

Broiler breeder Gumboro disease vaccination priming

by Stephane Lemiere DVM, Global Avian Technical Director, Merial

Broiler breeder parent stock vaccination against Gumboro disease or infectious bursal disease (IBD) is usually based on the injection of at least one inactivated vaccine in oil adjuvant, typically included in a combined product. Priming using one or several live IBD vaccine has been the most common way to immunise breeders so far.

Protection against vvIBD challenge in chicks of one commercial genetic line vaccinated in ovo with the HVT-IBD vector vaccine Vaxxitek HVT+IBD was demonstrated in the context of an experimental station in Europe.

The parents' IBD vaccination program, using Vaxxitek HVT+IBD, Vaxxitek HVT+IBD plus IBD inactivated vaccine, or inactivated IBD vaccine alone, did not impair their progeny's in ovo Vaxxitek HVT+IBD take and subsequent protection against vvIBD virus challenge. An advantage in terms of immunisation of

control. At 20 weeks of age, the birds were divided into groups and vaccinated intramuscularly in the breast with various inactivated IBD vaccines. Serum samples were collected at 20, 26, 30, 40, 50 and 60 weeks of age and tested for IBD antibodies using ELISA and virus neutralisation (VN) tests.

When the breeders were 30 and 50 weeks of age, 14 day-old progeny from the various breeder groups were challenged intra-ocularly with USA pathogenic IBD virus isolates IBD-E, AVS-SU and AVS-DL to evaluate maternal immunity. Protection against challenge was evaluated by calculating the bursa to body weight ratio seven days after challenge.

Serum samples and broiler chicks from a breeder flock vaccinated with a typical IBD program were included in the study as an industry reference. Serum samples were collected four and eight weeks post-vaccination to meas-

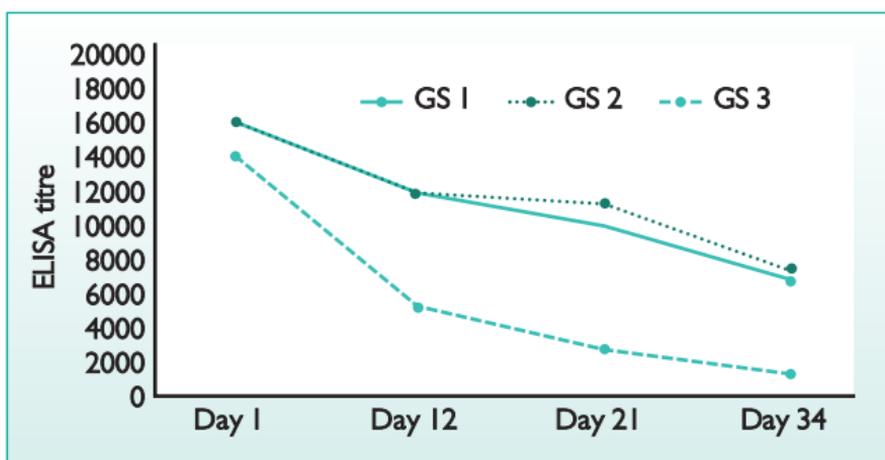


Fig. 1. Maternally-derived IBD plus ELISA antibody waning monitoring (GS = groups for serology).

the progeny against vvIBD was shown in chicks born to breeders vaccinated with Vaxxitek HVT+IBD as a primer, as compared to breeders vaccinated with the inactivated vaccine alone.

A high level of IBD maternally-derived antibodies transmitted to the progeny (Fig. 1) by their parents, together with an earlier onset of immunity by in ovo injection of the HVT-IBD vector vaccine, induced clinical protection, as monitored on bursas, after vvIBD virus challenge.

In the context of an experimental station in the USA, commercial broiler breeders were vaccinated subcutaneously at one day of age with Vaxxitek HVT+IBD.

