Innovax®-ILT and Innovax®-ND-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]

KEY POINTS

- In a university research study,[1] broilers vaccinated with Innovax®-ILT or Innovax®-ND-ILT and challenged at 28 days of age with a virulent ILT strain showed:
  - Strong protection against clinical respiratory signs (competitor rHVT-ILT vaccine group not significantly different from positive controls)
  - Strong protection against mortality (much more death loss in competitor rHVT-ILT group)
  - Maintenance of normal broiler growth rates under conditions of heavy ILT challenge (gains significantly lower in competitor rHVT-ILT group)
  - Lower in-bird replication of the ILT challenge virus (significantly greater viral load in competitor rHVT-ILT group)
- Both Innovax vaccines provided similar, superior protection against the clinical impacts of severe ILT challenge.

ILT virus envelope glycoproteins expressed in commercial HVT vectors play major functions in HVT infection and replication:[7,8]

- glycoprotein I forms heterodimers with glycoprotein E, favoring cell-to-cell virus spread while avoiding host immune defenses;
- glycoprotein B is essential for infectivity (membrane fusion and virus penetration);
- glycoprotein D binds to target host cell receptors and has a superior envelope incorporation and cell surface expression, leading to induction of a superior protective immune response than glycoprotein B.

INNOVAX®-ILT & INNOVAX®-ND-ILT

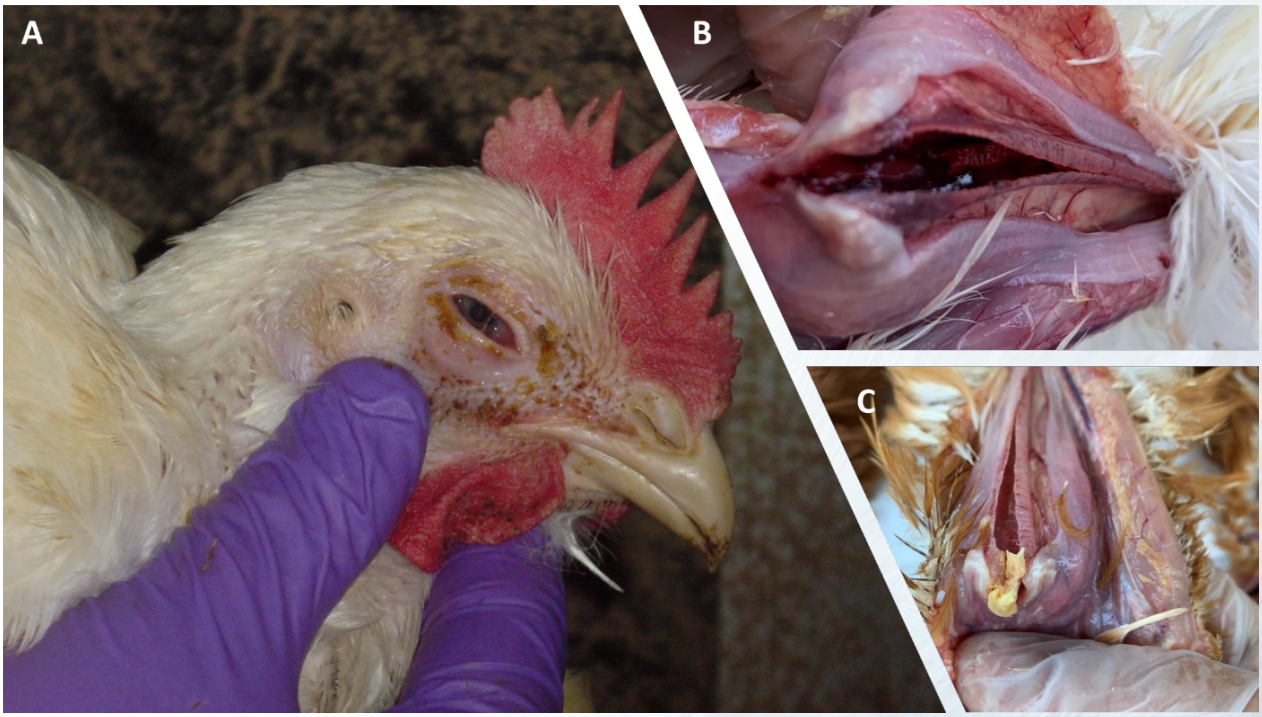
Innovax®-ILT and Innovax®-ND-ILT (Merck Animal Health) are HVT recombinant (rHVT) vaccines that express immunogenic glycoproteins I and D of ILT virus. Because the ILT gene insertions in Innovax-ILT code for glycoproteins I and D, post-vaccination production of antibodies against these proteins stops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

A recent research study compared the ability of Innovax-ILT, Innovax-ND-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 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 35-week-old broiler-breeder flock. The eggs were randomly divided into 5 treatment groups, with 3 of the groups manually vaccinated *in ovo* at 18.5 days of embryonation with a full dose of either Innovax-ILT, Innovax-ND-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 a real-time, HVT-specific polymerase chain reaction (PCR).

