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ASSESSMENT OF NEWCASTLE DISEASE VACCINATION OF HOUBARA BUSTARD BREEDERS (*CHLAMYDOTIS UNDULATA UNDULATA*)

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ABSTRACT: The houbara bustard (*Chlamydotis undulata undulata*) is endangered in North Africa. Through a captive-breeding program established in Morocco by The Emirates Center for Wildlife Propagation, wild populations are being supplemented by the releasing of captive-reared birds. Newcastle disease, which is caused by *Newcastle disease virus* (NDV; Avian paramyxovirus type 1), can infect houbara bustards and is a significant threat through contact with backyard poultry and possibly wild birds. Three vaccination schedules for Newcastle disease were evaluated by serologic monitoring to assess the efficiency and safety of various types of vaccines (live vs. inactivated), vaccine strains (Hitchner B1 and Clone 30), and administration routes (intranasal vs. injection). We evaluated antibody titers in 211 adult houbara bustards for 10 mo. Antibody titers to NDV in both sera and egg yolks were monitored by hemagglutination inhibition test. The inactivated vaccine provided a high, homogeneous, and durable serologic response in breeders; titers were higher than $\log_2$ 11 after 4 wk and remained higher than $\log_2$ 7 after 10 mo. The response to the two live vaccines was similar, and antibody titers did not exceed $\log_2$ 6 at seroconversion. Maternally derived antibodies were efficiently transmitted in vitellus, further confirming that offspring of females hyperimmunized with the inactivated vaccine received high titers of maternal antibodies.

Key words: Avian paramyxovirus type 1, *Chlamydotis undulata undulata,* houbara bustard, maternal antibodies, Newcastle disease, *Newcastle disease virus,* vaccination.

INTRODUCTION

The houbara bustard *Chlamydotis undulata undulate* is an endangered species living in North Africa. It is a sedentary bird living in arid environments, where it rains less than 200 mm annually (Goriup, 1983). The Emirates Center for Wildlife Propagation (ECWP) sustains wild populations by releasing captive-produced bustards (Lacroix, 2003). The Emirates Center for Wildlife Propagation is situated in Morocco, where Newcastle disease (ND) is routinely diagnosed in both commercial and backyard poultry, despite the absence of officially reported ND cases since 1986 (Bell, 1984; OIE, 2003). Macqueen's bustard (*Chlamydotis macqueeni*) also is susceptible to ND (Bailey et al., 1996a, b, 1997).

Newcastle disease is caused by *Newcastle disease virus* (NDV; avian paramyxovirus-1), tentatively included in the genus *Rubulavirus* within the *Paramyxoviridae* (Van Regenmortel et al., 2000); NDV infection has been documented in many wild and migratory bird species (Kaleta and Baldauf, 1988; Takakuwa et al., 1998). Transmission occurs either directly (through contact with infected birds or pseudo-vertically through eggshell contamination) or indirectly (via respiratory aerosol, fecal contamination of food and water). Clinical signs are polymorphic, depending on the tropism and virulence of the virus strain, target species, and vaccinal immunity (Alexander, 2003). The most pathogenic pathotypes, referred to as "velogenic viscerotropic," are routinely isolated in Morocco (Bell, 1986; Bell and Mouahid, 1987; Facon, 2002). These strains are typically associated with hemorrhagic intestinal lesions. This classification should be considered with caution, however, as variation in clinical response can occur between viruses within the same pathotype (Alexander, 2000).

Although NDV vaccines are not specifically produced for houbara bustard, com-

TABLE 1. Experimental design of the vaccine trials. For each population of birds (A, B, and C), three groups were sampled and submitted to a vaccine program.

Populations of birds	Vaccine group 1: live Hichner B1		Vaccine group 2: live Clone 30		Vaccine group 3: inactivated vaccine	
	Group	No. birds	Group	No. birds	Group	No. birds
Group A: males hatched in 2001	A1	30	A2	30	A3	30
Group B: females hatched in 2001	B1	30	B2	30	B3	30
Group C: females hatched in 2000	C1	15	—	—	C3	16

mercial vaccines are licensed in most countries for use in domestic chickens and turkeys. Prior to this study, NDV vaccination of bustards at the ECWP in Morocco was based on a live Hitchner B1 vaccine, administered biannually by nasal instillation. While both live Hitchner B1 and Clone 30 (derived from the strain La Sota) are innocuous when administered to poultry species, dramatic differences in their efficacy have been reported (Alexander, 2000).

