AVIAN DISEASES 44:138–144, 2000

Protective Immunity Against Newcastle Disease: The Role of Antibodies Specific to Newcastle Disease Virus Polypeptides

D. L. Reynolds and A. D. Maraqa

Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

Received 23 June 1999

SUMMARY. Studies were performed to determine if passive immunization with hyperimmune sera generated to specific Newcastle disease virus (NDV) proteins conferred protection against virus challenge. Six groups of 3-wk-old chickens were passively immunized with antiserum against either hemagglutinin-neuraminidase/fusion, (HN/F) protein, nucleoprotein/phosphoprotein (NP/P), Matrix (M) protein, a mixture of all NDV proteins (ALL), intact ultraviolet-inactivated NDV (UVNDV), or negative sera. Blood samples were collected 2 days postimmunization, and the birds were challenged with Texas GB strain of NDV. Antibody titers were detected from those recipient birds that had received the antisera against the HN/F, ALL, or UVNDV by a hemagglutination inhibition test, an enzyme-linked immunosorbent assay (ELISA), and a virus neutralization test. Antibodies were detected only by the ELISA from the birds that had received antisera against NP/P and M protein. Antibody titers in the recipient birds dropped by two dilutions ($\log_2$) after 2 days postinjection. Birds passively immunized with antisera against HN/F, ALL, and UVNDV were protected from challenge, whereas chickens passively immunized with antisera against NP/P and M protein and specific-pathogen-free sera developed clinical signs of Newcastle disease. The challenge virus was recovered from the tracheas of all passively immunized groups. The presence of neutralizing antibodies to NDV provided protection from clinical disease but was unable to prevent virus shedding from the trachea.

RESUMEN. Inmunidad protectora contra el virus de la enfermedad de Newcastle: Papel de los anticuerpos específicos contra los polipéptidos del virus de la enfermedad de Newcastle.

Se realizaron estudios para determinar si la inmunización pasiva con suero hiperinmune generado contra proteínas específicas del virus de la enfermedad de Newcastle confería protección contra el desafio. Seis grupos de pollos de tres semanas de edad fueron inmunizados pasivamente con antisuero contra las proteínas Hemaglutinina-Neuraminidasa/fusión, nucleoproteína/fosfoproteína, Matrix, contra la mezcla de todas las proteínas del virus, contra el virus intacto de Newcastle inactivado con rayos ultravioleta ó con antisuero negativo. Se tomaron muestras de sangre dos días después de la inmunización y se desafiaron las aves con la cepa Texas GB del virus de Newcastle. Se detectaron anticuerpos de las aves que habían recibido antisuero contra las proteínas Hemaglutinina-Neuraminidasa/fusión, todas las proteínas ó con el virus intacto de Newcastle inactivado. Los anticuerpos se detectaron por medio de la inhibición de la hemoaglutinación, el inmunoensayo con enzimas asociadas (ELISA) y la virus neutralización. Se detectaron anticuerpos mediante la prueba ELISA únicamente en las aves que habian recibido antisuero contra la nucleoproteína/fosfoproteína y la proteína matriz. Las aves inmunizadas pasivamente con antisuero contra las proteínas nucleoproteína/fosfoproteína y matrix y con suero de aves libres de patógenos específicos, desarrollaron signos clínicos de la enfermedad de Newcastle. El virus de desafio fue recuperado de las tráqueas de todas las aves inmunizadas. La presencia de anticuerpos neutralizantes contra la enfermedad de Newcastle suministró protección contra la enfermedad clínica pero no previno la eliminación del virus de la tráquea.

Key words: Newcastle disease virus, passive immunization, humoral immune response, protection

Abbreviations: ALL = a mixture of all Newcastle disease virus proteins sera; CMI = cell-mediated immunity; ELD_{50} = 50% embryo lethal dose; ELISA = enzyme-linked immunosorbent assay; F = fusion; HI = hemagglutination inhibition; HN = hemagglutinin-neuraminidase; IBV = infectious bronchitis virus; L = large; M = matrix; ND = Newcastle disease; NDV = Newcastle disease virus; NP = nucleoprotein; P = phosphoprotein; PBS = phosphate-buffered saline; SPF = specific-pathogen free; UVNDV = ultraviolet-inactivated Newcastle disease virus; VN = virus neutralization