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)



At 28 days of age, all chickens in the Innovax-ILT, Innovax-ND-ILT, competitor rHVT-ILT, and positive control groups were challenged with a virulent ILT virus strain (1874C5). Each bird 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 and could thus monitor for any background infections that might impact study results.

Vaccine protection was evaluated based on reduction of clinical signs of the disease, replication of the challenge virus in the trachea, mortality, and maintenance of weight gains.

- Clinical signs of ILT were quantified daily from 3 to 7 days post-challenge 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 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 7 days post-challenge.
- Body weight gain was measured for 7 days after 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, Innovax-ND-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 among the 3 vaccinated groups. As expected, no HVT viral loads were detected in the negative control or positive control groups since they did not receive a HVT vaccine.

Protection against clinical signs

Significant differences in the severity of ILT clinical signs were observed among the 3 groups of vaccinated and challenged broilers (Figure 2). Clinical signs scores were significantly ($P < 0.05$) lower at days 3 to 6 post-challenge in broilers vaccinated with Innovax-ILT or Innovax-ND-ILT compared to those that received the

Figure 2: Mean clinical signs scores (0-6) following ILT challenge at 28 days of age. (clinical signs absent in non-challenged negative controls on all days)

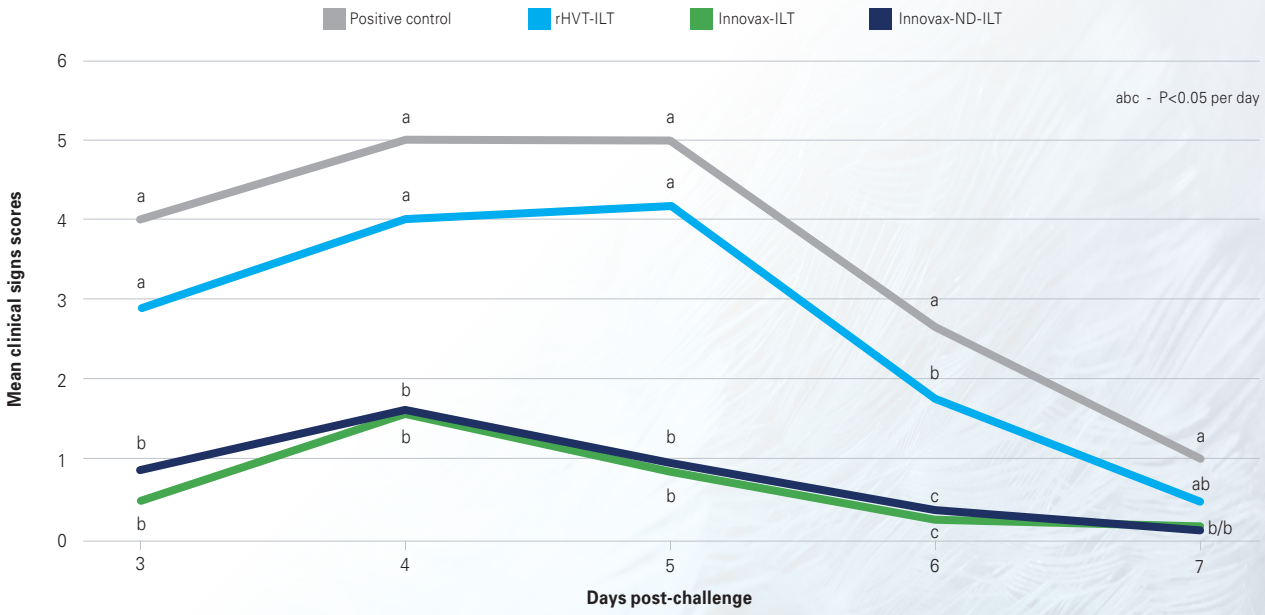


Figure 3: Mean viral load of the ILT challenge strain in tracheal swabs at 3 and 5 days post-challenge. (virus absent in non-challenged negative controls on all days)