Protection against an experimental challenge with NDV cannot be correlated with the serologic response to vaccination with live vaccines administered through mucosal (mostly intranasal) routes; protection against an intranasal challenge can occur in cases in which the serologic response is poor. In contrast, the immune response following a parenteral vaccination, using an inactivated vaccine, is mostly humoral and is highly protective against an intramuscular challenge (Alexander, 2003). In the same way, protection of the day-old chick is strictly conferred through maternally derived antibodies, and serologic monitoring of breeders provides a reliable indication of NDV immunity in day-old chicks (Meulemans, 1988).

Currently, different breeding centers for houbara bustards are not using uniform NDV vaccination protocols (Bailey et al., 2000). Since the extent of captive breeding of this species is expanding in North Africa and the Middle East, a rational assessment of vaccination against NDV is required. Here we report a comprehensive study of NDV vaccination strategies applied to houbara bustard breeders. Three vaccination schedules for ND were evaluated by serologic monitoring of both sera and egg yolks to assess the efficiency and safety of various types of vaccines (live and inactivated), vaccine strains (Hitchner B1 vs. Clone 30), and administration routes (intranasal and injection).

MATERIALS AND METHODS

Birds

Houbara bustards ($n=180$) were divided into three groups of 60 birds, each consisting of an equal number of males and females. Males and females hatched in 2001 are designated as groups A and B, respectively (Table 1). All of the bustards were hatched in 2001 and maintained in ECWP facilities; each bird was housed separately. In the same manner, 31 females hatched in 2000 and maintained in ECWP facilities were included in the study and comprised group C (Table 1).

Vaccines

Three vaccines were used, including a Hitchner B1 live vaccine (Hitchner B1 Nobilis®, Intervet, Boxmeer, The Netherlands) applied by nasal instillation after reconstitution in NaCl solution buffer; a Clone 30 live vaccine (Clone 30 Nobilis®, Intervet) applied by nasal instillation after reconstitution in NaCl solution buffer; and an inactivated Clone 30 water-in-oil adjuvanted vaccine (Newcavac Nobilis®, Intervet) injected subcutaneously (SC) at the dorsal base of the neck. These three vaccines are referred to as vaccine groups 1, 2, and 3, respectively (Table 1).

Vaccination schedules

All birds received two doses of vaccine at 1 and 2 mo of age using a double dose of Hitchner B1 vaccine. Females hatched in 2000 received a booster dose every 6 mo with the same vaccine. A booster vaccination was administered as follows: 1) The live Hitchner B1 vaccine was applied to vaccine group 1, consisting

of 30 males (group A1), 30 females (group 1B) hatched in 2001, and 15 females hatched in 2000 (group C1); 2) The live Clone 30 vaccine was applied to vaccine group 2, consisting of 30 males (group A2) and 30 females (group B2) hatched in 2001; and 3) The inactivated vaccine was applied to vaccine group 3, consisting of 30 males (group A3), 30 females (group B3) hatched in 2001, and 16 females hatched in 2000 (group C3).

Clinical response to vaccination

Following vaccination, birds were monitored daily for 10 days for changes in behavior, food intake, and weight and were subjected to a complete clinical examination. The safety of live Clone 30 and inactivated vaccine had been previously determined in a trial on 18 bustards conducted before this study.

Blood sampling and serology

For collection of sera, birds were bled every 4 wk after booster vaccination for a period of 4 mo at week 9 (T1), week 13 (T2), week 17 (T3), and week 21 (T4), respectively. In addition, serum was collected from males at week 40 (T5) and week 49 (T6). Blood was collected into silicone tubes (evacuated blood collection tubes, Terumo Europe, Belgium), by venepuncture of the right brachial vein. Blood was allowed to clot at 4 C and was then centrifuged for 10 min at 1,000 × G. Sera were stored at −20 C and sent to the Laboratoire de Développement et d'Analyses des Côtes d'Armor (LDA22) in France. Antibody titers to NDV were determined using the hemagglutination inhibition test (HI), which is conventionally used to detect and quantify NDV antibodies (Allan et al., 1978; Piela et al, 1984; OIE, 2000).

Collection of egg yolks and immunoglobulin Y purification

For all females, the first egg of the laying period was not inseminated and was devoted to extraction of immunoglobulin Y (IgY) and subsequent HI NDV assay. Immunoglobulin Y was purified using a commercial kit (Eggcellent Chicken IgY Purification Kit, Pierce, Rockford, Illinois, USA). Aliquots of purified proteins were frozen at −20 C and then sent to the LDA 22 for serology.