One group received HVT+SBI Marek's disease vaccine as a con-

ure IBD antibodies using the ELISA and VN test. All progeny from breeders that received Vaxxitek HVT+IBD showed superior protection against challenge with the various IBD virus isolates tested. Hens vaccinated with the vectored vaccine had increasing levels of IBD antibodies between 20 and 60 weeks of age, while normally IBD antibody levels decrease by 50 weeks of age in birds vaccinated with typical commercial programs used in the USA.

In conclusion, Vaxxitek HVT+IBD can be considered as the reference vaccine for IBD priming in broiler breeders. ■



Hatchery spray vaccination against Newcastle disease and IB

by Stephane Lemiére DVM, Global Avian Technical Director, Merial

Vaccinations by spray against both Newcastle disease (ND) and infectious bronchitis (IB) disease have been performed in hatcheries for decades, and today remain the standard in many countries.

Usually these vaccinations are considered as primers, but in low incidence countries or areas, like in North America and Europe, there may be a single vaccine application for control of both diseases. In some of those countries, only IB vaccination may be performed, as ND could be considered at low risk. Nevertheless, dual vaccination of ND and IB is currently widely used across the globe. The standard vaccination program is based on the use of a low post-vaccination reaction ND live vaccine, such as Hitchner B1.

The main concern is about respiratory post-vaccination reactions that may occur with other types of ND vaccine strains, especially when they are administered together with an IB live vaccine. Some ready-to-use vaccine associations already exist in different markets worldwide, but extemporaneous mixture of vaccines is also common practice.

An immunogenic IB vaccine strain is generally recommended at day old, to build up immunity against this disease.

Massachusetts (Mass) strain based IB vaccines are mostly used and have been shown to be fully compatible with low pathogenicity ND vaccine virus strains.

These vaccines are usually administered using hatchery sprayers delivering rather low quantities of vaccine suspensions to day-old chicks; the objective is to get chicks in contact with the live vaccine suspension, and to make it in contact with the conjunctiva, the nasal mucosa and the upper respiratory tract.

The vaccine viruses mainly replicate locally and stimulate the Harderian gland, a main immune system outpost involved in development of a local immune response to the vaccines. As such vaccines induce an immunity of several weeks, and re-vaccinations in the field may be necessary according to epidemiological conditions and disease incidence.

For long-living birds, a more complete vaccination program will also be based on the use of

adjuvanted inactivated vaccines against ND and IB in this context. The main advantage of using the Merial ND respiratory/enteric tropism live vaccine (AVINEW VG/GA) in association with a Mass IB vaccine is to benefit from its safety besides its proven efficacy. Post-vaccination reactions after the use of ND respiratory/enteric vaccine are almost non-existent in field conditions, as widely recorded since almost 20 years of use worldwide, and its association with an IB Mass live vaccine leads to negligible post-vaccination respiratory reactions.

The main advantage as compared to classical low pathogenic ND live vaccines applicable at day old is that the Merial ND respiratory/enteric vaccine protects against ND velogenic strain challenges, as already demonstrated in many scientific publications.



ND and IB field challenges usually occur at very early stages, and passive immunity, especially against IB, is very limited. An early immunisation is therefore needed in order to protect against both diseases of the chickens: ND and IB. The respiratory/enteric live ND vaccine is registered in most countries and may be used at day old for ND control, in association with a Mass live vaccine.

In some countries, a frozen presentation of this live ND vaccine is available for administration at the hatchery. In Asia, a bivalent frozen presentation or a freeze-dried presentation of the respiratory/enteric ND live vaccine with a Mass IB strain, are available.

Elsewhere, any extemporaneous use of the respiratory/enteric ND live vaccine with a live IB Mass vaccine may be recommended according to current local legal constraints on the vaccine use. ■



New routes of administration for Newcastle disease vaccination

by Francisco Perozo DVM, MS, PhD, Full Professor,
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Newcastle disease (ND) is a main concern for endemic countries and an economical and epidemiological threat for countries declared free from the disease.

The poultry industry relies on management and biosecurity measures, multiple vaccination protocols (live, vector-based and inactivated vaccines) and the control of immunosuppressive factors to diminish the detrimental effects of the disease.

