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Newcastle disease viruses in birds in the Atlantic flyway: isolations, haemagglutination-inhibition and elution-inhibition antibody profiles

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Summary — The study involved 15 avian species with 5 012 attempts to isolate Newcastle disease viruses (NDV) from their faeces over a three-year period (1977–1979). NDV were isolated from asymptomatic adult Canada geese, nestling Royal terns, a juvenile European Mute swan, and adult Tundra swans on the Eastern flyway. Ring-billed gulls were negative for haemagglutination-inhibition (HI), elution-inhibition (EI) (anti-neuraminidase) antibodies, and NDV despite 3 403 isolation attempts. The EI antibody assay used a strain isolated from a Mute swan. The prevalence of EI antibodies in the swan and geese ranged from 3–41%, while the geometric mean titre (GMT) varied from 20–36. The prevalence of HI antibodies in the swans and geese ranged from 4–62%, while the GMT varied from 16–42. In each of three years (1977–1979), the adult Mute swans had a higher HI antibody prevalence than the juveniles ($P < 0.01$). Among the Mute swans the HI and EI assays detected serologic conversions and persistent antibodies over the three-year period. The HI and EI assays were effective in showing differences in antibody prevalences in populations of feral birds of different species and age. The EI assay is applicable for population studies of the anti-neuraminidase antibody.

Newcastle disease virus / isolation from birds / haemagglutination-inhibition antibody / elution-inhibition antibody / antineuraminidase antibody

Résumé — **Virus de la maladie de Newcastle chez les oiseaux du trajet migratoire atlantique : isolements et profils d'anticorps inhibant l'hémagglutination et inhibant l'éluion.** *L'étude a concerné 15 espèces aviaires et a mis en œuvre 5 012 essais d'isolement de virus de la maladie de Newcastle (NDV) à partir de leurs fèces, sur une période de trois années entre 1977 et 1979. Des virus NDV ont été isolés à partir d'oies du Canada adultes, de jeunes sternes royaux au nid, de cygnes*

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muets européens juvéniles et de cygnes de la toundra adultes au cours de leur migration vers l'est. Aucun de ces oiseaux ne présentait de signes cliniques. Les goélands à bec cerclé n'avaient pas d'anticorps inhibant l'hémagglutination (HI), ni d'anticorps inhibant l'élu-tion (EI) ; ils étaient également négatifs en isolement de virus NDV malgré 3 043 essais. Le test de titrage des anticorps inhibant l'élu-tion utilisait une souche isolée d'un cygne muet. La prévalence des anticorps EI chez les cygnes et les oies variait de 3 à 41 % alors que leur moyenne géométrique (GMT) variait de 20 à 36. La prévalence des anticorps HI dans ces mêmes espèces oscillait de 4 à 62 % avec une GMT comprise entre 16 et 42. Au cours de chacune des trois années (1977-1979), le cygne muet adulte montrait une prévalence d'anticorps HI supérieure à celle observée chez les juvéniles ($p < 0,01$). Chez le cygne muet, les réactions HI et EI ont permis de détecter au cours de cette période de trois années des séroconversions et des anticorps persistants. Les tests d'inhibition de l'hémagglutination et d'inhibition de l'élu-tion ont permis de révéler des différences dans la prévalence des anticorps, chez les populations d'oiseaux sauvages d'espèce et d'âge différents. Le test d'inhibition de l'élu-tion trouve des applications pour le suivi des anticorps antineuraminidase au sein des populations.

virus de la maladie de Newcastle / isolement à partir d'oiseaux / anticorps inhibant l'hémagglutination / anticorps inhibant l'élu-tion / anticorps antineuraminidase

INTRODUCTION

The studies of Newcastle disease virus (NDV) (paramyxovirus type 1) in the Atlantic flyway have examined a variety of ducks and Canada geese (*Branta canadensis*) (Debbie, 1971; Rosenberger et al, 1974, 1975; Deibel et al, 1985; Hinshaw et al, 1980, 1985). A review of NDV in each species and region has been compiled by Vickers and Hanson (1982).

In the present study swans and geese have been emphasized. Also, the possible role of gulls in the natural history of NDV has been examined. Predominant in the study was the European Mute swan (*Cygnus olor*) along the eastern shore of MD, USA in the upper Chesapeake bay. The locations and demographic information for the Mute swans was compiled by Reese (1980).