Newcastle disease (ND) is a highly contagious viral disease of poultry (1,2,3). It causes hemorrhagic intestinal lesions, severe respiratory distress, nervous disorders, decreased egg production, and high mortality (1,2,3). Newcastle disease virus (NDV) was the prototype member of the *Paramyxovirus* genus and was designated PMV1 (1,2). Currently, NDV has been placed in the *Rubulavirus* genus and has been designated Newcastle disease virus (3,26). A recent study suggests that NDV should be placed in a separate genus on the basis of its genome sequence (8). NDV is an enveloped virus with negative-stranded RNA. The NDV virion contains six proteins: nucleoprotein (NP), phosphoprotein (P), matrix (M) protein, fusion (F) protein, hemagglutinin-neuraminidase (HN), and large (L) proteins (3,4,26). The HN and F are glycosylated and form two projections on the lipoprotein envelope of the virion. Studies with monoclonal antibodies to these proteins have demonstrated neutralization to NDV *in vivo* and *in vitro* by preventing virus attachment and cell fusion activity (21). The M protein is located on the inner surface of the envelope and provides structural integrity for the virion. The NP, P, and L proteins are associated with genomic RNA to form the nucleocapsid (3,26). The role of antibodies to NP/P and M protein in protection against NDV infection is unclear.

After vaccination with NDV, antibodies directed toward the various components of the virus are produced to protect the birds against ND infection (3). Although high levels of antibodies have always been associated with protection against ND (3,5), reports have suggested that serum antibodies are not directly correlated with the resistance of chickens to experimental NDV challenge (9,10,11,14). One study found that a low level of antibodies was capable of preventing infection (11), whereas another (12) found that an inactivated NDV vaccine given intramuscularly induced little resistance despite a high concentration of serum antibody. Studies with other paramyxoviruses have indicated that neutralizing antibodies may not be sufficient to protect against the disease (18,32). Neutralizing antibodies have also been reported to be unable to prevent infection, although the clinical disease may have been less severe (18).

In this study, we used passive immunization to determine the role of antibody directed against specific NDV polypeptides (or whole virus) in protecting the bird from virulent NDV challenge.

MATERIALS AND METHODS

Embryonated eggs and chickens. Specific pathogen-free (SPF) eggs (HY-Vac Co., Gowrie, IA) were used for virus propagation and hatching. Chicks were reared on wire-floored cages. Feed and water were provided *ad libitum*.

Virus strains. The lentogenic type B1, strain B1, of NDV was propagated in 9-day-old SPF embryonated eggs by the chorioallantoic route. The allantoic fluid was harvested and centrifuged at 3000 × *g* for 30 min. Then the virus was purified and concentrated from the allantoic fluids.

The velogenic Texas GB strain of NDV was propagated in embryonic eggs and used as an inoculum at 10^2 50% embryo lethal dose (ELD_{50})/bird (23) and used as the challenge virus (see below).

Virus propagation and purification. NDV purification was performed as previously described (25).

Preparation of NDV proteins. The procedure for isolating NDV glycoproteins (HN/F), nucleoprotein and nucleocapsid-associated protein (NP/P), and M from the NDV virus was performed as described previously (28). Briefly, purified NDV (approximately 0.5 mg protein/ml) was diluted 1:5 (v/v) in 4% Triton X-100 (Sigma Chemical Co., St. Louis, MO) in 0.01 M Tris buffer (pH 7.2) in the presence of 1 M KCl. The solution was mixed gently at room temperature for 45 min. The suspension was centrifuged at 10,000 × *g* for 35 min, and a clear supernatant fluid containing HN, F, and M polypeptides and a pellet containing NP/P were obtained. The pellet was washed repeatedly in phosphate-buffered saline (PBS)

and pelleted by centrifugation at 10,000 × *g* for 1 hr at 4 C. The supernatant from the 10,000 × *g* centrifugation was centrifuged at 200,000 × *g* for 1 hr to remove any remaining nucleocapsid or incompletely disrupted virus. Then the pellet was discarded and the supernatant fluid was dialyzed against 0.01 M PBS for 16 hr in order to remove potassium chloride. After dialyzing, the M protein was separated from the glycoproteins (HN and F) by centrifugation at 10,000 × *g* for 30 min. The pellet (M protein) was suspended in PBS. The proteins that remained in the supernatant fluid after removal of M protein were centrifuged repeatedly at 10,000 × *g* for 10 min at 4 C to remove any other protein contaminates. The purity of the collected proteins was assayed by Coomassie blue-stained polyacrylamide gel electrophoresis (17).

