

# Immunogenicity of the associated administration of Evanovo® and a commercial HVT–ND–IBD vaccine in commercial broiler chickens

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# Background and objectives

EVANOVO® is a live attenuated parasitic vaccine specially developed for in-ovo (IO) administration of chickens against coccidiosis. Combining in-ovo vaccination against coccidiosis with other commercial vaccines in a single injection can optimize and streamline hatchery vaccination processes without compromising the effectiveness of the co-administered vaccines. Therefore, the objective of this study was to assess the safety and immunogenicity of the associated administration of EVANOVO® and a Turkey herpesvirus double construct (HVT – ND – IBD) vaccine when injected IO to 18-day-old chicken embryonated broiler eggs.

### Materials and methods

18-day-old commercial broiler chicken embryonated eggs from the same batch were randomly distributed into three groups of 98 embryonated eggs balanced by egg weight. The IO co-administration of EVANOVO® (HIPRA, Spain) and the HVT – ND – IBD vaccine was compared to IO vaccination with HVT – ND – IBD vaccine followed by a coarse-spray vaccination with EVANT® (HIPRA, Spain) at one-day of age, as a common approach used in broiler vaccination programs. The co-administration of the vaccines was performed with the solvent supplied by the HVT – ND - IBD producer. The storage and preparation of the vaccines was erformed according to their Summary of Product Characteristics (SPC). The vaccines were co-administered within the shelf life after reconstitution according to the SPC of the HVT – ND – IBD vaccine. After hatching, ninety chicks from each group were included to the study. Feed and water were supplied *ad libitum*. All the chicks were housed on different floor pens in the same room under similar conditions for 45 days.

Table 1. Experimental design

Groups	IO administration	One-day of age adminis- tration	Total number of eggs (day -3)	No. Animals
A	EVANOVO® + HVT – ND – IBD	_	98	90
В	HVT – ND – IBD	EVANT®	98	90
С	HVT – ND – IBD	<b>-</b>	98	90

The safety of the co-administration of EVANOVO® and an HVT – ND – IBD vaccine was evaluated by means of the percentage of hatchability and viability and the observation of adverse reactions during the entire study period.

Specific antibody levels against Newcastle disease (ND) and Infectious Bursal disease (IBD) were measured to determine the immunogenicity of the HVT – ND – IBD vaccine at 45 days after hatching. The oocyst counts to assess EVANOVO® and EVANT® vaccination were performed weekly up to 35 days after hatching.

Hatchability and viability results were analyzed by a Chi Square test. Serology data were analyzed by Kruskal-Wallis. The SPSS® (SPSS Inc.) program was used to perform statistical analysis. Values with P≤ 0.05 were considered statistically significant.