A strain of NDV which responded to elution-inhibition (EI) antibodies (Appleyard and Oram, 1977; Graves, 1992) was isolated from a Mute swan (swan 575) (Graves, 1979, 1996). The assay is not an estimate of the inhibition of neuraminidase (NA) activity using added substrates (Aminoff, 1961). The substrate on red blood cells (RBC) is used in measuring the anti-NA antibody.

The strain 575 provided the means to study the inhibition of the NA in a sufficient number of sera to estimate the prevalence of EI antibody (Graves, 1996).

To further explore and expand the natural history of NDV on the Atlantic flyway, birds were tested for antibodies and viruses. This report presents the isolation rates of NDV for 15 species and the profiles of haemagglutination-inhibition (HI) and EI antibody in adult and juvenile Mute swans, Tundra swans (*Cygnus columbianus*), and Canada geese. The capacity to measure prevalence and differences in prevalences of EI and HI antibody was tested using these populations of birds.

MATERIALS AND METHODS

Populations tested

Temporarily flightless Mute swan adults and juveniles, 13–16 weeks of age, were bled, faecal samples or cloacal swabs were collected, and the sex was determined during banding of family units in late summer in the Eastern bay and the Choptank river, MD, USA. Wintering adult Tundra swans and Canada geese were sampled at US Fish and Wildlife Service, Mattamuskeet and

Pungo Lake National Wildlife Refuges in eastern NC. Pen-reared quail (*Callipepla squamata*), Mallard ducks (*Anas platyrhynchos*) and adult Canada geese were sampled in the summer near Chestertown, MD; sera were drawn from captive geese in the Baltimore area and from feral geese in NC. Royal tern (*Thalasseus maximus*) nestlings, 5–6 weeks old, were sampled in mid-summer on Metomkin Island, VA. Adult Ring-billed gulls were sampled in central Baltimore city, MD. The number of samples assayed each month is listed elsewhere (Graves, 1992). Great Blue heron (*Ardea herodias*) nestlings, 5–6 weeks old, were sampled during mid-summer at Watts Island, VA. Herring and Laughing gull (*Larus argentatus*, *Larus atricilla*) nestlings, 5–6 weeks old, were sampled in mid-summer on Clam island, NJ.

Adult songbirds and doves sampled in Baltimore, MD during July–August, 1977 included 84 House finches (*Carpodacus mexicanus*), 65 Song sparrows (*Melospiza melodia*), 35 Northern cardinals (*Cardinalis cardinalis*), 27 European starlings (*Sturnus vulgaris*), and 70 Rock doves (*Columba livia*). The number, location, season and year in which sampling occurred for each species appear in tables I and II.

Isolation and identification of NDV, anti-sera, RBC, HI and EI antibody assays

Isolation of NDV from avian faeces in embryonated chicken eggs used standard methods (Graves, 1992; Lennette and Schmidt, 1989; Subcommittee on Avian Diseases, 1971). The Center for Disease Control, Atlanta, GA, provided the Roakin strain and homologous antiserum. The Roakin antiserum was used to identify NDV in HI tests (Graves et al, 1974; Graves, 1992, 1996).

The antiserum and all avian sera were heat-inactivated at 56 °C and treated with a receptor-destroying enzyme before use (Graves et al, 1974, 1992; Graves, 1992). The antisera were adsorbed with chicken red blood cells (RBC). The control test for nonspecific agglutinins was avian sera and RBC.

The RBC used in the HI and EI assays were from two White Leghorn chickens. The four- to five-year-old birds had been involved in studies of long-term growth of fibroblasts (Gey et al, 1974). The RBC used in the EI tests require screening as elution from certain RBC may be prohibitively

slow. As discussed elsewhere, many factors govern the haemagglutinin (HA) and NA activities. For example, in mixtures of NDV with different genotypes, the slow-eluting component determined the time required for the elution from RBC (Spalatin et al, 1970). Still, what factors determine the suitability of the RBC for the EI assay have not been described.

The HI assays were conducted in microtitre plates using RBC and eight HA units of the Roakin strain and standard methods (Graves et al, 1974; Graves, 1992; Subcommittee on Avian Diseases, 1971).

