# Efficacy of Innovax®-ND-ILT and LT-IVAX® for Protection Against Virulent Infectious Laryngotracheitis Virus in Long-Life Birds

## INTRODUCTION

Infectious laryngotracheitis (ILT), a disease caused by a *Gallid alphaherpesvirus* 1, is an economically relevant respiratory disease affecting the poultry industry worldwide.[2] In a severe presentation, ILT causes gasping, expectoration of bloody mucus, and high mortality due to asphyxia. Biosecurity and vaccination are the pillars of successful ILT control programs.[3,4]

Vaccination programs rely on the use of live, modified attenuated vaccines produced in chicken embryos (CEO) or tissue culture origin (TCO), and recombinant vaccines using the herpesvirus of turkey (HVT) as a vector to express ILT virus immunogenic proteins.[5] TCO vaccines provide protection against respiratory signs and mortality associated with ILT, and reduce replication and shedding of field challenge strains. Unlike CEO products, TCO vaccines are highly attenuated and do not regain virulence after bird-to-bird passage, thus inducing robust protection while limiting horizontal transmission.[6] As a response to the frequent ILT outbreaks associated with CEO-related viruses, recombinant HVT expressing immunogenic ILT virus proteins (rHVT-ILT) have been increasingly used worldwide.[3,7] These rHVT-ILT vaccines reduce clinical signs of the disease and maintain bird performance, but they are not as effective for reducing the shedding of challenge viruses when compared with live attenuated vaccines.[5,8]

## KEY POINTS

- In a university research study,[1] chickens were vaccinated with Innovax®-ND-ILT on day 1, LT-IVAX® at 10 weeks of age, or both (Innovax-ND-ILT on day 1 + LT-IVAX at 10 weeks). Birds were then subjected to virulent ILT challenge at 15 weeks of age.
- Superior protection against clinical signs and mortality was observed for birds vaccinated with Innovax-ND-ILT + LT-IVAX. Post-challenge clinical signs and survival rates in this dual-vaccination group did not significantly differ from non-challenged, non-vaccinated control birds.
- Naive, susceptible birds in contact with Innovax-ND-ILT + LT-IVAX vaccinates showed no statistical differences for clinical signs or mean viral loads compared to naive birds in contact with non-challenged controls.

Vaccination programs that include administration of an rHVT-ILT vaccine at the hatchery followed by live attenuated vaccines applied in the field have been shown to improve safety of the live vaccine and provide optimal protection against ILT in multi-age layer and heavy-weight broilers.[3,9]

## INNOVAX®-ND-ILT

### LT-IVAX®

Innovax®-ND-ILT (Merck Animal Health) is an rHVT vaccine expressing the D and I glycoproteins of ILT virus plus fusion protein of Newcastle disease virus, thus offering protection against Marek's disease, Newcastle disease, and ILT after hatchery in ovo or subcutaneous (SC) vaccination. In contrast, LT-IVAX® (Merck Animal Health) is a highly attenuated TCO vaccine that provides solid and uniform protection against ILT when administered via eye-drop to chickens 4 weeks of age or older.

A recent research study[1] investigated the synergistic protection provided by Innovax-ND-ILT and LT-IVAX against virulent ILT challenge in Leghorn-type birds. The study also explored the reduction of post-challenge viral shedding and horizontal transmission to susceptible birds as additional benefits of adding LT-IVAX to an Innovax-ND-ILT-based vaccination program.

