MERCK ANIMAL HEALTH
TECHNICAL SERVICES BULLETIN

Innovax®-ILT: Comparative Protection Against Virulent Infectious Laryngotracheitis Virus in Commercial Broilers

INTRODUCTION

Infectious laryngotracheitis (ILT) is a common respiratory disease of chickens that inflicts significant economic losses to the global poultry industry. Severe forms of the viral disease are characterized by gasping, expectoration of bloody mucus, and moderate to high mortality, mainly due to respiratory blockage caused by tracheal plugs (Figure 1). Illness and death rates vary depending on the virulence of the circulating strain of ILT virus and the presence of other respiratory infections.

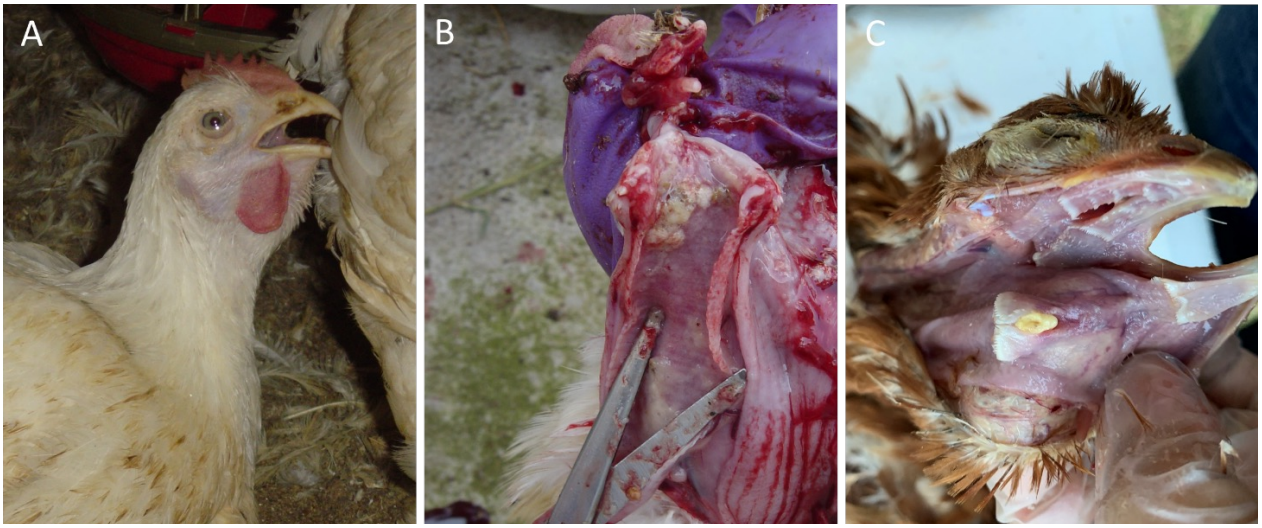
Live commercial vaccines are often used to help control ILT outbreaks. Unfortunately, conventional chicken-embryo-origin (CEO) vaccines can easily spread bird-to-bird and thereby allow the strains to regain virulence.[2] CEO ILT vaccines have been identified as the origin of ILT outbreaks in broilers as well as endemic pathogenic ('hot') ILT on multi-age layer farms. As a response to the frequent ILT outbreaks related to CEO vaccines, a new generation of *recombinant vaccines* using fowl poxvirus and herpesvirus of turkey (HVT) as *vectors* were developed.[3] Like other HVT vector vaccines, ILT vaccines using this technology ('rHVT-ILT') are not eliminated from the bird, but instead persist to induce long-lasting cell-mediated immunity (e.g., full protection still exhibited at 60 weeks post-vaccination under challenge).[4-6]

ILT virus envelope glycoproteins expressed in commercial HVT vectors play major functions in HVT infection and replication.[7,8] Two rHVT-ILT vaccines currently are commercially available in the US, expressing both glycoproteins I and D, or only glycoprotein B, respectively.[5,6]

KEY POINTS

- In a university research study,1 broilers vaccinated with Innovax®-ILT and challenged at 21 or 40 days of age with a virulent ILT strain showed greater protection against ILT clinical signs compared to challenged birds vaccinated with a competitor rHVT-ILT vaccine.
- After challenge at 21 days of age, Innovax-ILT vaccinates demonstrated lower in-bird replication of the ILT challenge virus compared to the rHVT-ILT vaccine group.
- Superior protection against ILT-associated mortality was consistently observed in broilers vaccinated with Innovax-ILT compared to the competitor rHVT-ILT group.