The EI antibody inhibited the elution of strain 575 NDV from the RBC. Inhibition resulted in permanent agglutination patterns if sufficient HA were present in the EI assay. Other strains do not respond to EI antibodies. The HI antibody obscures the inhibitory effect of the EI antibody if insufficient HA units are used. The EI assay required 16 HA units of strain 575 (Graves, 1996). After the HI results were recorded the assays were incubated. In most instances the EI assay attained end points in 16 h or less of humidified incubation at 37 °C. However, the EI assays were incubated for an additional 24 h to ensure that the agglutination pattern was permanent. If the RBC haemolyzed with incubation, viewing the stroma using indirect light to examine the microtitre plates allowed an accurate estimation of end points.

The EI titre is defined as the reciprocal of the highest dilution of serum where a permanent agglutination pattern was maintained (Graves, 1992, 1996). If the end point did not occur at a specific dilution, the next higher dilution, which showed a permanent although a partial agglutination pattern, was used to calculate an average of the two dilutions in question (Graves, 1996). HI and EI titres of 1/16 or greater were considered positive and were used to calculate the geometric mean titre (GMT). Results from duplicate tests were averaged. A fourfold increase in titre was considered to be a seroconversion.

Statistical analyses

The differences in HI or EI antibody prevalences between juvenile and adult swans, comparisons of adult Canada geese, Mute and Tundra swans as well as the findings from other studies were determined by corrected χ^2 values (Mantel and Haenszel, 1959).

For the following reasons the comparisons between HI versus EI prevalences as well as their respective GMT lack accuracy. The HI and EI assays in the present study required different strains and concentrations of HA. Further, the ratio of the EI/HI determinants for each NDV strain may not be constant (Graves, 1996). Thus the HI and EI antibody titres estimated with a combination of strains would not give comparable results.

The HI or EI prevalences were compared. If differences existed in the prevalence of HI antibodies in a population or populations, an expectation would be that the EI assay should also detect a difference. To evaluate the EI antibody assay, the prevalences in three populations and the differences in prevalences in the juvenile and adult Mute swans were used.

RESULTS

NDV isolations and clinical signs

NDV was isolated from asymptomatic Tundra swans, a Mute swan, Canada geese,

and Royal terns in 1977–1978; no isolations were made in any species in 1979 (table I). Strain 575 used in the EI assay was isolated from a juvenile Mute swan. The swan was resighted with no apparent clinical disease for 24 months after NDV was isolated.

The isolation rate in Royal terns was high (11%) whereas among the other species yielding NDV the rates ranged from 0.35–0.66%. Ring-billed gulls were negative throughout the three years of testing despite 3 403 isolation attempts. Four species (ten animals) were positive and 11 species were negative for NDV (table I). Paramyxoviruses other than NDV were not isolated.

Profiles of HI and EI antibody among swans and geese, 1976–1979

HI antibody prevalence varied from 4–62% with the GMT ranging from 16–42; EI antibody prevalence varied from 3–41% with

Table I. Newcastle disease virus isolations from birds of the Atlantic flyway (1977–1979).

<i>Species^a</i> (age)	<i>Time of sampling</i>	<i>Area</i>	<i>NDV isolations^b</i>			<i>Total</i>	<i>Detection rate (%)</i>
			1977	1978	1979		
Tundra swan (A)	W	NC	1/84	2/230	0/136	3/450	0.66
Mute swan (A, J)	LS	MD	1/123	0/69	0/90	1/282	0.35
Canada geese (A)	S	MD	0/21	2/261	0/66	2/282	0.57
Royal tern (N)	LS	VA		4/36		4/36	11
Ring-billed gull (A)	Y	MD	0/577	0/1128	0/1698	0/3403	0
Great Blue heron (N)	MS	VA		0/26	0/28	0/54	0
Herring gull (N)	MS	NJ		0/70		0/70	0
Laughing gull (N)	MS	NJ		0/26		0/26	0
Mallard duck (A)	MS	MD		0/30		0/30	0
Quail (A)	MS	MD		0/30		0/30	0
Dove and songbird ^b (A)	MS	MD	0/281			0/281	0

Time of sampling: W = winter, S = summer, MS = mid summer, LS = late summer, Y = year-long except May–June. Area: MD = Maryland, VA = Virginia, NC = North Carolina, NJ = New Jersey. Age: A = adults, J = juveniles 13–16 weeks old, N = nestlings 5–6 weeks old. ^a The species and number tested are listed in Materials and Methods. ^b Number of Newcastle disease viruses isolated/total samples tested.