Preparation of antiserum. Hyperimmune sera to purified NDV proteins were prepared by emulsifying the separated HN/F, NP/P, and M polypeptides with an equal volume of incomplete Freund's adjuvant. The inocula were injected subcutaneously into 3-wk-old SPF chickens (two birds per group) at a dose of 4 μg protein/inoculum/bird. The chickens were booster vaccinated 2 wk later, and then blood was collected weekly for 5 wk.

Hyperimmune sera to purified NDV were also prepared by subcutaneous injection of 40 μg protein/inoculum/bird of ultraviolet-inactivated NDV (UVNDV) emulsified with incomplete Freund's adjuvant into 3-wk-old SPF chickens. The chickens were booster vaccinated 2 wk later, and then blood was collected weekly for 5 wk.

Enzyme-linked immunosorbent assay (ELISA). An avidin–biotin dot immunobinding assay was used as previously described (7). Briefly, 0.12-inch nitrocellulose membrane disks (Trans-Blot Transfer Medium; Bio-Rad Laboratories, Hercules, CA) were cut and placed at the bottom of each of the wells of a 96-well, flat-bottom tissue culture plate (Becton Dickinson and Co., Lincoln Park, NJ). One microliter of purified NDV (100 μg protein/ml) was dotted in the middle of each disk, and the plate was incubated overnight at 37 C to dry the dot. One hundred microliters of diluent buffer was added to each well. Then, 100 μl of 1:100 diluted serum was added to each well of the first column of wells. Afterward, twofold serial dilutions were performed across the plate to achieve a dilution range of 1:100 to 1:204,800. Positive and negative sera were also included in the test. After 1 hr of incubation, the secondary biotinylated antibodies (biotinylated anti-chicken immunoglobulin G; Vector Laboratories, Burlingame, CA) at a dilution of 1:5000, streptavidin at a dilution of 1:2000, and the chromogen (4-chloro-1-naphthol; Sigma) were subsequently added. The nitrocellulose membrane was washed and blocked between steps. Finally, the plates were incubated in the dark until dark purple dots appeared against the white (nitrocellulose) background, which was considered a positive reaction.

Table 1. Antibody titers of donor sera obtained from chickens vaccinated with NDV or various NDV polypeptide preparations.

Sera[A]	Antibody titer reciprocal $\log_2$ HI[B]	ELISA[C]	VN[D]
HN/F	6	8	7.84
NP	0	9	<2
M	0	6	<2
ALL	6	9	7.46
UVNDV	7	9	8.7

[A]Sera collected from birds vaccinated with HN/F, NP/P, M, or UVNDV.

[B]HI geometric mean titers expressed as reciprocal $\log_2$.

[C]ELISA was performed as reciprocal $\log_2$ dilution × 100.

[D]The neutralization titer was the reciprocal of the dilution of antisera that neutralized 50% of virus.

Western blot. Western blots were performed as previously described (25).

Hemagglutination inhibition (HI) test. The HI tests were performed as previously described (29).

Virus neutralization (VN) test. The VN test was performed with swine testicular cells as described previously (24).

Sample collection for virus isolation. Cotton-tipped applicators were used for swabbing the trachea and sample collection as previously described (25).

Experimental design. Six groups (10 chickens/group) of 3-wk-old SPF chickens were passively immunized subcutaneously with 4 ml of either anti-HN/F sera, anti-NP/P sera, anti-M sera, a mixture of all NDV proteins sera (ALL), anti-UVNDV sera, or negative SPF sera. Blood samples were collected 2 days postimmunization, and the birds were challenged with 10^2 ELD_{50} of the Texas GB strain of NDV per bird administered intramuscularly. Tracheal swabs were taken at 4 days postinoculation and used for virus isolation. All the chickens were observed for 14 days after challenge. Two trials were conducted, and in each trial, 10 birds per group were used.