## DESIGN

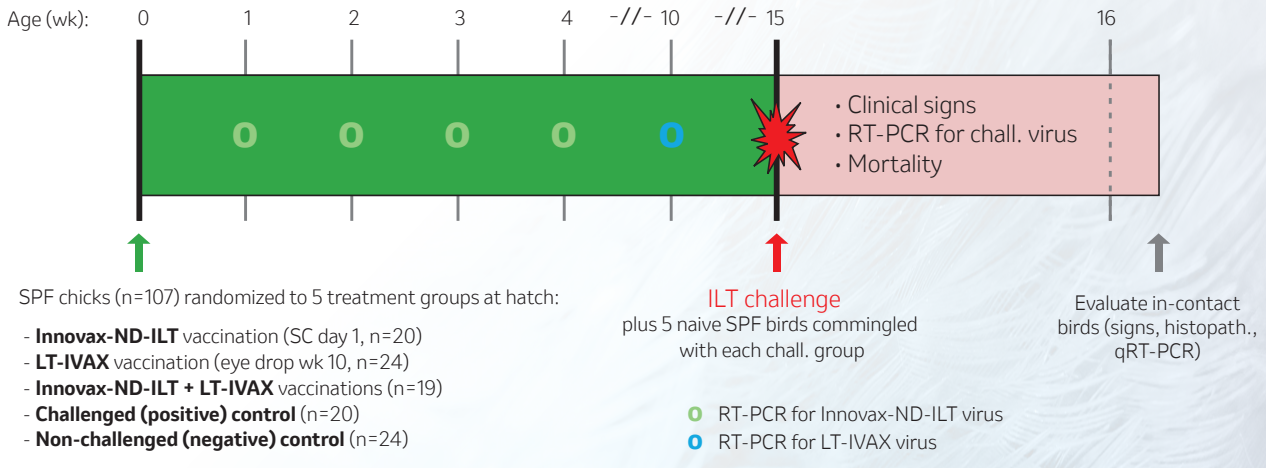
The challenge study was conducted at a major Southeast US university and involved specific pathogen-free (SPF) chicken eggs obtained from a commercial source. At hatch, 107 SPF chicks were randomly distributed into 5 treatment groups and managed as follows (Figure 1):

- Innovax-ND-ILT vaccination via SC injection at day of age (n=20)
- LT-IVAX vaccination via eye drop at 10 weeks of age (n=24)
- Innovax-ND-ILT and LT-IVAX vaccinations (n=19)
- Challenged (positive) non-vaccinated control (n=20)
- Non-challenged (negative) non-vaccinated control (n=24)

Replication of Innovax-ND-ILT vaccine virus in vaccinated birds was assessed using real-time polymerase chain reaction (RT-PCR) for detection of HVT vector in DNA samples extracted from wing primary feathers of 15 randomly selected birds at 1-, 2-, 3-, and 4-weeks post-vaccination. Replication of LT-IVAX vaccine virus in vaccinated birds was assessed by detection of the ILT viral UL44 gene via RT-PCR from DNA samples extracted from tracheal swabs collected 4 days post-vaccination. DNA from feather pulps and trachea/conjunctiva samples extracted from non-vaccinated birds were included in the evaluation as negative controls.

At 28 days of age, all vaccinated chickens and the positive control group were challenged with a highly virulent ILT virus strain (1874C5). Each bird received a total volume of 200 µL containing $10^4$ $TCID_{50}$ of the challenge ILT virus (50 µL delivered in each eye, 100 µL delivered intratracheally).

*Figure 1: Summary of study design.*



Chickens in the negative control group remained unchallenged (mock challenged) and could thus monitor for any background infections that might impact study results.

Efficacy of the 3 different vaccination programs was evaluated based on reduction of clinical signs of the disease, decreased replication of the challenge virus in the trachea, and protection against mortality.

- Clinical signs of ILT were evaluated 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 vaccinated group per time point was calculated.
- Replication of the ILT challenge strain was determined by acquiring tracheal swabs at days 3 and 5 post-challenge for analysis by quantitative RT-PCR (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.

The capacity of the different vaccination programs to maximize protection and reduce the risk of horizontal transmission was evaluated by adding naive SPF birds to isolation units with challenged birds. Groups of 5 naive SPF chickens were allowed to commingle with the 4 ILT-challenged groups. Eight days later, clinical signs were evaluated in the susceptible in-contact birds, and trachea samples were collected for histopathological examination and detection of the ILT challenge virus by RT-PCR.

Collected data were statistically analyzed using appropriate standard methods (e.g., ANOVA, Kruskal-Wallis/Dunn, Tukey, log rank), with comparisons between treatment groups declared significant at $P < 0.05$.

## RESULTS

### ILT vaccine virus replication

Innovax-ND-ILT vaccine virus was detected in 93% of the feather follicle samples collected from vaccinated birds from 1 to 4 weeks of age, with no significant differences in the level of replication from 1 to 3 weeks post-vaccination. The presence of LT-IVAX vaccine virus in tracheas of vaccinated birds was assessed at 4 days post-vaccination, with 46% and 32% positive results from LT-IVAX vaccinates and Innovax-ND-ILT + LT-IVAX vaccinates, respectively. No significant differences in level of LT-IVAX virus replication were observed between the groups vaccinated with LT-IVAX and Innovax-ND-ILT + LT-IVAX.