Table II. Haemagglutination-inhibition and elution-inhibition antibodies for Newcastle disease virus in adult and juvenile European Mute swans, Tundra swans and Canada geese of the Atlantic Flyway (1976–1979).

Year	Age	(No positive/no tested)			Prevalence ^a of antibodies (%)	GMT ^b
		Male	Female	Total		
<i>Haemagglutination-inhibition antibodies (HI)</i>						
Mute swan						
1977	A	9/18	8/10	17/28	61	39
	J	2/40	1/39	3/79	4	25
1978	A	5/12	6/14	11/26	42	32
	J	4/20	1/9	5/29	17	24
1979	A	6/12	7/9	13/21	62	42
	J	5/40	9/38	14/78	18	25
Tundra swan						
1976	A			33/121	27	38
1978	A			65/223	29	28
Canada geese ^c						
1977	A			5/19	26	21
1978	A			1/9	11	16
<i>Elution-inhibition antibodies (EI)</i>						
Mute swan						
1977	A	3/18	1/10	4/28	14	24
	J	0/40	2/39	2/79	3	23
1978	A	3/12	3/14	6/26	23	32
	J	9/20	3/9	12/29	41	22
1979	A	3/12	2/9	5/21	24	24
	J	2/40	5/38	7/78	9	20
Tundra swan						
1976	A			16/121	13	35
1978	A			20/223	9	36
Canada geese ^c						
1977	A			4/19	21	22
1978	A			3/9	33	25

Age: A = adult; J = juvenile Mute swans 13–16 weeks old. ^a The prevalence (%) is the (number positive/total tested) x 100. ^b HI and EI titres $\geq 1/16$ were considered positive and used to calculate the geometric mean titre (GMT). HI tests used the Roakin strain; EI tests used the 575 strain. ^c The 19 Canada geese (1977) were captive from the Baltimore area whereas the 9 (1978) were feral and were from North Carolina. The feral Tundra swans were from North Carolina whereas the Mute swans were from Maryland.

the GMT ranging from 20–36 among adult and juvenile Mute swans, Tundra swans and Canada geese (table II).

The prevalences of HI antibodies in adult and juvenile Mute swans were 54.6% (41/75) and 11.8% (22/186) respectively, and were significantly different ($\chi^2 = 29.08$, $P < 0.01$). The prevalences in EI antibody among adults and juveniles were 20% (15/75) and 11.3% (21/186) respectively, and were not significantly different ($\chi^2 = 2.5$, NS). The distributions of the prevalence of HI and EI antibodies were not significantly different between male and female Mute swans; respectively 21.8% (31/142) and 26.9% (32/119) for HI antibody, 14.1% (20/142) and 13.4% (16/119) for EI antibody (table II). Nor were sex differences in prevalences of HI or EI antibodies present in adult or juvenile Mute swans.

Seroconversions and persistent antibodies in Mute swans throughout time, 1977–1979

As shown in table III, among the ten recaptured adults, swan AA 84 had a fourfold increase in titre for both HI and EI antibody between 1978 and 1979, and swan AC 12 became seropositive for HI antibody between 1977 and 1978. Swan AA 96 had a twofold increase in HI and a possible seroconversion for EI antibody between the years 1977–1979. Persistent HI antibody was present in five swans and declining HI or EI antibody titres were observed in two swans.

Antibody in other family members of Mute swan 575

Although NDV was isolated, no antibody was found in swan 575. The female and male parents of swan 575 had HI titres of

1/16 and 1/32 respectively. Also, a female sibling had a 1/32 HI titre; the remaining male sibling was not tested.

Species negative for HI and EI antibodies

In 1978, all Ring-billed gulls (82), Laughing gulls (26), Herring gulls (70), Royal terns (36), and Great Blue herons (26) were negative for antibodies. In 1979, 25 Great Blue herons were negative for antibodies.

Differences in the HI and EI antibody prevalences between the different species

As shown in table IV, the differences in the prevalence of HI or EI antibodies among adult Mute swans when compared with the Tundra swans were significant ($P < 0.01$ and $P < 0.04$). The differences in the prevalence of HI antibody among adult Mute swans when compared with Canada geese were significant ($P < 0.05$); the difference in the EI antibody prevalence was not significant. Canada geese when compared with Tundra swans were significantly different for EI ($P < 0.05$) but not for HI antibody prevalence.