Figure 1: Clinical signs and macroscopic lesions in broilers and layers affected by ILT. A. presence of lacrimation and swollen eyelids; B. blood in trachea; C. tracheal plugs. (pictures courtesy of Dr. Andres Montoya and Dr. Ivan Alvarado)



INNOVAX®-ILT

Innovax®-ILT (Merck Animal Health) is a HVT recombinant vaccine that expresses immunogenic glycoproteins I and D of ILT virus. Because the ILT gene insertions in Innovax-ILT code for glycoproteins I and D, post-vaccination production of antibodies against these proteins stops ILT virus from attaching to chicken cells and spread of the virus from cell to cell, thereby preventing infection and supplying long-term immunity without post-vaccine reactions. Innovax-ILT offers protection against ILT and Marek's disease after single-dose *in ovo* or day-1 subcutaneous vaccination at the hatchery.

A recent research study compared the ability of Innovax-ILT or a competitor rHVT-ILT vaccine (which instead codes for glycoprotein B) to provide ILT protection in broilers that were vaccinated *in ovo* and subsequently challenged at 21 or 40 days of age with a virulent field strain of ILT virus.[1]

DESIGN

The challenge study was conducted at a major Southeast US university and involved 14-day-old broiler embryos acquired from a 37-week-old broiler-breeder flock. The eggs were randomly divided into 4 treatment groups, with 2 of the groups manually vaccinated *in ovo* at 18.5 days of embryonation with a full dose of either Innovax-ILT or a competitor rHVT-ILT vaccine. The remaining 2 groups were similarly handled but not vaccinated and would serve as negative and positive control groups. Embryos remained in incubators until hatch. Replication of the vaccine strains in birds was confirmed at 14 days of age in primary feather follicles using HVT-specific polymerase chain reaction (PCR).

At 21 or 40 days of age, all chickens in the Innovax-ILT, competitor rHVT-ILT, and positive control groups were challenged with a virulent ILT virus strain (1874C5). Individual birds received a total volume of 200 µL containing $10^{3.8}TCID_{50}$ of the challenge ILT virus (50 µL delivered in each eye, 100 µL delivered intratracheally). Chickens in the negative control group remained unchallenged.

Vaccine protection was evaluated based on reduction of clinical signs of the disease, reduction of replication of the challenge virus in the trachea, and reduction of ILT-associated mortality.

- Clinical signs of ILT were quantified daily from 3 to 6 days after each challenge event using a scoring system. Birds were scored from 0 to 3 for signs of conjunctivitis, dyspnea, and lethargy (normal=0; mild=0.5-1; moderate=1.5-2; severe=2.5-3), while dead birds received a score of 6. The total clinical signs score was estimated for each bird and the mean clinical sign score per group was calculated for each post-challenge observation day.
- Replication of the 1874C5 ILT challenge strain was monitored by acquiring tracheal swabs and analyzing quantitative real-time PCR at days 3 and 5 post-challenge (individual and average genome load expressed as the $\log_{10}2^{-\Delta\Delta Ct}$).

- Mortality (and by inverse, survivability) was recorded from 1 to 6 days post-challenge. .

Collected data were statistically analyzed using appropriate standard methods (e.g., ANOVA, Tukey test for post hoc analysis), with comparisons between treatment groups declared significant at $P < 0.05$.

RESULTS

Replication of the rHVT vaccines

The presence of Innovax-ILT or the competitor rHVT-ILT vaccine was detected at 14 days of age in all broilers respectively vaccinated *in ovo* at 18.5 days of embryonation. No significant differences ($P > 0.05$) in HVT viral loads were observed between the 2 vaccinated groups. As expected, no HVT viral loads were detected in the negative control or positive control groups since they did not receive any HVT-ILT vaccine.