Hatchery vaccination is of foremost importance because handling of one-day-old chicks represents (based on expected compliance with standard operation procedures) the most homogenous and controlled segment of the poultry production chain, hence it is in the industry's best interest to take advantage and generate a successful immunisation of the birds at this moment.

Live ND hatchery vaccination is currently implemented worldwide using spray boxes specially designed to vaccinate 100 chicks in a few seconds. Based on the stringency of their field challenge, some countries will require an additional inactivated vaccine to be applied subcutaneously at the hatchery (0.1 or 0.2ml into the neck area) followed by live virus field revaccination using several routes (eye drop, spray or drinking water).

Currently, single needle concomitant subcutaneous (SQ) application of vector HVT-IBD vaccines (Vaxxitek HVT-IBD) and killed ND, is a trend in ND endemic countries. Additionally, early literature reports efforts to improve live ND immunisation programs using oil as adjuvant for live vaccines.

Peleg and co-workers, in 1993, reported that live in oil vaccines, prepared immediately prior to the vaccination, were shown to be 30-50 times more effective than either the same vaccines reconstituted in water or killed in oil vaccines.

Hence, in order to take advantage of the benefits of live plus killed ND vaccination, adding live ND virus to the vector HVT-IBDV, and apply SQ concomitantly with a killed ND, can improve the efficacy of the vaccination program against ND and

was tested in the following experiment.

The study aimed to assess the protection provided by concomitant subcutaneous (SQ) hatchery application of live-plus-killed ND vaccines (Avinew and Galimmune ND), compared with protocols including two commercial vector HVT-ND vaccines in broilers.

Seven groups (24 one-day-old broilers) were used: hatchery SQ live plus killed ND vaccination with and without boost, two commercial vHVT-ND vaccines with and without live NDV boosts (day 1 and 10) and an unvaccinated control group, were challenged at 28 days of age with a Venezuelan velogenic viscerotropic NDV strain belonging to genotype VII. Serological response and percentage of survival were used as efficacy criteria.

Significantly higher ($P < 0.05$) ND antibody titers at 28 days were observed when SQ live ND vaccine was added to the standard vaccination protocol. The protection in the SQ vaccinated birds, with and without field boost, was 100 and 95.8%, respectively. HVT-ND vector vaccines I and II reached 83.4 and 79.2%, respectively, when applied alone. After two live NDV revaccinations, the vector vaccines protection reached 91.6%.

The observed protection levels and the differences in serological responses, suggest the suitability of including the Avinew strain SQ vaccination for ND endemic areas with high field challenge, and the necessity of live boosts to complement protection in vector HVT-ND vaccines currently available.

Overall, the benefits could come from increasing the antigenic load in the chicks by adding a live SQ vaccine, from taking advantage of the live+ killed improvement principle, and/or from including Vaxxitek HVT-IBD to protect the immune status of the flock, allowing an efficacious immune response.



Evaluation of dual repeated day-old Marek's vaccine programs in future broiler breeders*

by Stephane Lemiere

Hatchery future breeder vaccination is about Marek's. A common practice is to associate a serotype 3 (SR-3), HVT strain and a serotype 1 (SR-1), Rispens strain to elaborate a breeder Marek's vaccination program. The SR-3 vaccine could be either the native HVT strain vaccine, or the HVT vector of infectious bursal disease protective gene VP2.

Some poultry multiplication operations use a dual vaccine application at day-old, SR-3 and SR-1 injected by subcutaneous route, and then another SR-1 using another route, intramuscular for example. Dual vaccine application at day-old was studied in commercial future heavy broiler breeders (Table 1). SPF chickens were included into the study in order to perform a Marek's disease challenge.

The primary parameter of study monitored throughout the studies was a virus recovery technique, a quantitative polymerase chain reaction (qPCR) performed on feather pulp samples and spleen samples. SR-3 and SR-1-specific qPCRs were used as the techniques for virus recovery. Pro-

tection against Marek's disease challenge used the standard RB1B strain; monitoring of protection against mortality and clinical signs related to Marek's throughout the study, as well as tumor formation, as visualised at necropsy was performed.

