from the president…

Newcastle Disease (NDV)

Dave Zacek, President of Lohmann Animal Health International, Gainesville, Georgia, USA

The scientific article in this issue has Newcastle disease (NDV) as its focus. We at LAHI have a complete line of Newcastle vaccines, both live and killed.

Our live line contains both Hitchner B1 and LaSota strains alone or in combination with Infectious Bronchitis strains. Additionally, we are the manufacturer for the new in-ovo vaccine, Newplex™ from Embrex, Inc.

We offer a complete line of inactivated Newcastle vaccines for all aspects of poultry production, day old chicks, replacement pullets and turkeys. Our Chick ND Gold offers an innovative oil emulsion to offer superior protection in areas challenged by velogenic NDV. This product is applied subcutaneously at the hatchery with a concurrent live vaccination and followed by additional field applications later in the broiler's life to offer complete protection.

Our killed Newcastle vaccines for growing chickens are available in various combinations with some or all of the following antigens: Infectious Bronchitis (Mass. and Ark. strains), Salmonella enteritidis, Reoviruses, Infectious Bursal Disease Viruses (standard and variant) and Mycoplasma gallisepticum. For turkeys, we offer an inactivated NDV vaccine presented in combination with Paramyxovirus-3, an essential part of any turkey breeder vaccine program to prevent devastating egg production losses from these 2 viruses.

Our Area Managers and Technical Service Veterinarians can help organize LAHI Newcastle vaccines to best fit your particular Newcastle prevention needs whether for boilers, breeders, table egg layers or turkeys. Please contact us for help.

Lohmann Animal Health International is a leading manufacturer and supplier of poultry biologics for the U.S. and international vaccine markets. Based in Gainesville, GA, LAHI maintains production facilities in Vineland, NJ, and Waterville, ME. LAHI is a member of the PHW Group , a 32-company enterprise that produces avian vaccines and poultry for the global market.

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Evaluation of commercial live and inactivated Newcastle disease virus vaccines for protection of chickens and turkeys against the 2002-03 California exotic Newcastle disease virus.

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Editors Note: This article represents a summary of Dr. Kapczynski's presentation at the 53rd Western Poultry Disease Conference; March 2004, Sacramento, CA.

Introduction

Respiratory disease control is a high priority economic concern to the poultry industry internationally. Paramyxovirus infections, primarily Newcastle disease virus (NDV) in chickens are frequent etiologies of respiratory disease that cause major production losses. Moreover, outbreaks of virulent Newcastle disease (ND), termed exotic Newcastle disease (END) in the U.S., are categorized as notifiable diseases that must be reported to the Office International des Epizooties and can result in trade barriers that limit commercial export of poultry products from affected countries.

END is one of the most serious infectious diseases of poultry. The etiology, NDV, belongs to the Avulavirus genus within the family Paramyxoviridae, and is designated avian paramyxovirus 1 (APMV-1). Nine serotypes (APMV-1 to APMV-9) of avian paramyxovirus, of which APMV-1 is the most economically important, have been identified. APMV-1 infects approximately 236 species of pet and free living birds in addition to domestic avian species. All NDV (APMV-1) isolates, including the variant isolates from pigeons, are members of a single serotype even though minor antigenic differences among NDV isolates can be detected with monoclonal antibody binding assays. This broad cross-reactivity is the reason that broad cross-protection has been observed in vaccine studies to the present. The fusion (F) protein and hemagglutinin-neuraminidase (HN) surface glycoproteins are the principal antigens that elicit a protective immune response.