Protection against clinical signs

Significant differences in the severity of ILT clinical signs were observed between the 2 groups of vaccinated and challenged broilers (Figure 2). After challenge at 21 days of age, broilers vaccinated with Innovax-ILT demonstrated significantly ($P < 0.05$) lower clinical signs scores than positive controls on all sample days, and significantly lower clinical scores than the competitor rHVT-ILT group on days 4, 5 and 6 after ILT challenge. Similar outcomes were observed for broilers challenged at 40 days of age, as Innovax-ILT vaccinates exhibited significantly lower clinical signs scores than competitor rHVT-ILT vaccinates on days 3, 4, and 6 post-challenge. Further, clinical signs scores in the Innovax-ILT group were even similar ($P > 0.05$) to scores of the unchallenged negative control group (0) on all post-challenge sample days. After either challenge event, the highest clinical signs scores were observed in the positive control group, indicating the ILT challenge was sufficient to cause serious disease and thus differentiate vaccine efficacy.

Replication of the challenge virus

Differences in viral load of the challenge ILT strain in tracheal swabs were observed among treatment groups for broilers challenged at 21 days of age (Figure 3). At 3- and 5-days post-challenge, significant ($P < 0.05$) reductions in viral genome loads were detected in broilers vaccinated with Innovax-ILT. However, viral genome loads in broilers vaccinated with the competitor rHVT-ILT reached levels similar ($P > 0.05$) to the quantity observed in the positive control group. For broilers challenged at 40 days of age (Figure 3), no significant differences ($P > 0.05$) in viral loads were observed among treatment groups.

Survival rates

The severe virulence of the challenge ILT strain used in the study was demonstrated by the low survival rate (high mortality) observed in the positive control group (Figure 4). Only 35% of these non-vaccinated birds remained alive at 6 days post-challenge for birds challenged at 21 days

Figure 2: Mean clinical signs scores (0=normal; 6=death) following ILT challenge at 21 & 40 days of age. (clinical signs absent in non-challenged negative controls on all days)

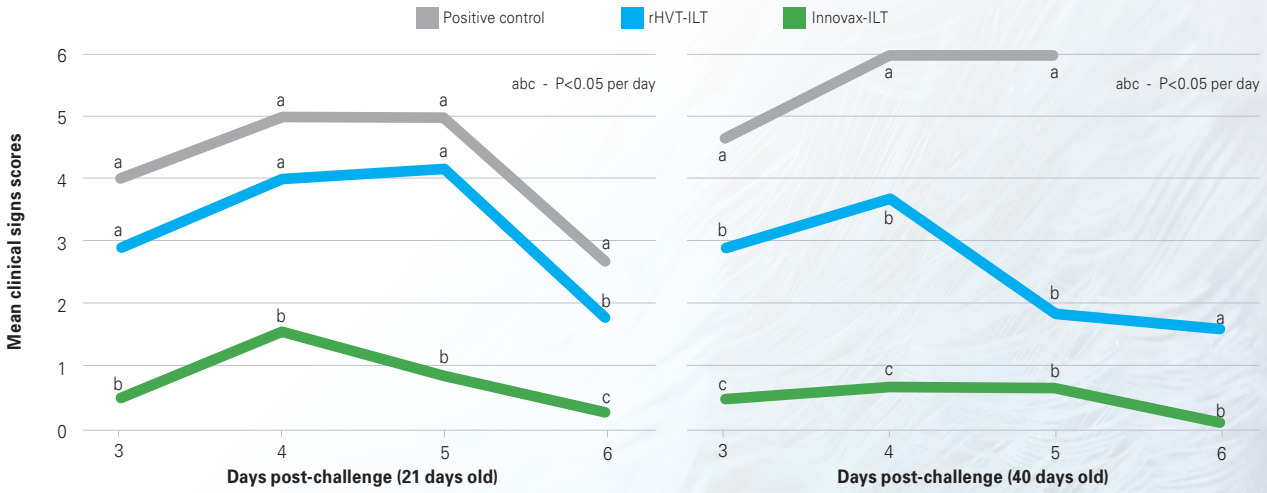


Figure 3: Mean viral load of the ILT challenge strain in tracheal swabs at 3 & 5 days post-challenges. *(virus absent in non-challenged negative controls on all days)*