RESULTS

The antibody titers of serum donor birds vaccinated with various NDV polypeptides are shown in Table 1. Those birds that were vaccinated with the HN/F glycoproteins and UVNDV had detectable levels of specific antibodies as measured by the HI, ELISA, and VN

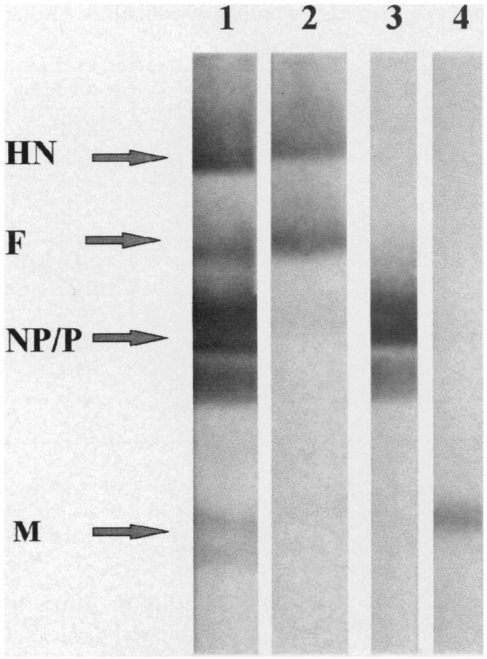

Fig. 1. Western blot results from chickens vaccinated with (1) UVNDV; (2) NDV HN/F glycoproteins; (3) NDV NP/P proteins, and (4) NDV M proteins.

tests, whereas birds vaccinated with NP/P and M proteins had antibodies that were detected by the ELISA but not by the HI or the VN test. A combination of anti-HN/F, NP/P, and M sera had antibodies that were detected by the HI, ELISA, and VN tests. The western blot results revealed that all vaccinated groups had a positive antibody response to the respective polypeptides to which they had been vaccinated (Fig. 1).

Table 2 displays the results of serum titers from trials 1 and 2 of the recipient birds 2 days postadministration. The results revealed that recipient birds that were passively immunized with anti-HN/F sera, a combination of ALL sera, or UVNDV sera had detectable antibody titers as measured by the HI, ELISA, and VN tests, whereas recipient birds passively immunized with anti-NP/P or anti-M sera had detectable antibody titers only in the ELISA and not by the HI or VN tests. The antibody titers were found to be two to three $\log_2$ dilutions lower than the original sera used for administration.

Table 2. Antibody titers of recipient birds passively immunized with NDV hyperimmune sera.

Trial	Sera[A]	*n*	Antibody titer $\log_2$ HI[B]	ELISA[C]	VN[D]
1	HN/F	10	3.4	5.2	4.3
	NP	10	0	6.3	<2
	M	10	0	3.6	<2
	ALL	10	3.8	5.3	4.6
	UVNDV	10	4	6.7	4.5
	C	10	0	0	<2
2	HN/F	10	3	5.3	4.42
	NP	10	0	5.5	<2
	M	10	0	3.9	<2
	ALL	10	4	5	4.70
	UVNDV	10	4	6.5	4.88
	C	10	0	0	<2

[A]Donor sera obtained from SPF chickens immunized with separated NDV proteins, UVNDV, or unimmunized SPF negative sera.

[B]HI geometric mean titers expressed as reciprocal $\log_2$.

[C]Geometric mean titer expressed as reciprocal $\log_2$ dilution × 10.

[D]The neutralization titer was the reciprocal $\log_2$ of the last dilution of antisera that neutralized 50% of the virus.

The challenge results from trials 1 and 2 are shown in Table 3. The results revealed that the majority of recipient birds passively immunized with anti-HN/F sera, ALL sera, and UVNDV sera were protected from challenge. However, birds passively immunized with anti-NP/P sera, anti-M sera, and negative SPF sera developed clinical signs of ND. Clinical signs of ND and/or death appeared by the third day postchallenge in birds that were not protected. The challenge virus was recovered from all groups of passively immunized birds.

