Protection from Clinical Disease Against Three Highly Virulent Strains of Newcastle Disease Virus After In Ovo Application of an Antibody–Antigen Complex Vaccine in Maternal Antibody–Positive Chickens

Author(s): Darrell R. Kapczynski, Alison Martin, Eid E. Haddad, and Daniel J. King
Source: Avian Diseases, 56(3):555-560.
Published By: American Association of Avian Pathologists
DOI: http://dx.doi.org/10.1637/9980-110311-Reg.1
Protection from Clinical Disease Against Three Highly Virulent Strains of Newcastle Disease Virus After In Ovo Application of an Antibody–Antigen Complex Vaccine in Maternal Antibody–Positive Chickens

Darrell R. Kapczynski, AE Alison Martin, BC Eid E. Haddad, BD and Daniel J. King A

A Southeast Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 934 College Station Road, Athens, GA 30605

B Pfizer Animal Health Global Poultry, Durham, NC 27703

Corresponding author. E-mail: darrell.kapczynski@ars.usda.gov

Received 11 January 2012; Accepted 6 May 2012; Published ahead of print 7 May 2012

SUMMARY. Worldwide, Newcastle disease (ND) remains one of the most economically important diseases of poultry. Current vaccination strategies for commercial poultry include the use of inactivated and live ND vaccines that typically induce protection against virulent field viruses. Here, we tested the efficacy of an antigen–antibody complex (AAC) ND vaccine delivered in ovo. Commercial maternal antibody–positive broiler chickens (Gallus domesticus) were vaccinated in ovo with an AAC vaccine composed of live B1-LaSota Newcastle disease virus (NDV) complexed with NDV-specific antisera, and then they were challenged at weekly intervals after hatch. Challenge viruses included three exotic ND disease (END) viruses: the neurotropic strain Texas GB NDV-92-01 (TxGB) and two viscerotropic isolates, one isolate from the 2002–2003 outbreak in California (California 2002 isolate S212676 [CA]) and the other isolate from a 1997 END outbreak in South Korea (South Korea 94-147 [SK]). Results demonstrate that maternal antibody was able to provide approximately 50% protection in either vaccinated or control chickens at 7 days of age after TxGB challenge. However, with challenge at ≥14 days, most control birds died, whereas all AAC-vaccinated birds were protected. Challenge with the CA or SK viruses in chickens at 28 days of age resulted in 100% protection of vaccinated birds, whereas all control birds died. In addition, AAC-vaccinated birds displayed decreased incidence of viral shedding in oral and cloacal swabs than control birds. Antibody titers were significantly (P < 0.05) higher in vaccinated chickens, as determined by enzyme-linked immunosorbent assay and hemagglutinin-inhibition tests, than in nonvaccinated controls. Together, these results demonstrate the efficacy of AAC vaccines delivered in ovo to protect commercial poultry.

RESUMEN. Protección contra la enfermedad clínica inducida por tres cepas altamente virulentas del virus de la enfermedad de Newcastle después de la aplicación in ovo de una vacuna con base en complejos antígeno-anticuerpo en pollos con inmunidad materna.

La enfermedad de Newcastle sigue siendo una de las enfermedades económicamente más importantes en la avicultura a nivel mundial. Las estrategias actuales de vacunación para las aves comerciales incluyen el uso de vacunas inactivadas y vivas que normalmente inducen protección contra los virus de campo virulentos. En este trabajo, se analizó la eficacia de una vacuna aplicada in ovo basada en complejos antígeno-anticuerpo contra la enfermedad de Newcastle. Se vacunaron pollos de engorde (Gallus domesticus) por in ovo y que tenían anticuerpos maternos con una vacuna compuesta de complejos inmunes conteniendo un virus vivo de la enfermedad de Newcastle cepa B1-LaSota adicionada con un antisero específico, y luego fueron desafiados a intervalos semanales después de la eclosión. El desafío de los virus incluyó tres virus de la enfermedad de Newcastle exóticos: la cepa neurotropica Texas GB NDV-92-01 (TxGB) y dos cepas viscerotrópicas, un aislamiento del brote en California durante los años 2002–2003 (aislamiento de California 2002, S212676 [CA]) y otro aislamiento de un brote a finales de 1997 en Corea del Sur (Corea del Sur 94-147 [SK]). Los resultados demuestran que los anticuerpos maternos fueron capaces de proporcionar aproximadamente el 50% de protección, ya sea en los pollos vacunados o en los controles a los 7 días de edad después de la exposición con la cepa TxGB. Sin embargo, con los desafíos los ≥14 días, la mayoría de las aves del grupo control murieron, mientras que todas las aves vacunadas con la vacuna con complejos inmunes estuvieron protegidas. El desafío con las cepas CA o SK en los pollos a los 28 días de edad indujo una protección del 100% en las aves vacunadas, mientras que todas las aves de control murieron. Además de las aves vacunadas con la vacuna con complejos inmunes mostraron menor incidencia para la excreción del virus en las muestras orales y cloacales en comparación con las aves del grupo control. Los títulos de anticuerpos fueron significativamente (P < 0.05) mayores en los pollos vacunados, según lo determinado por el ensayo de inmunoadSORción con enzimas ligadas y por las pruebas de inhibición de la hemaglutinación, en comparación con los controles no vacunados. En conjunto, estos resultados demuestran la eficacia de las vacunas por complejos inmunes aplicadas in ovo para proteger aves de corral comerciales.

