overlay to cultures at 78 to 80 hours postinoculation instead of 72 hours postinoculation, 2.0 to 3.0 mm larger plaque diameters were observed in control cultures. Variations of one to three hours prior to or after 72 hours postinoculation for application of the second overlay could effect a decrease or an increase in plaque diameters of 0.5 mm. This was seen for NDV isolates from backpassage chickens exposed to isolates A 99-C/84, B 8-C/12, and B 22-C/112. Therefore, an increase or a decrease of 0.5 mm in plaque diameters for backpassage chicken isolates, when they were compared to the pet bird isolates used for exposure would not be considered a significant change according to the NDV plaque-assay technique.

The minor changes observed in plaque subpopulations could not be considered as a definite indication that a virus had changed by backpassage in susceptible chickens. Consequently, the other *in vitro* assays used in these characterization studies were needed to determine if a shift in viral subpopulations had occurred. Accordingly, a dramatic reversion of the hemagglutination-elution and hemagglutinin-thermostability characteristics for backpassage chicken isolate IC-4145 as compared to B 1-C/312 NDV supported the change detected by plaque assay. Results obtained for IC-4145 indicated that a major change in ND viral subpopulations did occur for parrot isolate B 1-C/312 by backpassage in a susceptible chicken.

By plaque assay different viral subpopulations were observed for parrot isolate B 9-0/376 and conure isolate Co 8-0/181 after backpassage in susceptible chickens. Backpassage isolate CEC 1-3776 had slightly diminished red-plaquing viral subpopulations when assayed as compared to B 9-0/376. A more dramatic "shift" was observed for backpassage isolate CEC 1-1926. No clear-plaquing viral subpopulation was detected, whereas for Co 8-0/181 NDV, 57% of the virions assayed produced clear plaques. However, little change was noted for the two backpassage isolates according to the three remaining *in vitro* assays used to characterize the viruses. The mean death times were virtually identical to those of the parrot and conure isolates. Similarly, the backpassage isolates were still slow-eluting viruses with heat-stable and heat-labile hemagglutinins, respectively.

Therefore, the last two backpassage chicken isolates characterized as listed in Table 8 were relatively stable under the influence of propagation in susceptible chickens for two successive passages. The marked differences in plaque morphology and color for the two isolates when compared to the pet bird isolates used for chicken exposure were not unexpected. Rather, as noted by Estupinan and Hanson (20), different ND viral subpopulations are found to be in higher titers in inoculated chickens at different days postexposure. Apparently, the large red-plaquing viral subpopulations common to both the pet bird and backpassage isolates were primarily responsible for the mean-death time, hemagglutination elution, and hemagglutinin thermostability assay results.

The results of these studies of the impact of VVNDV on six pet bird species, and the virus-host interactions, have amply demonstrated the genetic plasticity of COPI-VVNDV. The virus readily adapted to five of the six pet bird hosts investigated. Through passage in those birds the initial 1% clear-plaquing viral subpopulations were favored and generally were replicated to a higher overall population density as demonstrated by plaque assay of later pet bird isolates. Characterization of selected viral isolates indicated that hemagglutination elution was the most consistent biomarker for COPI and subsequent NDV pet bird isolates. With one exception all viruses tested were slow eluters, and upon backpassage in a susceptible chicken the backpassage isolate for that virus was also a slow eluter. The presence of sporadic large red-plaquing viral isolates among later pet bird isolates indicated that the viscerotropic velogenic viral subpopulation apparently was maintained in pet birds with chronic NDV infections. However, the site for maintenance of the chronic ND viral infection could not be determined by organ culture studies. Thus, the mechanism by which VVNDV is maintained in pet birds with chronic NDV infections remains an enigma.

In conclusion, five of the six pet bird species used in these studies, the budgerigar, parrot, conure, mynah, and nun, were highly susceptible to VVNDV. Ample serologic evidence was obtained to confirm that these species were infected with NDV during the studies. Duration of infection as detected by sampling two orifices, the oropharynx and cloaca, was greatest for the parrots. Parrots shed NDV in excess of one calendar year. Budgerigars, conures, mynahs, and nuns all sporadically shed NDV in excess of 83 days after exposure to COPI-VVNDV. The bulk of the later isolates appeared to be mesogenic strains of NDV that had been derived from COPI-VVNDV during passage through the pet birds. However, VVNDV strains were also present among the later viral isolates, indicating that once infected with VVNDV birds of these five species would pose a serious threat to the poultry industry.