Statistical analysis

Data were analyzed using the Student's independent *t*-test. Systat software (SPSS, Inc. Chicago, Illinois, USA) was used for all statistical calculations. Statistical significance was set to $P=0.05$.

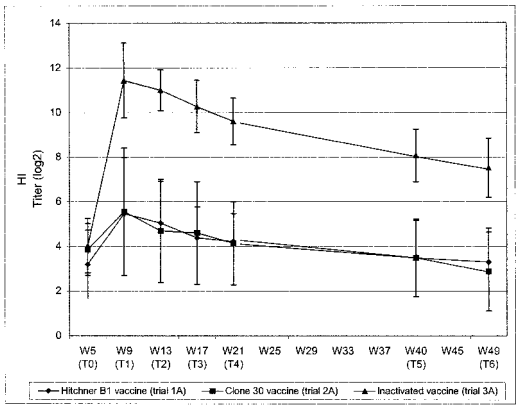

FIGURE 1. Serologic response to booster vaccination of male houbara bustards (group A), during 44 wk. *Newcastle disease virus* antibody hemagglutination (HI) titers are expressed in $\log_2$ (±SD).

RESULTS

Throughout the trials, no changes in behavior, food intake, or reduced weight gain were observed, and birds vaccinated with live Clone 30 showed no nasal discharge or dyspnea. After the SC injection of inactivated vaccine, two birds showed an erythema of less than 1 cm in diameter for 3–4 days. Body weight was monitored at each sampling period, and mean weights for females and males were 1,224±119 g and 1,722±172 g, respectively. No clinical signs suggestive of ND were observed in birds during the experiment.

Because our experimental conditions did not allow the inclusion of a nonvaccinated group, results were analyzed in reference to the standard vaccination schedule routinely used in ECWP, using HB1 live vaccine (groups A1, B1, and C1, respectively). The serologic responses to the three vaccines were compared for male and female breeders born in 2001 and for both groups; responses to HB1 (vaccine groups A1 and B1) and Clone 30 (vaccine groups A2 and B2) were low and mostly equivalent (Figs. 1, 2). Antibody titers reached $\log_2$ 5.5±2.8 for both A1 and B1 groups and $\log_2$ 5.8±3.3 for combined A2

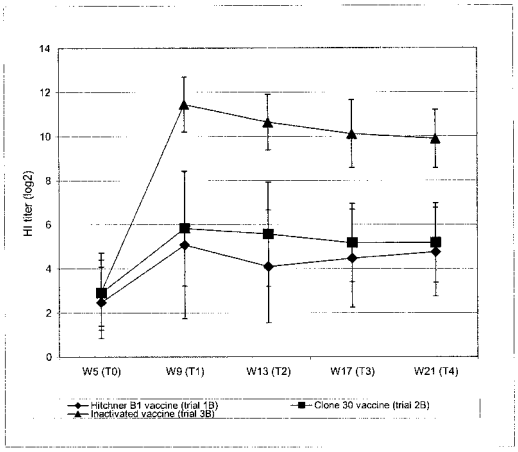

FIGURE 2. Serologic response to booster vaccination of female houbara bustards, born in 2001 (group B), during 16 wk. *Newcastle disease virus* antibody hemagglutination (HI) titers are expressed in $\log_2$ (±SD).

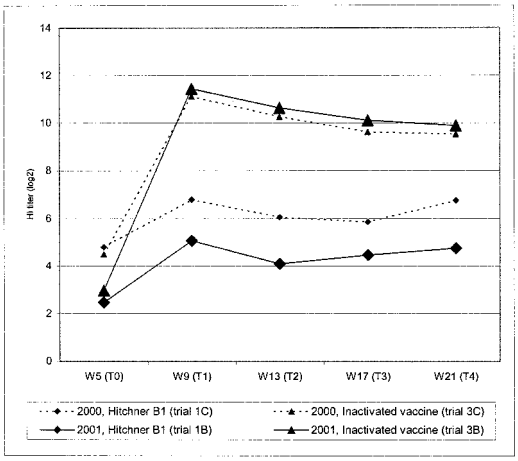

FIGURE 3. Hemagglutination (HI) *Newcastle disease virus* antibody titers, $\log_2$ (±SD), of houbara bustards females: comparison of HI response between generation 2000 and generation 2001, during 16 wk.

and B2 groups. In contrast, the inactivated vaccine induced a higher serologic response, with less variation in titer observed among individual birds; HI titers were elevated to $\log_2$ 11.4±1.2 at T1 and were still high ($\log_2$ 9.6±1.0) at T4 (Figs. 1, 2).