DISCUSSION

The NDV isolations from both Tundra and Mute swans confirm the previous serologic indication that five of six species of captive swans in Michigan had been infected with NDV (Friend and Trainer, 1970). The Royal terns had the highest rate of isolation in the study (11%). The number of terns assayed was small, leading to a possible sampling artifact. Despite the NDV isolations, no antibody was found in the young terns or the juvenile Mute swan 575. The swan's par-

Table III. Haemagglutination-inhibition (HI) and elution-inhibition (EI) antibody titres for Newcastle disease virus in ten European Mute swans throughout time (1977–1979).

Swan no	1977		1978		1979	
	HI	EI	HI	EI	HI	EI
AA84	ND	ND	32 ^a	< 16	128 ^b	64
AC12	<16	16	32	< 16	ND	ND
AA52	64	<64	ND	ND	64	<16
AA96	16	<16	ND	ND	32	24
AA02	64	64	ND	ND	64	24
AA55	ND	ND	32	<16	64	<16
AC80	16	<16	<16	<16	<16	<16
AA21	<16	ND	<16	<16	ND	ND
AF49	<16	ND	<16	<16	ND	ND
AF07	<16	ND	ND	ND	<16	<16

^a Reciprocal of the serum dilution where the HI and EI antibody end points were observed. ^b Serologic conversion is defined as a fourfold or greater increase in antibody titre as illustrated by swan AA84 and perhaps AC12 and AA96. ND = Not determined.

Table IV. Differences in prevalences of haemagglutination-inhibition (HI), and elution-inhibition (EI) antibody titres of Newcastle disease virus in populations of adult Mute swans, Tundra swans, and Canada geese.

	HI			EI		
	Mute swan	Tundra swan	Canada geese	Mute swan	Tundra swan	Canada geese
	41/75 ^a (55%)	98/344 (28%)	6/28 (21%)	15/75 (20%)	36/344 (10%)	7/28 (25%)
Mute swan	–	$P < 0.01$	$P < 0.05$	–	$P < 0.04$	NS
Tundra swan	–	–	NS	–	–	$P < 0.05$

^a Number of positive adult birds/total tested. The frequency for each year appears in table II. The percent positive appears in brackets. NS: not significant.

ents and a sibling had HI antibody which suggests that there may have been insufficient time for detectable levels of antibody to develop in 575. Still, transmission studies in ducks have shown no detectable circulating HI antibody although NDV was isolated (Vickers and Hanson, 1982).

The isolations confirm the susceptibility of the *Sterna* genus to NDV. Sooty terns (*Sterna fuscata*) in Australia were the host where the initial isolation of NDV was made (Mackenzie et al, 1984). Regarding Canada geese in New York the adults, yearlings and juveniles all had HI antibody in 1967 and 1969 (Debbie, 1971). The present isolations of NDV from Canada geese and also in 1974 by Rosenberger et al confirm the antibody findings.

The prevalence of HI antibodies among adult Mute swans in 1966 in Michigan was 54% (Bradshaw and Trainer, 1966), which agrees remarkably with the 55% found in this study. Also, others have found the prevalence among Tundra swan and Canada geese to be similar to the findings in this study (Friend and Trainer, 1970; Palmer and Trainer, 1970; Spalatin and Hanson, 1975).

No sex differences were found in the prevalence of HI and EI antibodies among Mute swans. In the Mississippi flyway the excess antibody found in captive female Mallard ducks attained significance when immature females (8/64) and males (0/40) were compared statistically ($\chi^2 = 4.74$, $P < 0.03$); when adults and immature females (11/119) were combined and compared with males (1/60) significance was not attained ($\chi^2 = 3.26$, $P < 0.07$). With captive, immature or adult female Canada geese no differences with males were found (Bradshaw and Trainer, 1966). Also, in another study the comparison between female Mallard ducks (60/110) and males (9/35) did not attain significance ($\chi^2 = 3.51$, $P < 0.06$) (Friend and Trainer, 1970). In feral Mallard ducks no differences were found between

the male (8%) and female (10%) NDV prevalences (Vickers and Hanson, 1982). Similarly, no difference was found between female and male Canada geese (Palmer and Trainer, 1970). Considering the magnitude of these differences in HI antibody prevalence between male and female captive or feral birds, the finding that HI and EI antibody prevalence was not influenced by the sex of the Mute swans is not unusual.