Key words: Newcastle disease, antigen–antibody complex, vaccine

Abbreviations: AAC = antigen–antibody complex; Ag = agriculture; BSL = biosafety level; CA = California 2002 isolate S212676; EID = egg infective dose; ELISA = enzyme-linked immunosorbent assay; END = exotic Newcastle disease; HI = hemagglutination inhibition; ICPI = intracerebral pathogenicity index; ND = Newcastle disease; NDV = Newcastle disease virus; pc = postchallenge; SK = South Korea 94-147; TxGB = Texas GB NDV-92-01

Newcastle disease (ND) is a disease of chickens (Gallus domesticus) of economic importance throughout the world, impacting foreign trade. The highly virulent form of the virus is endemic in numerous countries and produces a lethal disease characterized by severe respiratory illness, conjunctivitis, neuropathy, enteritis, or a...
In ND outbreak situations, controlling clinical disease and reducing the shedding of virus from infected birds to susceptible cohorts are critical to controlling spread of disease. The objectives of the present study were to extend the knowledge of protection against highly virulent NDV in broilers vaccinated in ovo with an AAC NDV.
levels against NDV were expected to be present. Both groups of birds had maternal antibodies to NDV at 1 day of age (Fig. 1). The HI and ELISA titers for both treatment groups dropped from day 1 to day 7 of age, indicating a decrease in maternal antibody. After day of hatch, the anti-NDV serum antibody levels in unchallenged control birds declined to \(4\) (log \(2\)) as measured by HI, demonstrating a typical antibody decay pattern, and levels remained there throughout the study. In contrast, HI titers in vaccinated birds rose to \(6\) (log \(2\)) between 14 and 35 days of age, demonstrating a strong humoral immune response after \(in\ ovo\) vaccination. ELISA titers of control birds displayed similar antibody decay as observed in HI titers, with the highest ELISA titers found immediately after hatch and dropping thereafter (Fig. 1B). As stated by the manufacturer, titers \(>396\) are considered positive. Asterisk (*) indicates vaccinated birds significantly different from controls on that day by ANOVA \((P \leq 0.05)\).

**Response to TxGB challenge.** When challenged with TxGB at 7 days of age, 64% of control birds survived challenge, indicating the protective potential of maternal antibody (Fig. 2A). Among the ACC-vaccinated birds, only 50% were protected from clinical signs and mortality, despite significantly higher antibody levels (Fig. 1A). At 14 and 21 days of age, only 18% and 20% of control birds survived challenge without clinical symptoms, probably due to waning maternal antibody. After 21 days of age, all control birds succumbed to challenge. In contrast, 100% of vaccinated birds challenged at 14, 21, 28, or 35 days of age survived challenge, a level that was statistically different from that of the control group.

Experiments to determine incidence of viral shedding at 4 days pc demonstrated that 100% of control birds were shedding virus in both oral and cloacal swabs (Fig. 2B). In contrast, no NDV-positive swabs were recovered from vaccinated or unchallenged groups.

**Response to END challenge with isolates from California and South Korea.** To determine cross-protection against other END isolates, birds were challenged at 28 days of age with either isolates from outbreaks in California (2002) or Korea (1997). All unvaccinated control birds succumbed to challenge after inoculation with CA and SK strains of NDV (Fig. 3A). In contrast, \(in\ ovo\)–vaccinated birds were 100% protected against both CA and SK virus challenges.

Comparison of the prechallenge (day 0) and pc (day 14) HI titers demonstrated that vaccinated birds had protective antibody titers \((\geq 4)\) before challenge (Fig. 3B). An anamnestic response was observed in these groups to END challenge at day 14. No detectable
antibody titers were observed in unvaccinated birds before challenge, indicating maternal antibody was no longer present in these groups.