Females hatched in 2000 (group C) showed higher serologic titers following boosting, since they had received two additional HB1 vaccinations. Compared to females hatched in 2001, seroconversion titers after HB1 boosting were significantly increased (P=0.06), while the two groups of birds (3B and 3C) vaccinated using the inactivated vaccine showed similar serologic results (P=0.34) (Fig. 3). Antibody titers associated with the inactivated vaccine were not only higher but were also more consistent, as indicated by standard deviation shown in Figures 1 and 2.

Transmission of maternal antibody through eggs was determined through HI titration of egg yolks (Fig. 4). Mean HI titers in egg yolk after vaccination with inactivated vaccine, HB1 and Clone 30, were 8.4 (eggs from C3, n=9), 10.1 (eggs from B3, n=9); 5.7 (eggs from C1, n=7), 3.1 (eggs from B1, n=7); and 4.0 (eggs from B2, n=5). The ratios between egg yolk and serum HI titers were 0.85±0.14, 0.99±0.09, 0.95±0.11, 0.76±0.35, and 0.71±0.40, respectively, for these groups.

DISCUSSION

The aim of this work was to define an optimal vaccination schedule for houbara bustard breeders. In our hands, the inactivated oil–adjuvanted NDV induced a high and persistent antibody response, regardless of sex, age, or previous immune status. Similar results have been previously indicated, but these data were obtained from as few as six birds (Bailey et al.,

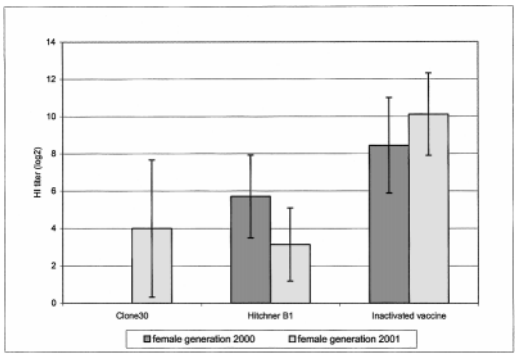

FIGURE 4. Hemagglutination (HI) *Newcastle disease virus* antibody titers, $\log_2$ (±SD), in egg yolks of females hatched in 2001 (group B) and in 2000 (group C).

1998a). In chickens, NDV HI titers greater to or equal than $\log_2$ 5 units are considered to be protective against a field challenge (Phillips, 1973). In our vaccination experiment, HI titers reached $\log_2$ 11.4 at seroconversion, and at 44 wk after the booster vaccine, titers still averaged $\log_2$ 7.5. Protective titers were maintained for up to 9 mo, as has been reported elsewhere for gallinaceous birds (Eidson et al., 1980; Gerlach, 1994). Our results indicate that protection against NDV was not produced with our previous vaccination schedule using Hitchner B1, since titers never exceeded $\log_2$ 5 units following seroconversion. The live vaccines (Hitchner B1 and Clone 30) induced similar serologic responses (groups 1 and 2). However, the efficacy of Clone 30 is uncertain, since clinical protection associated with this vaccine has not been thoroughly evaluated through challenge experiments.

Using the inactivated vaccine, antibody titers were not only higher than with Hitchner B1 and Clone 30, but they remained fairly consistent through the laying period. In a previous study (Bailey et al., 1998a), a dose of 1.0 ml/kg was recommended for inactivated vaccine in male bustards. However, houbara bustards show a significant sexual dimorphism; males were 41% heavier than females in our experiment. In our trial, performed on 30 males and 30 females, no difference in HI response was detected between males and females after administration of the same dose of 0.5 ml. One can hypothesize that the antigen load is so high (greater than 50 units of protective $dose_{50}$ for chicken) that no difference in HI response could be observed between males and females. These results indicate that the recommended dose of 0.5 ml inactivated vaccine per bird is sufficient for both male and female houbara bustard vaccination.