Infections with NDV can cause a wide range of disease symptoms depending on the virulence of the virus. Three pathotypes have classically defined the isolate grouping by virulence. Lentogenic strains have low virulence, cause mild or unapparent respiratory or enteric infections, and are utilized as live-virus vaccines. Mesogenic isolates of NDV mainly cause respiratory and nervous signs but not high mortality. Highly virulent NDV isolates are termed velogenic and infection can cause high mortality in chickens. Velogens can be further divided as neurotropic or viscerotropic depending on the clinical signs and lesions associated with infection. Until recently, the last major outbreak of END in commercial U.S. poultry occurred from 1971-1974 in southern California. However, an outbreak in turkeys in North Dakota during 1992 and in game chickens in California during 1998 have occurred. Infected poultry shed the virus which can be excreted from either or both the respiratory and the digestive tracts depending on the virus strain. Therefore bird-to-bird transmission can occur via aerosol and contaminated feces and droppings or various fomites.

In May 2002, END virus was isolated from ring neck pheasants in northern California, which preceded diagnosis of END from backyard game chickens in southern California (Los Angeles County) in October 2002. END virus was subsequently isolated from commercial poultry in December 2002, and determined to contain nucleotide sequence homology at the fusion protein cleavage site with the pheasant isolate. The first END quarantine zone was imposed in California in November, 2002. However, more than 19,000 premises would later be quarantined in 5 states, including California, Nevada, Arizona, Texas and New Mexico. The last positive isolation from commercial poultry was made on March 26th, 2003. The outbreak was deemed eradicated and the last quarantine lifted in September, 2003. More than 3 million birds, including approximately 150,000 backyard flocks were depopulated. Cost of the outbreak

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was estimated to be in excess of $200 million.

During an outbreak of END, the implementation of biosecurity measures to stop the spread of disease, eradication of infected flocks, and vaccination of poultry usually form the three-pronged attack considered as part of the intervention strategy. Current vaccination programs for NDV include the use of low virulent live-virus and inactivated vaccines designed to control infections from low virulence endemic field strains. The goal of these vaccines is to induce protective immunity while producing a minimal post-vaccinal reaction due to reduced rate of gain or complications from other infections. Currently available NDV vaccines are efficacious in protecting against disease from virulent NDV infections, but are ineffective at protecting against those infections. The recent outbreak of END in California underscores the need for continued development and testing of NDV vaccines and vaccination programs. Ideally the goal in controlling the spread of END is to prevent against infection as well as reducing the shed of virus from any infected birds.

The 2002-2003 outbreak of END in California raised the question again about the ability of current vaccines to provide protection against disease in poultry. Studies were undertaken by the U.S. Department of Agriculture at the Southeast Poultry Research Laboratory (SEPRL) to determine if existing commercial live and inactivated NDV vaccines could provide protection against the 2002 END virus isolate.

Materials and Methods

The commercial lentogenic live and inactivated NDV vaccines used for these studies were prepared by Lohmann Animal Health International from type B_1 strains. For challenge of immunity, the 2002 END California isolate (CA02; game chicken/US(CA)/S0212676/02) was used. The efficacy of these vaccines were tested in both SPF White Plymouth Rock chickens obtained at SEPRL (USDA, Athens, Georgia) and commercial Broad Breasted White turkeys obtained from Ridgeway Hatcheries, Inc. (LaRue, Ohio). Forty one-day-of-age SPF chickens were arbitrarily divided into 4 groups of 10 birds. Birds in groups 1 and 2 received 100 µl of phosphate-buffered saline (PBS, pH 7.4) via intranasal (IN;50 µl) and eye drop (ED;50 µl) routes at 14 days-of-age. Birds in group 3 received the live-virus B_1B_1 vaccine via ED and IN route according to the manufacturer's recommendations at 14 days-of-age. Birds in group 4 received 100 µl of inactivated oil-emulsion vaccine injected subcutaneously in the neck, according to the manufacturers recommendations at 14 days-of-age. Two weeks after vaccination, birds in group 2, 3 and 4 were challenged via ED and IN route with $10^{5.9}$ EID$_{50}$ / bird with CA02. Unchallenged birds were sham-challenged with 100 µl PBS via ED/IN route. For comparison, groups of twelve 10-day-old commercial turkeys (Ridgeway) receiving no services prior to testing were separated into four groups and vaccinated as above. Two weeks after vaccination, birds in group 1 received sham-challenge while birds in groups 2, 3, and 4 received $10^{5.9}$ EID^{50} / bird CA02. Following infection, chickens and turkeys were monitored daily for disease signs for 14 days post-challenge (pc). Birds displaying severe clinical signs of disease were euthanized by overdose of sodium pentobarbital. Serum samples were taken by wing bleed at 0 and 14 days pc and tested by hemagglutination-inhibition assay by standard methods. Oropharyngeal and cloacal swabs were collected into 2 ml brain-heart infusion (BHI) broth with antibiotics from each bird on day 0, 2, 4, 6 and 14 days pc for virus isolation. Virus isolation and titrations were performed in 9 - 11 day-old embryonated chicken eggs and fifty percent egg infectious dose (EID$_{50}$) titers were determined by standard methods.