### Protection against clinical signs

In the face of severe ILT challenge, groups vaccinated with Innovax-ND-ILT and/or LT-IVAX showed a reduction in clinical signs from 3 to 7 days post-challenge compared to the non-vaccinated/challenged control group (Figure 2). While clinical signs peaked in challenged controls at 5 days post-challenge, birds vaccinated with LT-IVAX or Innovax-ND-ILT + LT-IVAX demonstrated significantly lower clinical signs scores. The clinical signs score for the Innovax-ND-ILT + LT-IVAX group was not significantly different than the non-challenged negative control group.

*Figure 2: Mean clinical signs scores following challenge with virulent ILT virus at 15 weeks of age (different letters at 5 days post-challenge denote groups with significant differences at P < 0.05)*

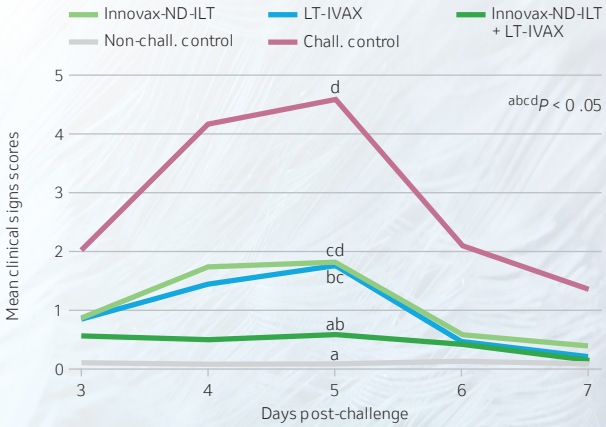
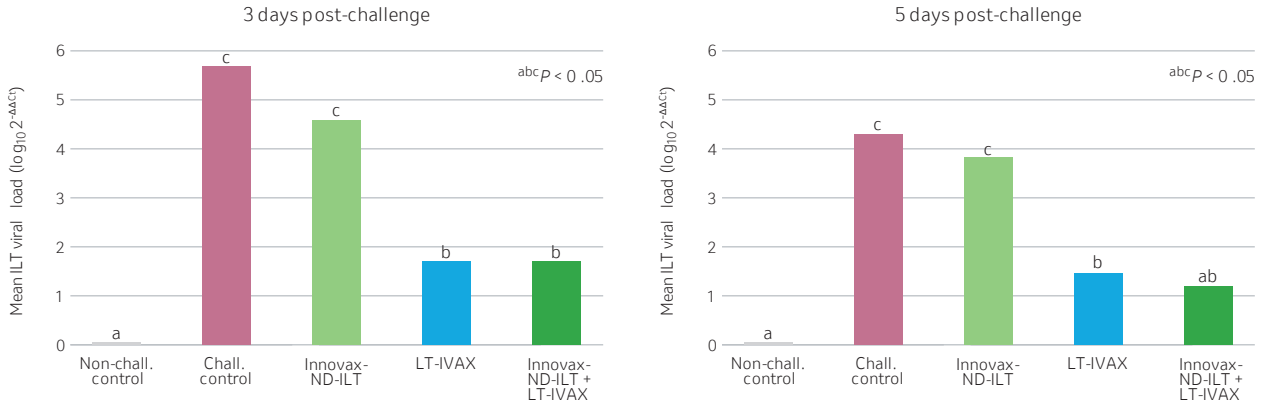


*Figure 3: Mean viral genome loads of ILT challenge strain in tracheal swabs at 3- and 5-days post-challenge . Challenge performed at 15 weeks of age (different letters above bars denote groups with significant differences at P < 0.05)*




## Challenge virus shedding

At 3 and 5 days post-challenge, significantly lower viral loads were observed in the LT-IVAX and Innovax-ND-ILT + LT-IVAX groups (Figure 3). At 5 days post-challenge, load of challenge virus in the Innovax-ND-ILT + LT-IVAX group was not significantly different than the non-challenged control group.

## Survival rates

High survival rates were achieved for all vaccinated groups compared with the non-vaccinated challenged control group during the post-challenge period (Figure 4).

A survival rate percentage of 87% was observed for the LT-IVAX vaccinated group, 95% for the Innovax-ND-ILT group, and 100% for the Innovax-ND-ILT + LT-IVAX group.