SUMMARY

Six pet bird species were exposed to a psittacine isolate of viscerotropic velogenic Newcastle disease virus (VVNDV) for evaluation of their clinical and serologic responses and viral excretion. Species examined were the budgerigar (Melopsittacus undulatus), yellow-headed Amazon parrot (Amazona ochrocephala oratrix), canary (Serinus canarius), halfmoon conure (Aratinga canicularis eburnirostrum), lesser hill mynah (Gracula religiosa indica), and black-headed nun (Lonchura malacca). To facilitate statistical evaluation of the results from these investigations, at least 42 birds were examined for each species.

The clinical response was most marked for the budgerigars, parrots, and conures. A minimal clinical response was noted for the nuns. By three to five days postexposure (DPE) some birds had ruffled plumage, conjunctivitis, and evidence of central nervous system damage. Symptoms of central nervous system dysfunction were ataxia, wing tremors, paralysis of the extremities, and tremors of the head accompanied by nodding and jerking movements.

Mortality was most severe in the halfmoon conures. Fifty-five percent of the birds died by 203 DPE. Fewer budgerigars, parrots, and nuns succumbed during the studies; 22, 29, and 21% respectively.

No clinical signs other than progressive death losses were noted for canaries and mynahs exposed to VVNDV. The two species had respective cumulative morality rates of 25 and 21%. Visceral lesions commonly seen

in chickens succumbing to VVNDV were not observed in dead budgerigars, parrots, canaries, conures, mynahs, and nuns.

Newcastle disease virus was detected for protracted periods in the oral and cloacal secretions of budgerigars, parrots, conures, mynahs, and nuns. Infected canaries rapidly eliminated the virus and did not develop chronic NDV infections.

Serologic evaluation using the hemagglutination-inhibition and neutralization tests also indicated that prolonged NDV infections were established in five of the six pet bird species examined. A minimal seroconversion rate of 13% was observed for canaries.

Following the _in vivo_ studies in the six pet bird species, 279 NDV re-isolates were selected for characterization by the embryonated-chicken-egg-mean-death-time, plaque-assay, hemagglutination-elution (at 4C), and hemagglutinin-thermostability (at 56C) techniques. Initially, the 279 isolates were screened by the mean-death-time and plaque-assay techniques, and five sequential isolates were chosen for each of three budgerigars and two parrots for characterization by the other two _in vitro_ assays to determine if viral mutation had occurred through pet bird passage.

Nineteen isolates were then selected for chicken backpassage studies. Fifteen of the 19 isolates were chosen for potential avirulence for eight-week-old domestic chickens. The four remaining isolates produced large red plaques when assayed and were therefore used as virulent virus controls likely to be VVNDV.

Eleven of the 15 isolates selected for potential avirulence established asymptomatic infections in backpassage chickens. A more extensive disease process was observed in chickens exposed to the four remaining potentially avirulent NDV isolates. Affected birds usually experienced a mild respiratory disease and several birds subsequently developed airsacculitis. One of the 12 chickens inoculated with one of the four isolates later developed torticollis. In summary, the clinical response observed for the three chickens used for each of the 15 isolates was consistent with the response seen for fully susceptible chickens vaccinated with mesogenic strains of NDV.

Three of the four isolates used as virulent virus controls were pathotyped as VVNDV when the backpassage chickens were necropsied. The fourth control virus produced an acute disease process in three backpassage chickens and was lethal for the two chickens exposed by contact with the first chicken that had been inoculated with the virus. Pneumonic and pneumoencephalitic lesions were noted at necropsy. The inoculated chicken eventually recovered. The isolate was classified as a probable strain of VVNDV.

Subsequent _in vitro_ characterization of selected backpassage chicken NDV isolates demonstrated little change in the four parameters originally evaluated for the pet bird isolates used for the backpassage studies. Although the psittacine isolate slowly evolved to relatively avirulent strains of NDV by passage in pet birds, a similar change did not occur during the chicken backpassage studies. Therefore, a rapid mutation of the 15 potentially avirulent pet bird isolates did not occur by backpassage through susceptible chickens. Accordingly, additional intensive studies are needed to clearly define the potential for rapid mutation of similar strains of NDV by serial passage in susceptible chickens.