Results and Conclusion

Protection from END challenge was determined by absence of clinical signs during the 14-day pc observation period. No clinical signs or mortality was observed in groups of chickens receiving either live or inactivated NDV vaccine (data not shown). In contrast, all sham-vaccinated END-challenged chickens displayed severe depression from day 2 to 4 pc and 100% mortality was observed 5 days pc. **Figure 1** demonstrates that vaccinated chickens had a significant reduction in titers of challenge virus shed from the oropharynx in comparison to sham-vaccinated group. Birds receiving live B_1 vaccine displayed the lowest levels of virus shed on day 2 and 4 pc ($< 1.2 \times 10_1$ EID$_{50}$/ ml) from oral swabs, indicating increased protection from infection compared to sham-vaccinated birds or birds receiving inactivated vaccine. In addition, viral shed in the live-vaccine group decreased such that only 3 chickens out of 10 were positive for END virus in the oropharynx, compared to 10 or 7 birds positive in either the sham-vaccinated or inactivated-vaccine groups, respectively, at day 4 pc. Virus recovered from cloacal swabs in the live-vaccine group was determined to be $< 1.0 \times 10^1$ EID$_{50}$/ ml on day 4 and 6 (data not shown). Birds receiving inactivated-vaccine displayed END titers of approximately 5×10^3 EID$_{50}$/ ml 2 days pc from oral swabs that decreased at day 4 pc. Viral titers recovered from

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cloacal swabs were highest at 4 days pc (2×10^2 EID$_{50}$/ ml). High titers of END virus were isolated from both the oropharynx and cloaca of sham-vaccinated birds. Viral titers in this group increased following challenge, with greater than 10^6 EID$_{50}$/ml recovered from each swabs at day 4 pc. Serum HI titers were determined to be >64 in vaccinated birds at day of challenge and >512 on day 14 pc. No HI titers were observed in the sham-vaccinated birds.

In the turkey trial, a small number from both sham-vaccinated and NDV-vaccinated groups developed clinical signs and death following challenge with END (data not shown). However, only 33% of unvaccinated turkeys succumb to END challenge, compared to 100% of chickens in the previous experiment. These observations may be due to differences in age of the animals tested or indicate a species tropism of the virus, or a combination of both. Vaccination of turkeys with either live or inactivated NDV increased survivability compared to sham-vaccinated birds. **Figure 2** illustrates that vaccinated turkeys had a significant reduction in titers of challenge virus shed from the oropharynx in comparison to the sham-vaccinated group for days 4 and 6 pc. Furthermore, viral shed in live-vaccine group decreased such that only 1 turkey of 12 was positive for END virus in the oropharynx, whereas all 11 sham-vaccinated controls still had high viral titers at day 6 pc. Interestingly, the turkeys were unable to completely clear the challenge virus over the 14 day pc sampling period. In the absence of clinical signs low levels of challenge virus was detected from both oropharyngeal and cloacal samples. HI antibody titers induced by either vaccine were similar as one dose of vaccine induced an HI response of 32 prior to challenge that increased over the course of challenge (mean 256).