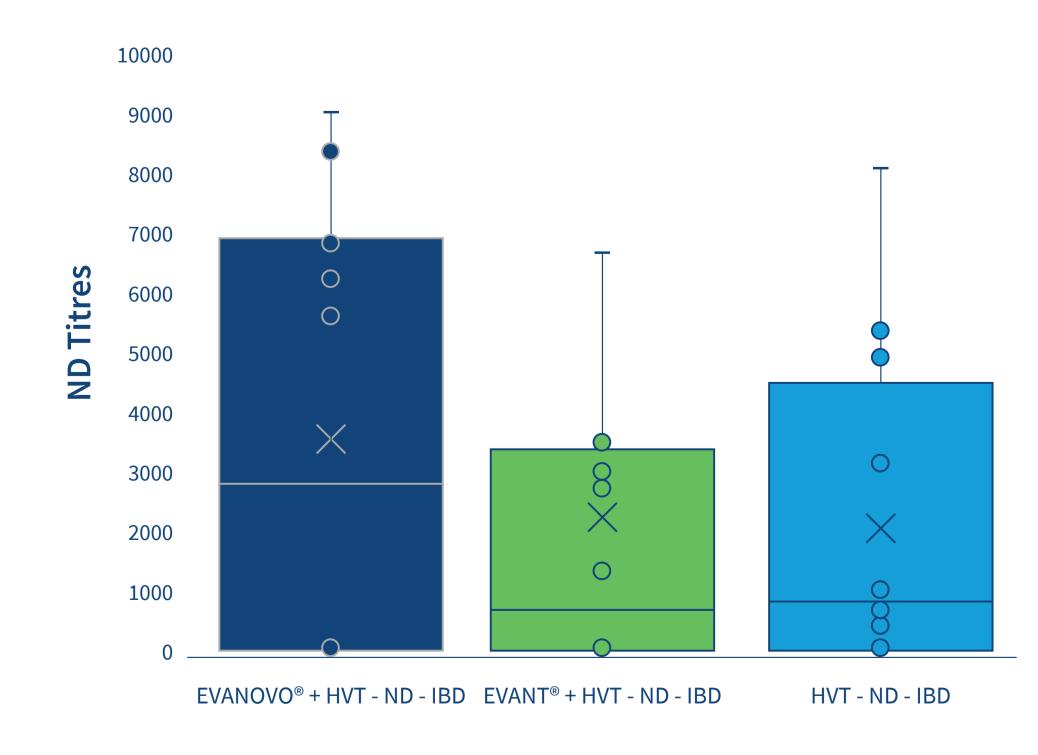
### Results

No adverse reactions were reported in any of the groups at hatchery level. In addition, no statistically significant differences were observed between groups on the hatching rates (Figure 1). On the other hand, no clinical signs were detected during the entire study period.

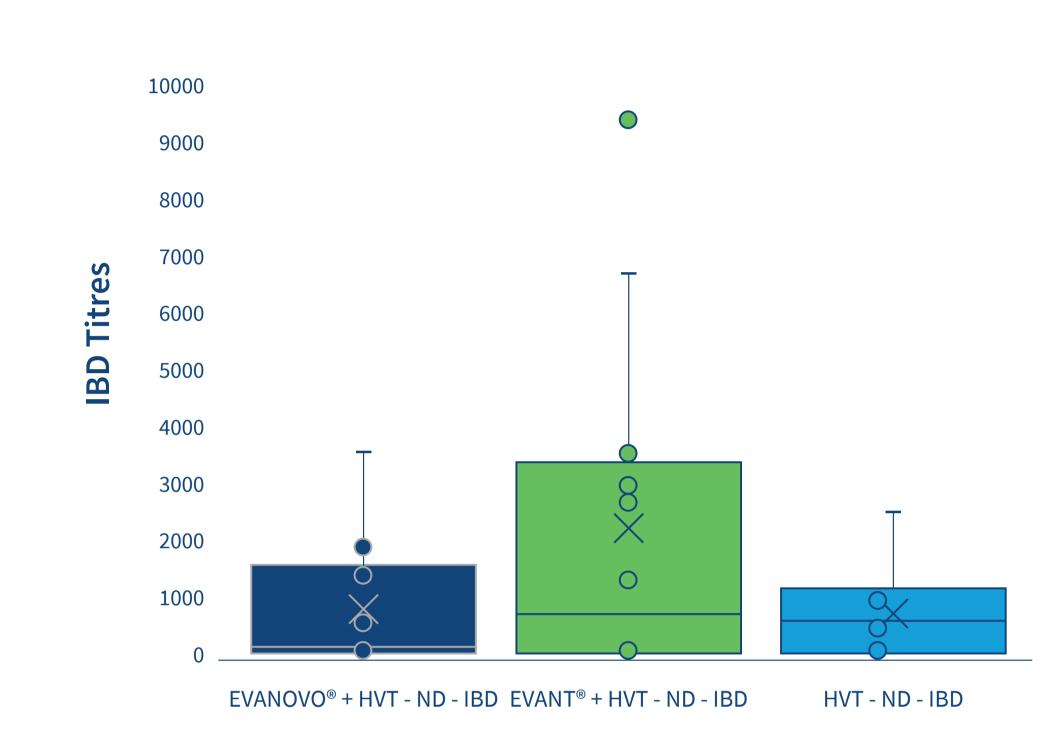


**Fig 1.** Hatching rates and viable birds according treatment.

There was no statistically significant effect on the seroconversion rate of antibodies against IBD and ND throughout the study period. IO co-administration of EVANOVO® and an HVT – ND – IBD vaccine (Group A) resulted in a similar rate of seroconversion when compared to IO vaccination with HVT – ND – IBD vaccine and EVANT® (Group B) at one-day of age (Figures 2 and 3).