As with other areas, the Atlantic flyway has many susceptible species with prevalences similar to the midwest. However, the failure to isolate NDV from 3 403 Ring-billed gulls and the lack of antibody in Ring-billed, Laughing, and Herring gulls suggests that at least these members of the *Larus* genus may not be important in the natural history of NDV. Still, gulls have been suggested to be a potential source of NDV in Australia (Garnette and Flanagan, 1989). The reason for the uniform negativity in the gull populations is unknown. In contrast to NDV, these Ring-billed gulls had a 2% rate of isolation wherein 70 influenza viruses containing seven NA and six HA subtypes, including the newly described HA 13, were recovered (Graves, 1992).

The Mute swans experienced a mean annual growth rate of 42% during the first decade of study (1962–1972) and 27% during the first seven years of the second decade (1973–1979). The swan populations during the years of sampling (1977–1979) were approximately 274, 315, and 354 respectively (Reese, 1980). This rapidly expanding population provided many non-immune young birds each year. With this expanding population an expectation is that with increasing age the EI prevalence should increase in a manner observed with the HI antibody prevalence. The 41% EI antibody frequency in 1978 among juvenile Mute swans caused the increase, with age, in antibody positivity to be statistically insignificant. There was no apparent reason for the high prevalence in 1978. If the 1978 results

are omitted, the increase in EI prevalence with age attains significance (9/49 versus 9/157; $\chi^2 = 5.90$, $P < 0.02$).

The increase in HI antibody prevalence with age was clearly illustrated by feral Canada geese in New York (Debbie, 1971). Combining the results from the two sampling areas and both sampling years (1967 and 1969), the juveniles when compared with yearlings (5/212 versus 15/35; $\chi^2 = 44.81$, $P < 0.000$) and the juvenile plus yearlings compared with adults (20/247 versus 54/101; $\chi^2 = 45.65$, $P < 0.000$) show the expected increase. Similarly, in a study of Canada geese from many sampling regions the juveniles compared with yearlings (200/1170 versus 64/237; $\chi^2 = 113.1$, $P < 0.000$), and yearlings compared with adults (62/237 versus 627/1431; $\chi^2 = 10.74$, $P < 0.001$), all showed the increase in HI prevalence (Palmer and Trainer, 1970). In contrast, the increase in HI antibody prevalence with age was not seen in Mallard ducks (Vickers and Hanson, 1982). Perhaps an early exposure to NDV eliminated the expected increase in antibody prevalence with age.

The HI and EI assays detected differences in prevalence among the Tundra and Mute swan adult populations in a like manner. Still, the HI and EI assays were only partially effective in detecting prevalence differences if the number of individuals tested was small. This occurred with Canada geese when compared with the larger Tundra and Mute swan populations.

Regarding the study of individual Mute swans the HI and EI assays detected seroconversions as well as persistent and declining antibody titres. Also, in rabbits as the immunization progressed the increase in HI and EI antibodies was detected (Graves, 1996). As with the HI assay, population studies of EI antibody prevalence is now possible with acceptable accuracy.

Here the isolations of NDV were in the cold and warm seasons as shown in many studies referenced above as well as with

those of Webster et al (1976) and Bahl et al (1977). Sentinel ducks in Minnesota showed increased NDV isolations during July–September (Kelleher et al, 1985). In the Baltimore area Ring-billed gull population, influenza virus isolations showed an increase of up to 11.5-fold in the warm months, which conforms to the findings in Sentinel ducks (Graves, 1992). It would appear that a study of NDV isolations may be initiated in any season although a greater rate of recovery may be expected in the warm months.

Estimating EI antibody titres and prevalences in large populations would aid evaluation of NDV strains. Another application would be attempting to understand what role the NA and its EI antibody play in the infectious process. It has been shown that the EI antibody does not react directly with the NA but with a heat-labile determinant resulting in 'foci' on RBC (Graves, 1996). If the foci are aggregates of NDV, as happens with vaccinia (Graves, 1969) and influenza viruses (Bucher and Palese, 1975; Stuart-Harris et al, 1985) the spread of NDV could be reduced. All birds in the present study were asymptomatic. The EI antibody profiles in virulent NDV infections (Alexander et al, 1970) await estimation.

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