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APPENDIX A: CHARACTERIZATION OF NEWCASTLE DISEASE VIRUS ISOLATES FROM SIX PET BIRD SPECIES PREVIOUSLY EXPOSED TO VISCEROTROPIC VELOGENIC NEWCASTLE DISEASE VIRUS

Table A1. Characterization of Newcastle disease virus isolates from six pet bird species previously exposed to viscerotropic velogenic Newcastle disease virus

Isolate[a]	MDT (hr)	Plaque assay[b] R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
A59-0/3	67	X				
A40-0/3	66		X			
A44-0/3	59	X				
A67-0/3	65	X				
A60-0/3	56	X				
A5-0/3	58	X				
A93-0/3	62	X				
A89-0/3	68	X			66	IS(5)
A78-0/3	56	X				
A100-0/3	46		X			
A10-0/3	64	X				
A86-0/5	57	X				
A2-0/5	65	X				
A83-0/5	57	X				
A76-0/5	60	X				
A47-0/5	51	X				
A53-0/5	72	X				
A97-C/5	64	X				
A105-0/5	48	X				
A72-0/5	74	X				

[a]Viral isolate code indicates the bird species and the individual number - oral (O) or cloacal (C) swab specimen/day postexposure of isolation, or tissue (T): A=budgerigar; B=parrot; C=canary; Co=conure; M=mynah; N=nun.

[b]R=~99% large red plaques; R/C=mixture of large red plaques and clear plaques; C=clear plaques of different sizes, usually ranging from 0.5 to 1.5 mm in diameter.

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
A39-C/5	65	X				
A61-0/5	59	X				
A76-C/5	53	X				
A59-0/5	ND[c]	X				
A73-C/8	63		X		39	IS(5)
A13-0/8	47	X				
A59-0/8	61		X			
A47-0/8	53		X			
A105-C/8	54	X				
A17-C/8	47	X				
A37-C/8	54	X				
A90-C/8	55	X				
A102-0/8	54	X				
A88-0/8	65	X				
A90-0/8	55	X				
A97-C/8	53	X				
A48-0/8	58	X				
A49-0/8	60	X				
A57-0/8	65	X				
A73-0/8	59	X				
A82-C/8	52	X				
A37-0/8	59		X			
A102-C/8	58	X				
A51-C/8	54		X			
A54-0/12	58	X				

[c]Not determined.

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
A89-0/12	63	X			68	IS(5)
A73-C/12	64	X			76	IS(10)
A24-0/12	72		X			
A69-0/12	50	X				
A102-0/12	66	X				
A72-C/12	50			X		
A49-0/12	58	X				
A42-0/12	60	X				
A87-0/12	61	X				
A3-0/12	53	X				
A37-0/12	46	X				
A102-C/12	68	X				
A53-C/12	57	X				
A63-0/21	70			X		
A70-C/21	57	X				
A63-C/21	70			X		
A95-0/21	50			X		
A94-C/21	61		X			
A85-C/21	65			X		
A92-0/21	62			X		
A31-0/21	42	X				
A44-C/21	46	X				
A38-C/21	65	X				
A97-C/21	55			X		
A99-C/28	65	X			64	IS(5)
A28-C/28	59	X				
A9-0/28	67			X		
A101-C/28	52			X		

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
A66-0/28	52		X			
A88-C/28	59		X			
A49-0/28	50			X		
A92-0/28	65	X				
A80-0/28	52	X				
A97-C/28	49	X				
A53-0/35	49	X				
A43-C/35	54		X			
A41-C/35	56	X				
A56-0/35	51		X			
A99-C/35	62		X		74	IS(<5)
A95-0/35	57			X		
A98-C/35	63	X				
A41-0/35	59	X				
A43-0/35	74			X		
A10-C/42	63	X				
A82-0/42	49	X				
A44-C/42	56	X				
A93-0/42	50	X				
A21-0/42	63	X				
A91-C/42	59	X				
A99-0/49	66		X		44	IS(5)
A58-0/49	69		X			
A1-C/49	60	X				
A58-C/49	63		X			
A47-0/49	58	X				
A45-C/49	57	X				