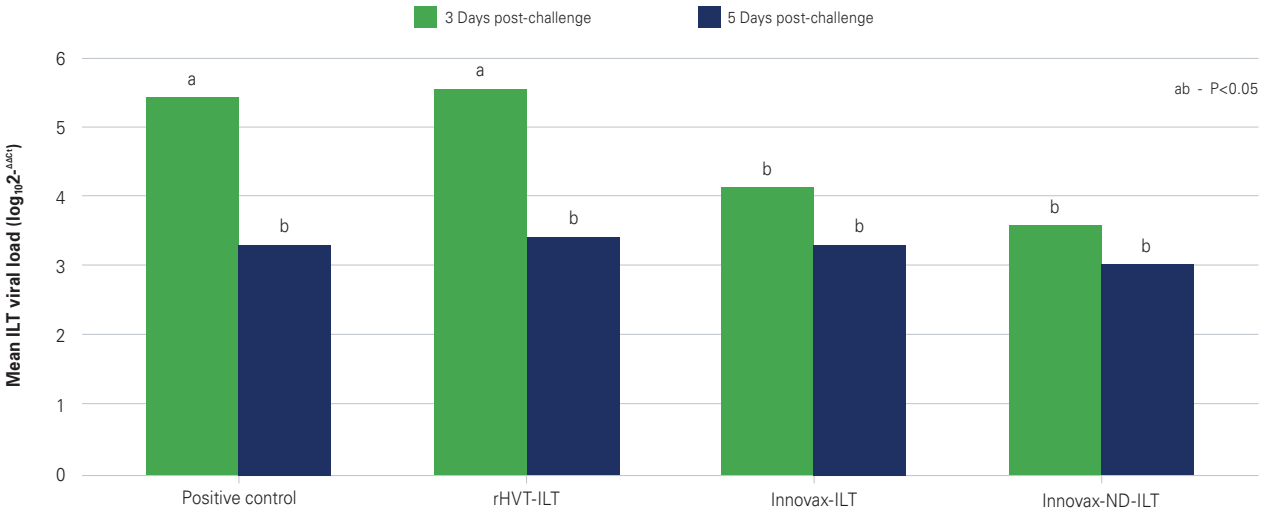
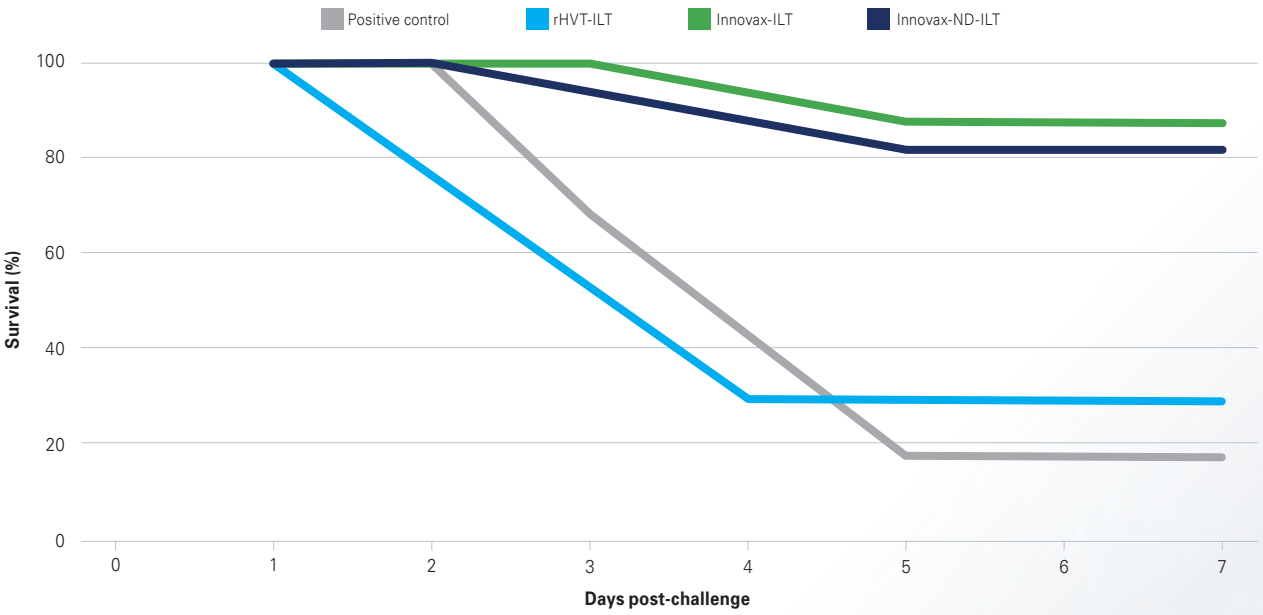


Figure 4: Survival rates during the week following ILT challenge at 28 days of age. (no ILT mortality in non-challenged negative controls on all days)



competitor rHVT-ILT vaccine or nonvaccinates (positive control). Clinical signs scores in the Innovax groups were even similar (P > 0.05) to scores of the unchallenged negative control group (0) on all post-challenge sample days. In contrast, broilers vaccinated with the competitor rHVT-ILT vaccine exhibited the highest clinical signs scores among vaccinated groups, and showed no difference (P > 0.05) vs positive controls on most days after ILT challenge.