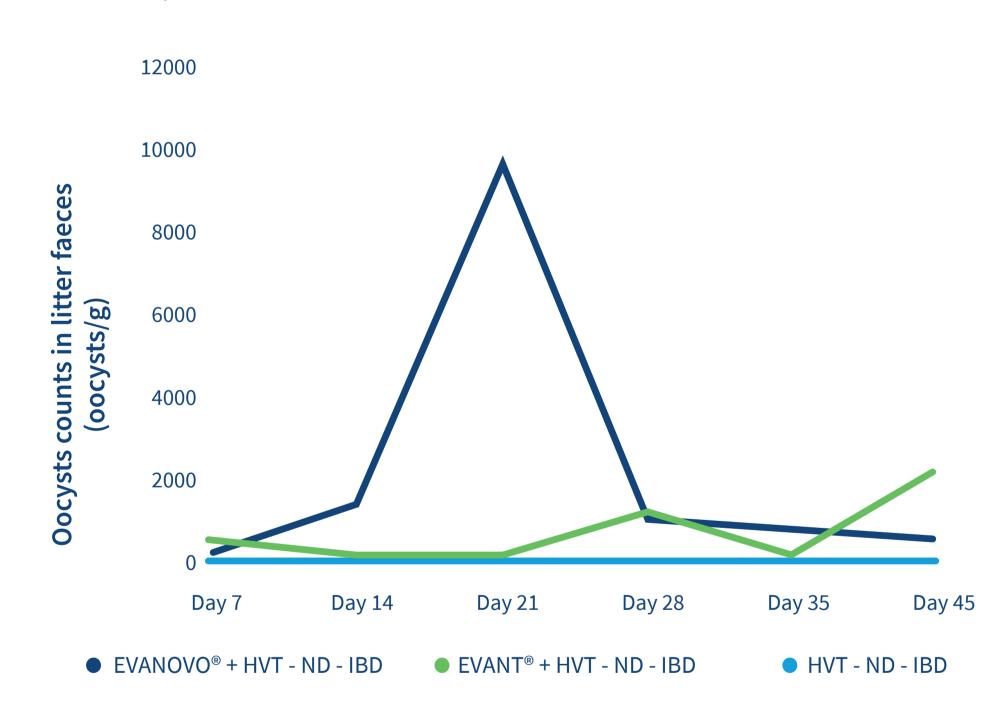


**Fig 2.** ND seroconversion titres at the end of the study period (day 45 post-hatching). A commercial diagnostic ELISA kit was implemented: BioCheck® NDV.



**Fig 3.** Titres of IBD seroconversion at the end of the study period (day 45 post-hatching). A commercial diagnostic ELISA kit was implemented: BioCheck® IBD CK113.

The elimination profile of oocysts in groups A and B indicated that both the administration and the post-vaccination *Eimeria* vaccinal strains replication was correct.



**Fig 4.** Average oocysts counts in litter faeces of each vaccinated group at different timepoints.

## Discussion and conclusion

The present study is the first one to describe the combinatorial use of EVANOVO® and HVT – ND – IBD vaccines. Birds immunized with EVANOVO® and HVT – ND – IBD vaccines by in-ovo route showed a normal profile of oocysts counts after vaccination and a similar antibody response against IBD and ND in comparison with the other vaccination schedule. The use of a commercial recombinant vaccine HVT – ND – IBD together with the EVANOVO® vaccine can provide an adequate and similar protection, in comparison with the in-ovo administration of the HVT – ND – IBD vaccine without the mixing of EVANOVO® regardless the implementation of a coarse spray coccidiosis vaccination at 1 day old chick with EVANT®.

In conclusion, these findings suggest that vaccination with the associated administration of EVANOVO® and HVT – ND – IBD vaccines are as safe and efficient in protecting birds from coccidiosis, Marek (MD), Newcastle (ND) and Gumboro (IBD) diseases as when each vaccine is administered alone.