Although NDV-vaccinated birds were shown to be protected against END, they continued to shed virus in the absence of clinical signs. Following an outbreak situation, this condition may prevent diagnosis of an infected flock and result in further spread of disease and duration of the outbreak. During the 1971-74 END outbreak, mass application of NDV vaccines were applied to commercial poultry to help improve immunity of the birds and eradicate the disease. However, evaluation of the vaccination program showed that although vaccination reduced mortality in END-infected flocks, it failed to stop the spread of

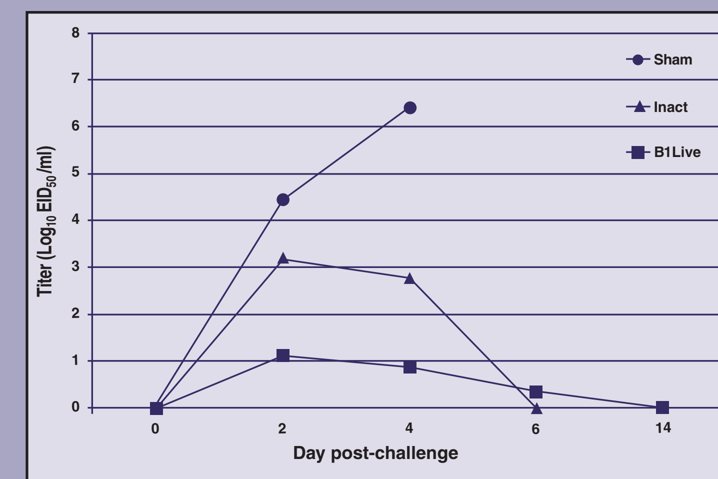

Figure 1. Oropharyngeal END Recovery From SPF Chickens

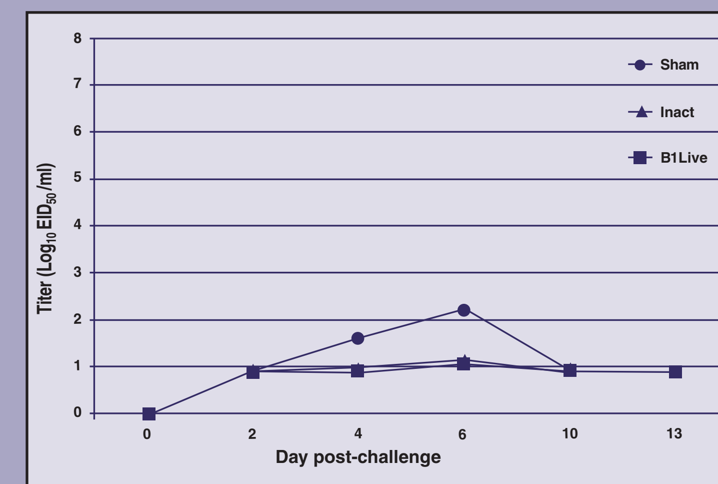

Figure 2. Oropharyngeal END Recovery From Commercial Turkeys

disease, regardless of the vaccine, route or frequency of use. In addition, the use of mass vaccination interfered with detection and diagnosis of infected flocks because the reduced virus shed from vaccinated birds increased the difficulty of isolating virus directly from tissues or swabs. Sampling susceptible sentinel birds placed in the flock was needed to detect the low virus shed from vaccinated birds. Although 30 years have passed since that outbreak, the poultry industry still relies heavily on vaccines available during that time. While these vaccines have served the industry well for many years, the development of improved vaccines and vaccination strategies to induce protection against infection and inhibit shed during outbreak situations are needed. Information provided by this research will provide affected states and poultry industries valuable information when considering vaccination as part of the control or eradication program for an END outbreak.