DISCUSSION

In a related study that addressed the role of cell-mediated immunity (CMI) in protection from ND, specific CMI to NDV, by itself, was not protective against a virulent NDV challenge (25). Additionally, in this study, a high correlation was found between the presence of HI or neutralizing antibody to NDV and protection, and the presence of specific antibodies, as determined by the HI or the VN test, was determined to be imperative for protection from ND. In this study, a passive immunization ap-

Table 3. Results of challenge and virus isolation from birds passively immunized with NDV antisera preparations.

Trial	Group	*n*	Morbidity[A]	Mortality	% Protection[B]	Virus shedding[C]
1	HN/F	10	3/10	1/10	70%	8/10
	NP	10	0/10	8/10	20%	2/2
	M	10	0/10	9/10	10%	1/1
	ALL	10	4/10	1/10	60%	5/10
	UVNDV	10	3/10	0/10	70%	6/10
	C	10	0/10	10/10	0	ND[D]
2	HN/F	10	3/10	1/10	70%	10/10
	NP	10	0/10	10/10	0	ND
	M	10	0/10	10/10	0	ND
	ALL	10	3/10	0/10	70%	8/10
	UVNDV	10	2/10	0/10	80%	7/10
	C	10	0/10	10/10	0	ND

[A]Number of birds displaying clinical signs of NDV.
[B]Percentage of surviving birds that did not display clinical signs of disease.
[C]Number of isolations/number of samples.
[D]ND = not done.

proach was used to delineate the role of specific NDV polypeptides in protection. The results of this study revealed that birds passively immunized with anti-HN/F sera, antisera from a mixture of all (ALL) proteins, and anti-UVNDV sera were protected from a lethal challenge of NDV, whereas, birds passively immunized with anti-NP/P and anti-M sera were not protected. Similar results have been reported in other studies (13,20,22,30). Umino *et al.* (30) found that passive administration of antiserum against intact NDV, or the surface glycoproteins HN/F, provided protection to susceptible chickens against NDV challenge. However, no report has been published on the protective role of the other NDV polypeptides. In studies with simian virus 5 (SV5), Randall *et al.* (22) found that *in vitro* neutralization was provided by antibodies directed against the surface HN glycoprotein, but neutralization was not achieved when antisera against the internal proteins NP, P, or M were used. Furthermore, similar findings have been reported for infectious bronchitis virus (IBV). Immunizing birds with purified nucleocapsid and membrane proteins of the IBV did not protect them against virulent challenge despite high antibody titers, whereas birds immunized with the surface S1 glycoprotein were protected (16).

The results of this study corroborate our previous findings that indicated humoral immunity was a key component in protective immunity (25). However, we found in this study that passively acquired antibodies had little effect on the ability to prevent tracheal viral shedding. The challenge virus was detected in the majority (but not all) of passively immunized protected birds 4 days postchallenge. Yoshaida (31) reported that the amount of neutralizing antibody titers was very low in tracheal samples from birds that had been passively immunized with anti-NDV hyperimmune sera. The transport of antibodies from the circulatory system was also different between the upper and lower respiratory tracts. The transudation of serum antibodies into the upper respiratory tract was reported to be very limited in chickens administered high-titered antiserum to avian IBV. However, transudation of antibodies to the lung correlated with the concentration of serum antibodies (12). Similar observations have been reported after influenza virus infections of neonatal ferrets. Husseini (15) found that ferrets with high maternal antibodies demonstrated complete protection in the lungs, however, virus replication occurred in the upper respiratory tract.

Many studies have addressed those conditions required to obtain the presence of antibody in the respiratory tract (9,10,12,19). Holmes (13) reported that either direct or indirect exposure of the respiratory tract to an antigen was necessary for inducing neutralizing antibody. In birds having maternal antibodies, the lacrimal fluid antibody titers ranged from 1% to 9% of the serum antibody titers. After

intraocular vaccination with live NDV, the lacrimal fluid antibody titers increased significantly. The increase in antibody titers was attributed to local replication of NDV in the Harderian gland (27). Ewert *et al.* (10) reported that birds that had been passively immunized with antisera to NDV showed a sharp rise of antibodies in the trachea 4 days after intratracheal viral inoculation. This rise was a result of transudation of antibodies from the serum that coincided with the course of viral pathology observed in the trachea of the infected birds.