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay			Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
		R	R/C	C		
A72-0/49	63	X				
A78-C/49	69	X				
A65-C/49	53		X			
A96-C/49	59		X			
A75-0/49	58	X				
A91-0/49	52	X				
A73-C/56	66		X		48	IS(5)
A22-0/56	63	X				
A103-C/56	57		X			
A28-C/56	61	X				
A56-C/56	68	X				
A93-0/56	67		X			
A51-0/56	58	X				
A102-0/56	58		X			
A89-0/56	66		X		55	15
A69-0/56	68		X			
A101-0/63	63	X				
A91-0/63	71	X				
A67-C/63	75		X			
A81-0/63	60	X				
A82-0/63	75		X			
A90-0/63	67		X			
A41-0/63	60	X				
A82-C/63	54	X				
A71-0/63	65			X		
A89-0/70	69			X	64	IS(5)

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
A25-0/70	55		X			
A9-0/70	57	X				
A25-C/70	65		X			
A39-0/70	64		X			
A56-0/70	60		X			
A37-C/70	64	X				
A44-C/70	61		X			
A75-0/70	61	X				
A92-0/70	63		X			
A96-0/70	56		X			
A30-C/77	61			X		
A9-C/77	62		X			
A20-C/77	60			X		
A73-C/77	58	X				
A22-C/77	64	X				
A75-0/77	72		X			
A63-0/77	63			X		
A88-0/77	56	X				
A99-C/84	62		X		66	IS(5)
A41-C/84	71	X				
A92-C/84	53		X			
A42-C/84	54		X			
A93-0/84	61			X		
A73-0/98	71		X		66	IS(5)
A65-0/98	75			X		
A10-0/98	55	X				
A73-0/112	68			X	120	IS(10)

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
A99-0/112	72			X	120	15
A80-C/112	63			X		
A79-C/112	70		X			
A94-C/112	59			X		
A71-C/119	ND			X		
A51-0/119	57			X		
A58-0/119	68			X		
A89-0/126	68			X	78	15
A28-0/126	64			X		
A83-C/126	68			X		
A21-0/133	82			X	120	20
A58-0/137	ND	X				
A64-C/137	67			X		
B22-C/3	67	X			46	IS(<5)
B8-C/3	62	X			120	IS(5)
B38-0/3	ND	X				
B22-0/3	ND	X				
B22-C/12	65		X		72	IS(5)
B8-C/12	65		X		113	IS(10)
B22-0/12	ND	X				
B38-C/12	ND	X				
B22-0/28	59		X		70	IS(10)
B22-C/28	64			X	70	IS(5)
B8-C/28	ND		X			
B38-C/28	ND			X		
B8-C/56	87			X	120	IS(10)

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
B31-0/56	ND			X		
B19-C/84	ND			X		
B8-C/112	71			X	120	IS(10)
B22-C/112	67			X	70	IS(5)
B8-0/112	ND			X		
B8-C/141	88			X	114	IS(<5)
B10-C/141	ND			X		
B19-C/141	ND			X		
B23-0/168	ND			X		
B25-C/168	ND			X		
B38-C/168	ND			X		
B22-C/196	70			X	46	IS(10)
B34-C/196	ND			X		
B15-C/196	ND			X		
B31-C/196	ND			X		
B31-C/217	ND			X		
B18-C/224	72			X	113	15
B14-C/224	ND			X		
B2-0/231	64	X			70	IS(<5)
B17-C/231	73	X			69	IS(10)
B1-C/238	96			X		
B10-C/238	82			X		
B6-0/238	ND	X				
B8-C/238	ND			X		

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
B23-C/245	98		X		142	IS(10)
B14-C/245	ND			X		
B1-C/259	66		X			
B17-C/312	91			X	120	IS(5)
B1-C/312	67			X	4	20
B9-O/376	73		X		43	30
B3-O/376	70		X		42	20
B13-C/376	65	X			45	IS(10)
C33-O/3	ND	X				
C46-O/3	ND	X			74	15
C82-C/3	ND	X				
C100-O/3	ND	X				
C123-O/3	ND	X				
Co1-O/3	63	X			42	IS(5)
Co23-C/3	ND	X				
Co45-O/3	ND	X				
Co29-O/3	ND	X				
Co20-C/3	ND	X				
Co16-O/12	71	X			45	IS(<5)
Co26-O/12	ND		X			
Co26-C/12	ND	X				
Co16-C/12	ND	X				
Co9-O/28	67	X			49	IS(10)
Co2-O/42	69	X			72	IS(5)
Co8-O/84	70			X	94	15