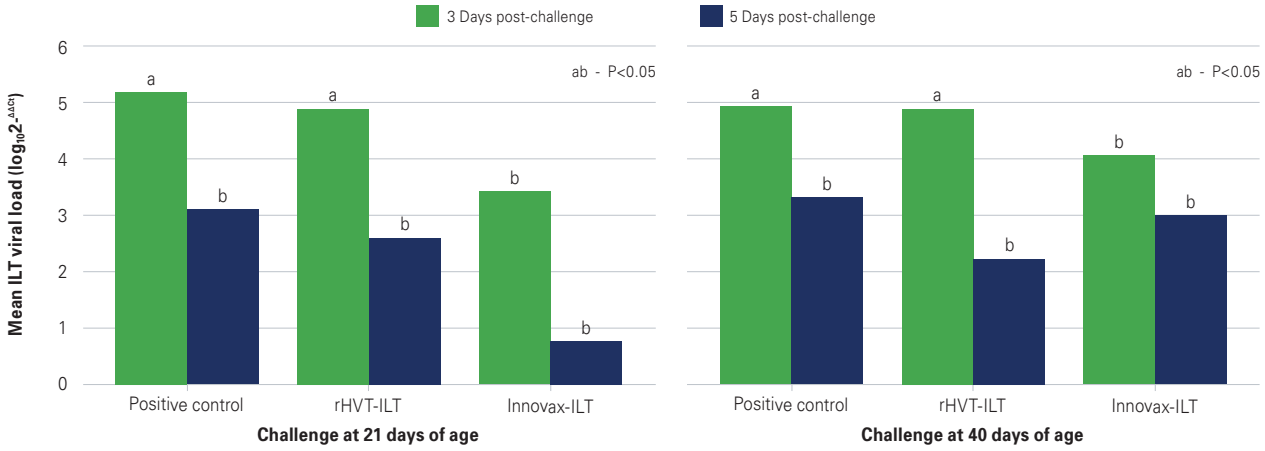
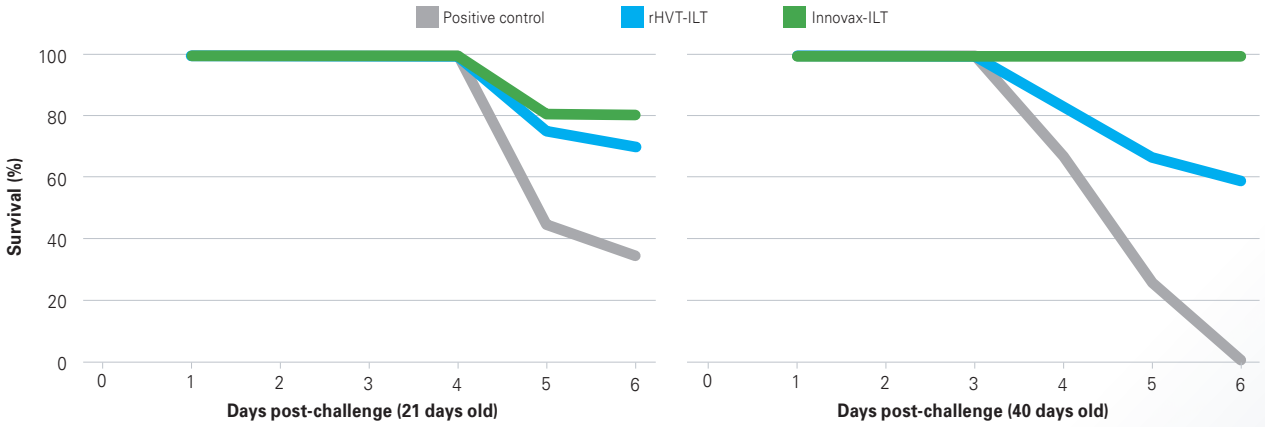


Figure 4: Survival rates during the 6 days following ILT challenge at 21 or 40 days of age. *(no ILT mortality in non-challenged negative controls on all days)*



(65% mortality), and no broilers (100% mortality) survived by 6 days after the day-40 challenge. However, even under such harsh conditions, high survival rates of 80% and 100% were observed for Innovax-ILT vaccinates that received ILT challenge at 21 and 40 days of age, respectively. Death losses were more severe for broilers vaccinated with the competitor rHVT-ILT vaccine, with survival rates of only 70% and ~60% for the 21- and 40-day challenges, respectively.

CONCLUSIONS

Innovax-ILT provided better protection than a competitor rHVT-ILT vaccine against clinical signs and mortality after challenge at 21 or 40 days of age with a virulent ILT strain. The immunity elicited by Innovax-ILT also delivered better protection against replication of the viral ILT strain in challenged birds.

In contrast, the competitor rHVT-ILT vaccine often failed to improve disease impacts compared to non-vaccinated challenged controls.

Reference.

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