No virus was recovered from oral or cloacal swabs before challenge (data not shown). After challenge, virus was recovered from 100% of nonvaccinated control birds in both oral and cloacal swabs at 4 days pc (Fig. 3C, D). In contrast, virus was identified in 15%–25% of swabs from vaccinated birds on day 4 pc.

**DISCUSSION**

Overall, the *in ovo*-administered ND vaccine provided full protection against all three virulent isolates of the virus when challenged at 28 days of age. Maternal antibodies alone were able to provide partial protection against TxGB up to 21 days of age, although *in ovo* vaccination clearly enhanced protection. We have demonstrated previously the protection achieved from TxGB challenge after *in ovo* vaccination with an AAC vaccine (10). Because TxGB is the virulent reference challenge strain for licensing vaccines in the United States, all commercially licensed NDV vaccines are expected to provide protection against it. As new viral strains emerge, however, it is important to determine vaccine protection against them. The END challenge strains CA and SK are not closely related phylogenetically to TxGB and offered an opportunity to determine efficacy against distant END isolates (17, 23). As demonstrated here, *in ovo* vaccination with AAC vaccines can induce protective levels of immunity in commercial chickens containing maternal antibody against more phylogenetically distant strains.

The rapid decline in antibody titers between 1 and 7 days of age indicates only a modest amount of maternal antibody at hatch. Nevertheless, survival of 64% of control birds to TxGB challenge at day 7 is indicative of the protection afforded by maternal antibody. Unlike inactivated or live NDV vaccines applied at day of age, the maternal antibody did not interfere with acquisition of protective immunity in *in ovo*-vaccinated birds. The HI titers of vaccinated birds reached fully protective levels by day 14, corresponding with 100% protection to TxGB challenge. Protection remained constant through 35 days of age.
An interesting observation was that ELISA titers developed more slowly than HI titers. This is in contrast with ELISA profiles for broilers vaccinated with conventional live virus vaccines at hatch and may be a feature of the immune-complexed ND vaccine (15). The earlier development of HI than ELISA titers also was observed under field conditions (11). HI titers, however, are more predictive of protection to virulent challenge (24).

Although in ovo–vaccinated birds were fully protected from challenge against all three virulent NDV strains, viral shedding was not eliminated. Although no virus was recovered from TxGB-challenged birds, virus was isolated from both oral and cloacal swabs of a few of the birds in the CA and SK groups 4 days pc, and this isolation may indicate decreased antigenic homology of the vaccine virus (B1-LaSota) to the challenge viruses. Decreased suppression of the challenge virus after CA and SK challenge also may be due to the slightly higher pathogenicity, as indicated by higher ICPI values of 1.79 and 1.78, respectively, than for TxGB strain (1.75).

Viral shedding after challenge may indicate the degree of local immunity in the respiratory system and digestive tract. Kapczynski and King (15) demonstrated that viral shedding in chickens challenged with CA strain virulent NDV was lower in birds vaccinated with a live vaccine by intraocular inoculation than in those vaccinated subcutaneously with an inactivated oil adjuvant ND vaccine (15). It is worth noting that the live vaccines used in our previous study (NEWHATCH-C2®, Merck Animal Health, Summit, NJ) differed from the vaccine used in these studies (B1-LaSota). The frequency of viral reisolation after CA challenge in the present study using in ovo vaccination is comparable to the published results for the live vaccine and is consistent with the presence of live virus in the AAC vaccine and indicates that the mechanism of protection may not be affected by complexing the virus with antibody. The role of local immunity to NDV in the respiratory tract has been demonstrated and is important for field protection against aerosol challenge (4). The challenges used in this study also mimic this route (administered intraocular) so as to match the desired conditions as well as possible. The in ovo route of vaccination presents antigen to the mucosal surfaces of the respiratory tract and digestive tract (14). This route favors development of mucosal immunity. In ovo vaccination for ND also elicits sufficient humoral immunity to afford protection against intramuscular challenge with TxGB as early as day 14 (10).

In summary, in ovo vaccination for ND broadens the options available to poultry producers and may offer a more efficient, cost-effective vaccination strategy. Whether in ovo NDV vaccination affects growth of broilers remains to be determined. Because full immunity against phylogenetically distant virulent viruses can develop at an early age in maternal antibody–positive chickens, this type of vaccine has an advantage when applied to commercial poultry. Finally, in ovo vaccination seems to stimulate both local and humoral immunity to NDV and may potentially enhance cell-mediated immunity, although that is the subject of future research.

REFERENCES


ACKNOWLEDGMENTS

We thank Tracy Smith-Faulkner and Phillip Curry for outstanding technical assistance and Roger Brock for animal care assistance. This research was supported by a research agreement with Embrex (58-6612-4-250) and USDA–ARS CRIS projects 6612-32000-062 and 6612-32000-049.