Because of the endangered status of houbara bustards, infectious challenges are not possible. The level of protection, therefore, represented a value extrapolated from data obtained from chickens, in which HI tests are routinely used to evaluate NDV vaccination results as an alternative to viral challenge (Allan et al., 1978). This assay (HI) is more accurate for assessment of field protection than are test results from enzyme-linked immunosorbent assay test kits (Alexander, 2003).

Hemagglutination inhibition titers in egg yolks were consistent with maternal antibody HI titers for all three vaccines, but higher antibody titers were detected in eggs from females immunized with the inactivated vaccine. The ratio between serum and egg yolk HI titers was also assessed and showed no significant differences between vaccines. The absorption of NDV antibodies from eggs to chicks is now well documented, and in gallinaceous birds, antibody titers of day-old chicks are equivalent to those of their hyperimmunized parents (Van Eck, 1990) and those resulting from parallel immunization of hens (Eidson et al., 1982). Yeo et al. (2003) demonstrated a good correlation between yolk- and chick-HI antibody titers, as well as between yolks and hens. Finally, absorption of maternal NDV antibodies from chicks of kori bustards (*Ardeotis kori*) has been reported (Bailey et al., 1998b).

Because initial NDV replication occurs in the respiratory and/or digestive tracts, one could theorize that vaccination should be focused on induction of mucosal immune response (Russel, 1993). However, maternal antibody transfer and subsequent protection of day-old chicks is a critical issue for NDV control. Furthermore, vaccination at day 1 is still possible in the presence of maternal antibodies, although interference may slightly reduce the immune response (Meulemans, 1988; Russel et al., 1995). If maternal antibody titers are high, passive protection is provided for at least the first 3 wk of life, and vaccination can occur after this period (Van Eck, 1990). In low-risk areas, protocols based on an initial vaccination at 3–4 wk after hatching are suitable if an inactivated vaccine is used for breeders.

In conclusion, our breeding flock of

houbara bustards was effectively immunized against NDV by using an inactivated oil emulsion vaccine, injected SC, with a booster dose before each laying period. This vaccine protocol results in a higher level of NDV maternal antibodies in eggs and likely in day-old chicks. An effective NDV vaccine program and related biosecurity are essential components for NDV control and the subsequent success of houbara bustard breeding programs.

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LITERATURE CITED

ALEXANDER, D. J. 2003. Newcastle disease and other avian Paramyxoviridae infections. *In* Diseases of poultry. 11th Edition, Y. M. Saif (ed.). CV Mosby Co.-Wolfe, Ames, Iowa, pp. 541–569.

———. 2000. Newcastle disease and other avian paramyxoviruses. *In* Revue Scientifique et Technique de l'OIE, pp. 443–462.

ALLAN, W. H., J. E., LANCASTER, AND B. TOTH 1978. Newcastle disease vaccines—Their production and use. FAO Animal Production Services, Rome, Italy.

BAILEY, T. A., J. H. SAMOUR, J. NALDO, J. C. HOWLETT, AND M. TARIK. 1996a. Causes of morbidity in bustards in the United Arab Emirates. Avian Diseases 40: 121–129.

———, U. WERNERY, R. E. GOUGH, R. MANVELL, AND J. H. SAMOUR. 1996b. Serological survey for avian viruses in houbara bustards (*Chlamydotis undulata macqueenii*). Veterinary Record 139: 238–239.

———, P. K. NICHOLLS, U. WERNERY, J. SAMOUR, J. E. COOPER, AND M. T. O'LEARY. 1997. Avian paramyxovirus type 1 infection in houbara bustards (*Chlamydotis undulata macquenii*): Clinical and pathologic findings. Journal of Zoo and Wildlife Medicine 28: 325–330.

———, U. WERNERY, J. H. SAMOUR, AND J. NALDO. 1998a. Antibody response of Kori bustards (*Ardeotis kori*) and houbara bustards (*Chlamydotis undulate*) to live and inactivated Newcastle disease vaccines. Journal of Zoo and Wildlife Medicine 29: 441–450.

———, ———, R. ZACHARIAH, J. H. SAMOUR, J. L. NALDO, AND J. C. HOWLETT. 1998b. Maternal transfer of paramyxovirus type 1 antibodies and antibody response to a live Newcastle disease vaccine in kori bustards. Journal of Wildlife Diseases 34: 472–478.

———, R., MANVELL, D. GOUGH, U. WERNERY, J. KINNE, AND J. HOWLETT. 2000. Review of PMV-1 (ND). *In* Bustards in the Middle East: Presentation, pathology and control; Results of vaccination trial. Abu Dhabi Falcon Hospital, NARC, Abu Dhabi, EAU.