The importance of the presence of a local antibody response in the respiratory tract of NDV-vaccinated birds in protection has been documented (6,13,14,15,18,19). Vaccination of birds with NDV via aerosol administration protected them against challenge given by the respiratory route but not against intramuscular challenge, whereas vaccination of chickens via the air sac protected the birds against air sac challenge but not against the intraocular route (6,18). The replication of the virus in the respiratory tract in the presence of circulating antibodies may provide ample antigenic stimulation to confer "full protection" to the host, that is, no clinical disease or virus replication in the trachea. Vaccinated birds derived from maternally immune chickens that had been vaccinated with NDV were more resistant to challenge than were corresponding unvaccinated birds. Surprisingly, no difference was found in the serum antibody response titers between vaccinated and unvaccinated birds (9).

In this study, the NDV viral polypeptides were separated by ultracentrifugation. Other techniques may have been used. However, separation by denaturing gels, such as sodium dodecyl sulfate, results in polypeptides that do not induce neutralizing antibodies representative of the intact viral polypeptides (25). For this reason, we chose to separate the viral polypeptides by ultracentifugation. However, the ultracentrifugation technique had limitations in separating the viral proteins. Because of the difficulties in separating the NP from the P proteins and the HN from the F, we elected to utilize these polypeptides in combination (i.e., NP/P and HN/F).

In conclusion, the results of this study indicated the importance of the presence of antibodies to the HN and F polypeptides in protection from NDV challenge. In addition, the demonstration of the challenge virus from the tracheas of protected birds is suggestive of the importance of the local immune response in protection.

REFERENCES

1. Alexander, D. J. Newcastle disease. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth, and J.E. Pearson, eds. Kendall/Hunt Publishing Co., Dubuque, IA. pp. 114–120. 1989.

2. Alexander, D. J. Newcastle disease and other avian paramyxovirus infections. In: Diseases of poultry, 9th ed. B. W. Calnek, ed. Iowa State University Press, Ames, IA. pp. 496–519. 1991.

3. Alexander, D. J. Newcastle disease and other avian paramyxovirus infections. In: Diseases of poultry, 10th ed. B. W. Calnek, ed. Iowa State University Press, Ames, IA. pp. 541–570. 1997.

4. Alexander, D. J., and M. S. Collins. The structural polypeptide of avian paramyxovirus. Arch. Virol. 67:309–323. 1981.

5. Beard, C. W., and M. Brugh. Immunity to Newcastle disease. Am. J. Vet. Res. 136:509–512. 1975.

6. Beard, C. W., and B. C. Easterday. The influence of the route of administration of Newcastle disease virus on the host response. 1. Serological and virus isolation studies. J. Infect. Dis. 117:55–61. 1967.

7. Cummins, D., D. L. Reynolds, and K. R. Rhoades. An avidin-biotin enhanced dot-immunobinding assay for the detection of Mycoplasma gallisepticum and M. synoviae serum antibodies in chickens. Avian Dis. 34:36–43. 1990.

8. de Leeuw, O., and B. Peeters. Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae. J. Gen. Virol. 80:131–136. 1999.

9. Eidson, C. S., S. H. Kleven, and P. Villegas. Efficacy of intratracheal administration of Newcastle disease vaccine in day-old chicks, Poult. Sci. 55: 1252–1267. 1976.

10. Ewert, D. L., C. S. Eidson, and D. L. Dawe. Factors influencing the appearance of antibody in tracheal washes and serum of young chickens after exposure to Newcastle disease virus. Infect. Immun. 18: 138–145. 1977.

11. Gough, R. E., and D. J. Alexander. The speed of resistance to challenge induced in chickens vaccinated by different routes with a B1 strain of live NDV. Vet. Rec. 92:563–564. 1973.

12. Holmes, H. C. Neutralizing antibody in nasal secretions of chickens following administration of

avian infectious bronchitis virus. J. Comp. Pathol. 43:235–241. 1973.

13. Holmes, H. C. Resistance of the respiratory tract of chicken to Newcastle disease virus infection following vaccination: the effect of passively acquired antibody on its development. J. Comp. Pathol. 89: 21–29. 1979.

