Field experiences with next generation sequencing and hemagglutination inhibition assays for monitoring vaccine take in broiler flocks vaccinated *in ovo* with an HVT-ND-IBD double construct vaccine

Magdalena Sitter[1], Geert Van Den Abeele[2], Tom Timmerman[3], Filip Boel[4], Joachim De Backere[4], Sjouke Van Poucke[5], Frank Verschuere[1] , Peter De Herdt[1]

In Belgium, broilers are increasingly being vaccinated *in ovo*, using a double insert HVT-ND-IBD vaccine. The vaccine consists of a Herpes Virus Turkey (HVT) backbone and both the F protein of Newcastle Disease virus (NDV) and the VP2 of Infectious Bursal Disease virus (IBDV) as inserts. Broilers vaccinated in ovo with the HVT-ND-IBD double construct vaccine are thereafter not revaccinated with traditional live vaccines against NDV and IBDV.
At the same time, there is a tendency towards on-farm hatching of broilers. In ovo vaccination and on-farm hatching are therefore often combined.

Broiler farmers and veterinarians are seeking tools that can confirm whether their birds have indeed been vaccinated *in ovo* with the vaccine of their choice and whether this vaccine has been replicating and conferring protection. In the present study, it was examined whether next generation sequencing (NGS) and hemagglutination inhibition (HI) assays are appropriate to reach these goals under field conditions.

The *in ovo* administration of the HVT-ND-IBD vaccine was consistently verified at the hatchery. After the vaccine inoculation, all eggs were transported to broiler farms for on-farm hatching using the NestBorn® system.

At the age of 25-27 days, approximately 15-30 feathers from the axillary region were collected from 20 birds per vaccinated flock. The pulp of the feathers of each broiler was then transferred to one circle of a Whatman® FTA card and submitted to the laboratory of Rapid Genomics (Gainesville, FL, USA) for examination of the vaccine DNA level in the samples with an NGS technique, using the specific detection of the HVT-ND-IBD vaccine used in this study. The load of chicken DNA was assessed simultaneously to prove the validity of the samples.
Between 5 and 6 weeks of age, sera were collected from 15-20 birds per flock. Antibody titers against NDV were determined in a certified lab by making twofold dilution series of the sera and using 8 hemagglutinating units of LaSota type antigen.
So far, 6 flocks have been examined in both NGS and HI. Additional to those, respectively 3 and 4 flocks were tested solely with NGS and HI. The study is ongoing and the results will be presented at the WVPAC2023.

The percentages of birds found positive for the presence of the HVT-ND-IBD vaccine in the feather pulp at 25-27 days of age, ranged from 65% to 100% per flock, the average being 82%. The mean HI titer per flock varied from 2.1 to 4.4 log2 units, the average being 3.28.

1… MSD Animal Health, Wim de Körverstraat 35, 5831 AN Boxmeer, NL
2… Dierenkliniek Venhei, Geelsebaan 95-97, 2460 Kasterlee, BE
3… Provet Dierenartsenpraktijk, Talpestraat 2, 8610 Kortemark, BE
4… Belgabroed broeierij, Steenweg op Hoogstraten 141, 2330 Merksplas, BE
5… Syn+, Aertstraat 9, 9870 Olsene, BE

Log2 titers 1, 2, 3, 4, 5, 6 or 7 were measured in 4%, 18%, 37%, 20%, 9%, 5% and 0.5% of the sampled chickens, respectively. Log2 titers below 1 were not observed.

Under the conditions of this study, both NGS and HI proved valuable tools to monitor the outcome of an HVT-ND-IBD double construct vaccine administered *in ovo*. The NGS tests specifically proved that the vaccine under consideration has been administered. HI examinations demonstrated that the birds have developed antibody titers to NDV. As Belgium is officially free from NDV and no other NDV vaccines had been used, it can be considered that the observed seroconversions resulted from the *in ovo* vaccination. Depending on the sampling timepoint, vaccine DNA levels present in the feather pulp on the day of sampling might not always reach the detection limit of NGS and low HI antibody titers might be due to unspecific reactions. It therefore could be wise to consider the results of both tests together for drawing conclusions on the quality of the in ovo vaccine administration.

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