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
Co40-0/84	ND	X				
Co18-0/181	ND	X				
Co8-0/181	67		X		94	IS(10)
Co41-T	ND	X				
Co13-T	ND	X				
Co1-T	ND	X				
Co28-T	ND	X				
Co47-T	ND	X				
Co14-T	ND	X				
Co29-T	ND	X				
M1-0/3	65	X			46	IS(10)
M16-C/3	ND			X		
M30-0/3	ND	X				
M43-0/3	ND	X				
M15-0/3	ND			X		
M30-C/3	ND	X				
M6-0/3	ND	X				
M22-C/8	ND		X			
M5-0/12	ND		X			
M26-C/12	ND	X				
M14-0/12	ND	X				
M4-C/12	75	X			46	IS(5)
M1-C/28	67		X		113	15
M33-C/28	71			X	55	IS(10)
M2-C/28	ND	X				
M35-C/134	ND		X			

Table A1. (Continued)

Isolate	MDT (hr)	Plaque assay R	R/C	C	Hemagglutination elution time (hr)	Stability of hemagglutinin at 56C (min)
M25-0/140	73			X	120	20
M10-C/140	67		X		66	IS(5)
M23-0/140	ND		X			
M11-C/140	ND			X		
N38-C/3	ND	X				
N52-0/3	72	X			73	IS(10)
N22-0/3	ND	X				
N7-0/3	ND	X				
N31-C/3	ND	X				
N22-0/12	72		X		44	IS(<5)
N33-0/12	72	X			72	IS(5)
N55-0/12	ND	X				
N55-C/12	ND	X				
N8-C/12	ND	X				
N24-0/28	73			X	120	15
N59-0/28	78	X			4	IS(5)
N36-0/28	76			X	75	IS(<5)
N56-C/56	94			X	120	IS(10)
N47-0/56	ND			X		
N48-C/84	77			X	120	15
N7-T	ND	X				
N32-T	ND	X				
N4-T	ND	X				
N38-T	ND	X				

APPENDIX B: BACTERIA ISOLATED FROM NECROPSY TISSUES NEGATIVE FOR NEWCASTLE DISEASE VIRUS ISOLATION

Table B1. Bacteria isolated from necropsy tissues negative for Newcastle disease virus isolation

Species	Day of death (DPE)[a]	Bacteria isolated
Budgerigar	87	NBG[b]
	19	Flavobacterium lutescens
	95	Escherichia coli
	77	NBG
	97	Citrobacter freundii
	119	Lactobacillus sp.
	62	NBG
	62	NBG
	52	NBG
	89	Klebsiella pneumoniae
	98	No test - tissue decomposed at necropsy
	30	NBG
	19	NBG
Parrot	42	Pseudomonas aeruginosa, Escherichia coli
	88	Citrobacter freundii, Escherichia coli
Canary	71	NBG
	97	NBG
	13	NBG
	81	Staphylococcus epidermidis
	56	Citrobacter freundii
	37	Streptococcus faecalis

[a]Day postexposure.

[b]No bacterial growth.

Table B1. (Continued)

Species	Day of death (DPE)	Bacteria isolated
	76	NBG
	52	Paracolobactrum intermedium, Escherichia coli
	52	NBG
	79	NBG
	54	Intermediate coliform bacilli
	82	NBG
	38	Streptococcus faecalis
	25	NBG
	8	NBG
	20	NBG
	28	NBG
	91	NBG
	47	Streptococcus faecalis
	77	Enterobacter aerogenes
	35	Streptococcus faecalis
	35	Streptococcus faecalis
Conure	51	Escherichia coli, Klebsiella pneumoniae, Lactobacillus sp.
	51	Escherichia coli, Group B, Salmonella sp.
	39	NBG
	71	NBG
	59	NBG
	28	NBG
	54	NBG
	12	Citrobacter freundii, Staphylococcus epidermidis
	36	NBG

Table B1. (Continued)

Species	Day of death (DPE)	Bacteria isolated
	58	*Klebsiella pneumoniae*
Mynah	21	NBG
	21	NBG
	21	NBG
	12	*Proteus vulgaris*, *Escherichia coli*
	15	NBG
	77	*Proteus mirabilis*
Nun	116	*Enterobacter cloacae*, *Klebsiella pneumoniae*
	56	NBG
	35	*Staphylococcus epidermidis*
	84	NBG
	103	NBG
	115	*Escherichia coli*
	28	NBG
	143	*Enterobacter agglomerans*, *Klebsiella pneumoniae*, *Enterbacter cloacae*, *Streptococcus lactis*