14. Holmes, H. C. Virus-neutralizing antibody in the sera and secretions of the upper and lower respiratory tract of chickens inoculated with live and inactivated Newcastle disease virus. J. Comp. Pathol. 89:21–25. 1979.

15. Husseini, R. H., C. Sweet, H. Overton, and H. Smith. Role of maternal immunity in protection of newborn ferrets against infection with a virulent influenza virus. Immunology 52:389–394. 1984.

16. Ignjatovic, J., and L. Galli. Structural proteins of avian infectious bronchitis virus: role in immunity and protection. In: Cornaviruses. H. Laude and J. F. Vautherot, eds. Plenum Press, New York. pp. 449–453. 1993.

17. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685. 1970.

18. Lamprecht, C. L., H. E. Krause, and M. A. Mufson. Role of maternal antibody in pneumonia and bronchiolitis due to respiratory syncytial virus. J. Infect. Dis. 134:211–217. 1981.

19. Malkinson, M., and P. A. Small, Jr. Local immunity against Newcastle disease virus in the newly hatched chicken's respiratory tract. Infect. Immun. 16:587–592. 1977.

20. Merz, D. C., A. Scheid, and P. W. Choppin. Immunological studies of the functions of paramyxovirus glycoproteins. Virology 109:94–105. 1981.

21. Muelemans, G., M. Gonze, M. C. Carlier, P. Petit, A. Burny, and L. Long. Protective effects of HN and F glycoprotein-specific monoclonal antibodies on experimental Newcastle disease. Avian Pathol. 15: 761–768. 1986.

22. Randall, R. E., D. F. Young, and J. A. Southern. Immunization with solid matrix–antibody–antigen complexes containing surface or internal virus structural proteins protects mice from infection with the paramyxovirus, simian virus 5. J. Gen. Virol. 69: 2517–2526. 1988.

23. Reed, L. J., and H. Muench. A simple method for estimating fifty percent end points. Am. J. Hyg. 27:439–497. 1938.

24. Reynolds, D. L., and A. D. Maraqa. A rapid virus neutralization assay for Newcastle disease virus using the swine testicular (ST) continuous cell line. Avian Dis. 43:564–572. 1999.

25. Reynolds, D. L., and A. D. Maraqa. Protective immunity against Newcastle disease: the role of cell-mediated immunity. Avian Dis. 44:145–154. 2000.

26. Rima, B., D. J. Alexander, M. A. Billeter, P. L. Collins, D. W. Kingsbury, M. A. Lipkind, Y. Nagai, C. Orvel, C. R. Pringle, and V. ter Muelen. Paramxyoviridae. In: Sixth report on the International Committee on Taxonomy of Viruses. F. A. Murphy, C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo, and M. D. Summers (eds.). Springer-Verlag, Vienna, Austria. pp. 268–274. 1995.

27. Russell, P. H. Newcastle disease virus: virus replication in the Harderian gland stimulates lacrimal IgA; the yolk sac provides early lacrimal IgG. Vet. Immunol. Immunopathol. 37:151–163. 1993.

28. Scheid, A., and P. W. Choppin. Isolation and purification of the envelope proteins of Newcastle disease virus. J. Virol. 11:263–271. 1973.

29. Thayer, S. G., and C. W. Beard. Serologic procedures. In: Isolation and identification of avian pathogens, 4th ed. D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 255–266. 1998.

30. Umino, Y., T. Kohama, M. Kohase, A. Sugiura, H. D. Klenk, and R. Rott. Protective effect of antibodies to two viral envelope glycoproteins on lethal infection with Newcastle disease virus. Arch. Virol. 94:97–107. 1987.

31. Yoshaida, I. Neutralizing antibody in the respiratory tract of chickens inoculated with Newcastle disease vaccines. Nat. Inst. Animal Health 11:75–82. 1971.

32. Young, D. F., R. E. Randall, J. A. Hoyle, and B. E. Soberielle. Clearance of a persistance paramyxovirus infection is mediated by cellular immune response but not serum-neutralizing antibody. J. Virol. 64:5403–5411. 1990.

ACKNOWLEDGMENTS

We thank Dr. Ali Akbar, Sevinc Akinc, and Joan Oesper for their technical assistance.