Replication of the challenge virus

Differences in viral load of the challenge ILT strain in tracheal swabs were observed among treatment groups (Figure 3). At 3-days post-challenge, significant (P < 0.05) reductions in viral genome loads were detected in broilers vaccinated with Innovax-ILT or Innovax-ND-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. No significant differences (P > 0.05) in viral loads were observed among vaccinated groups at 5 days post-challenge.

Survival rates

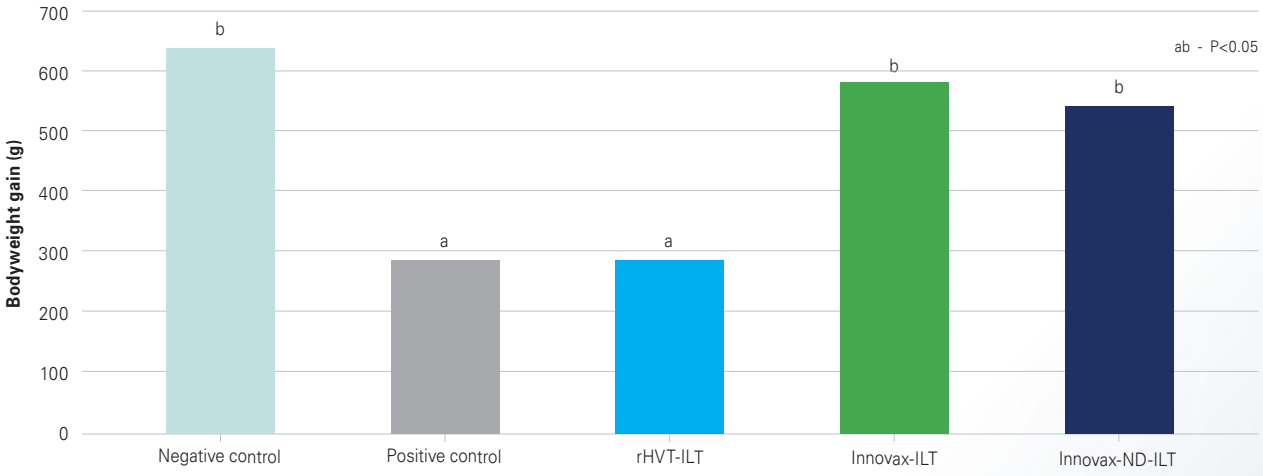
The severe virulence of the challenge ILT strain used in the study was demonstrated by the low survival rate (high mortality) observed in the

positive control group (Figure 4). Only 17% of these nonvaccinated birds remained alive at 7 days post-challenge. However, even under such harsh conditions, high survival rates of 89% and 82% were observed for broilers vaccinated with Innovax-ILT or Innovax-ND-ILT, respectively. Notably, death losses were severe for broilers vaccinated with the competitor rHVT-ILT vaccine as only 29% of these birds survived the challenge infection.

Body weight gain

Post-challenge weight gain results summarized in Figure 5 show that broilers vaccinated with Innovax-ILT or Innovax-ND-ILT achieved significantly ($P < 0.05$) better gains than the unprotected positive control group or birds vaccinated with the competitor rHVT-ILT vaccine. Furthermore, the Innovax groups experienced growth rates similar ($P > 0.05$) to the negative control group, indicating that Innovax-vaccinated birds exposed to severe disease challenge maintained growth rates consistent with 'normal' birds reared without ILT exposure. In contrast, growth rate of broilers vaccinated with the competitor rHVT-ILT vaccine was similar ($P > 0.05$) to that of positive controls, suggesting the competitor vaccine completely failed to provide protections against the growth-inhibiting impacts of ILT.

Figure 5: Body weight gain following ILT challenge at 28 days of age. *(no ILT challenge in negative controls)*



CONCLUSIONS

Innovax-ILT and Innovax-ND-ILT provided superior protection against clinical signs and mortality associated with a virulent ILT challenge strain compared to a competitor rHVT-ILT vaccine. The immunity elicited by Innovax-ILT and Innovax-ND-ILT also delivered better protection against replication of the viral ILT strain in challenged birds, and Innovax vaccinates maintained normal weight gains after challenge.

Conversely, the competitor rHVT-ILT vaccine failed to improve disease impacts compared to nonvaccinated challenged controls.

Reference.

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