BELL, J. G. 1986. Velogenic viscerotropic Newcastle disease virus strains in Morocco. Avian Diseases 30: 231–233.

———, AND M. MOUAHID. 1987. Vaccination against Moroccan strains of Newcastle disease virus. Tropical Animal Health and Production 19: 192–196.

———, J. BERRADA, R. WYFFELS, AND EL M. HOUADFI. 1984. Isolation and biological properties of some Moroccan strains of Newcastle disease virus. Avian Diseases 28: 319–322.

EIDSON, C. S., P. VILLEGAS, AND S. H. KLEVEN. 1980. Field trials with an oil emulsion Newcastle disease vaccine in broiler breeders. Poultry Science 59: 702–707.

———, S. G. THAYER, P. VILLEGAS, AND S. H. KLEVEN. 1982. Vaccination of broiler chicks from breeder flocks immunized with a live or inactivated oil emulsion Newcastle disease vaccine. Poultry Science 68: 1621–1629.

FACON, C. 2002. Health management of reproduction in captive houbara bustard: Assessment of viral risks and definition of a rational vaccination schedule. DMV Thesis, 4175, Toulouse, France, 103 pp.

GERLACH, H. 1994. Avian medicine: Principles and application. *In* Viruses. Wingers Publishing, Lake Worth, Florida, pp. 862–948.

GORIUP, P. D. 1983. The houbara bustard in Morocco. International Council for Bird Preservation. Oriental Press, Cambridge, UK.

KALETA, E. F., AND C. BALDAUF. 1988. Newcastle disease in free-living and pet birds. *In* Newcastle disease, D. J. Alexander (ed.). Kluwer Academic Press Publishers, Boston, Massachusetts, pp. 197–246.

LACROIX, F. 2003. The Emirates Center for Wildlife Propagation: Developing a comprehensive strategy to secure a self sustaining population of houbara bustards in eastern Morocco. Houbara News 5: 2. Available at: http://www.erwda.gov.ae/eng/pages/projects/special/houbara_wg.html.

MEULEMANS, G. 1988. Control by vaccination. *In* Newcastle disease, D. J. Alexander (ed.). Kluwer Academic Press Publishers, London, UK, pp. 161–163.

OIE [OFFICE INTERNATIONAL DES EPIZOOTIES]. 2000. Manual of standards for diagnostic test and vaccines. Office International des Epizooties, Paris, France, pp. 221–232.

———. 2003. Santé mondiale en 2002. Office In-

ternational des Epizooties, Paris, France, pp. 219–221.

PHILLIPS, J. M. 1973. Vaccination against Newcastle disease: An assessment of haemagglutination inhibition titers obtained from field samples. Veterinary Record 93: 577–583.

PIELA, T. H., C. M. GULKA, V. J. YATES, AND P. W. CHANG. 1984. Use of egg yolk in serological tests (ELISA and HI) to detect antibody to Newcastle disease, infectious bronchitis, and *Mycoplasma gallisepticum.* Avian Diseases 28: 877–883.

RUSSELL, P. H. 1993. Newcastle disease virus: Virus replication in the Harderian gland stimulates lacrimal IgA; The yolk sac provides early lacrimal IgG. Veterinary Immunology and Immunopathology 37: 151–163.

———, AND G. O. EZEIFEKA. 1995. The Hitchner B1 strain of Newcastle disease virus induces high levels of IgA, IgG and IgM in newly hatched chicks. Vaccine 13: 61–66.

TAKAKUWA, H., T. ITO, A. TAKADA, K. OKAZAKI, AND H. KIDA. 1998. Potentially virulent Newcastle disease viruses are maintained in migratory waterfowl populations. Japanese Journal of Veterinary Research 45: 207–215.

VAN ECK, J. H. 1990. Protection of broilers against Newcastle disease by hyperimmunisation of the dams. Veterinary Quarterly 12: 139–145.

VAN REGENMORTEL, M. H. V., C. M. FAUQUET, AND D. H. L. BISHOP (EDS.). 2000. Virus taxonomy. Classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, USA.

YEO, S. G., E. NAGY, AND P. J. KRELL .2003. Indirect method for prediction of hemagglutination inhibition antibody titers to Newcastle disease virus in chickens by titration of antibodies in egg yolk. Journal of Veterinary Diagnostic Investigation 15: 184–187.

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