## Horizontal transmission to in-contact birds

Naive, susceptible birds placed in contact with challenged vaccinates demonstrated reduced clinical sign scores (Figure 5) and mean viral loads (Figure 6) compared with birds commingled with challenged controls.

Naive birds in contact with Innovax-ND-ILT + LT-IVAX vaccinates showed no statistical differences for clinical signs or mean viral loads compared to non-challenged controls.

All the vaccination programs evaluated in this study significantly reduced the horizontal transmission of the challenge virus to naive birds. The most significant reduction in viral shedding and transmission was observed in birds vaccinated with Innovax-ND-ILT and LT-IVAX.

*Figure 4: Survival rates during the week following ILT challenge at 15 weeks of age (both the Innovax-ND-ILT + LT-IVAX group and the non-challenged control group had 100% survival)*

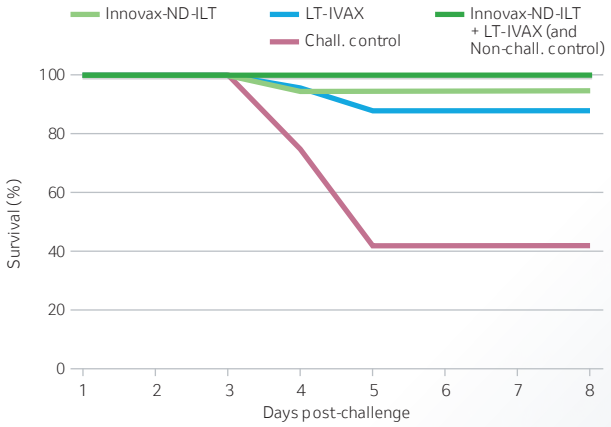



*Figure 5: Mean clinical signs scores in naive (susceptible) birds after 8 days in contact with challenged groups (different letters above bars denote groups with significant differences at P < 0.05)*

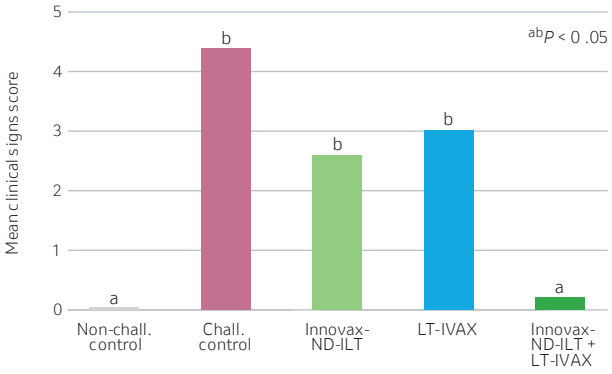
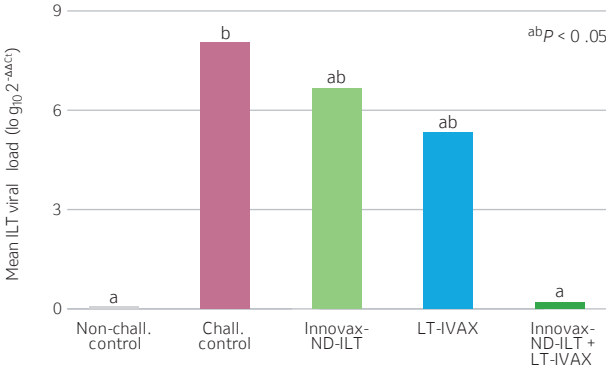



*Figure 6: Mean ILT challenge virus genome loads in naive (susceptible) birds after 8 days in contact with challenged groups (different letters above bars denote groups with significant differences at P < 0.05)*




## CONCLUSIONS

The 3 vaccination programs evaluated in this study [Innovax-ND-ILT on day 1; LT-IVAX at 10 weeks of age; Innovax-ND-ILT (day 1) + LT-IVAX (10 weeks of age)] showed reduced clinical signs, reduced mean viral load shedding, and good survival rates post-challenge at 15 weeks of age. Numerically lower clinical signs and mean viral load values were observed for susceptible birds commingled with vaccinated birds.

The most robust protection against virulent ILT challenge was observed in birds that received the dual vaccination program of Innovax-ND-ILT (day 1) followed by LT-IVAX (10 weeks). Chickens that received the dual vaccination program exhibited statistically less clinical signs and mean viral load shedding at 5 days post-challenge, along with the best survival rates. The dual program was also shown to be the best for the control of horizontal transmission of the challenge virus to susceptible birds.


Reference.

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