Correlation between vaccine take using the SR-3 PCR results and protection against Marek's challenge was established. In SPF chickens, the MD challenge did not cause any symptom in the vaccinated groups, whatever the vaccination program. Necropsy was performed at 54 days of age (Table 2), and no differences were shown between the groups. Only one positive chicken for MD in the single shot vaccination with HVT + Rispens group was found. The lesions and death in control group chickens validated the MD challenge operations.

A comparison between dual repeated Marek's vaccination based on either the native SR-3 HVT vaccine or the vector HVT-IBD VP2 vaccine gave results in favour of the use of such practices in the breeder hatcheries.

| | Day old 1st vaccination (SC) | Day old 2nd vaccination (IM) |
|---------|------------------------------|------------------------------|
| Group 1 | vHVT-IBD + Rispens | Rispens |
| Group 2 | HVT + Rispens | Rispens |
| Group 3 | HVT + Rispens | - |
| Control | - | - |

Table 1. Group study allocation.

Table 2. Necropsy examination results at D54 in SPF chickens.

| Group | No. of necropsied chickens on D54 | No. of chickens positive for MD lesions | Protection (%) |
|---------|-----------------------------------|---|----------------|
| 1 | 26 | 0 | 100 |
| 2 | 25 | 0 | 100 |
| 3 | 26 | 1 | 96.2 |
| Control | 25 | 19** | 24.0 |

**4 chickens were found dead with MD lesions before D54

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Newcastle disease control toolbox: The Villegas-Glisson/University of Georgia strain

by Francisco Perozo DVM, MS, PhD, Full Professor, University of Zulia Veterinary College, Venezuela

In the modern poultry industry the approach for Newcastle disease virus (NDV) control includes four basic aspects that need to work together and that require a well provided toolbox for the practitioners to implement in different areas:

- Obtain a correct diagnosis (viral isolation, pathotyping and molecular epidemiology).
- Maintain the immune system health (control immunosuppressive factors and proper management).
- Decrease the challenge (biosecurity, cleaning/disinfection and effective downtime between flocks).
- Diminish susceptibility (vaccination).

After vaccination, the replication of the live virus in the respiratory tissues induces a respiratory reaction that, when uncomplicated, is usually overcome by the natural immunogenic defence mechanisms of the chicken. A problem arises, however, when the respiratory reaction becomes complicated, due to negative influences found in the chicken breeding environment (ammonia, other

proven to replicate both in the respiratory and intestinal tract, inducing protection equivalent to LaSota strain, with lower reactivity in the respiratory tract. The vaccine is utilised worldwide for hatchery spray vaccination, alone or in combination with infectious bronchitis vaccine. In the field it is being applied by eye drop, spray or drinking water, in broilers layers and breeder flocks.

The VG/GA strain was isolated in 1987 from the intestinal tract of turkeys showing no signs of respiratory disease and was characterised serologically and phenotypically as a naturally attenuated lentogenic strain. After vaccination with the VG/GA strain, solid immunity to virulent NDV is conferred to inoculated chickens as evidenced by resistance to virulent NDV challenge.

The strain tissue tropism has been evaluated by both immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR), confirming that it can be detected both in the respiratory and intestinal tract of chickens. The replication pattern of VG/GA

In addition to the threat represented by a velogenic ND outbreak, the major concern of the poultry industry with low field challenge, is represented by the post-vaccine reactions induced by the more respirotropic strains. The 'rolling' reactions and secondary complications are very expensive and can be prevented with good vaccination techniques and the selection of the most suitable strain. The inclusion of the VG/GA strain in these countries' vaccination programs has improved these losses while providing full protection in the event of an exotic NDV outbreak. More recently, experimental application by the subcutaneous route at the hatchery, has provided promising results and is currently being tested in different countries endemic for the velogenic form of NDV.

To summarise, field experiences worldwide have shown that the VG/GA strain contained in AVINEW is efficacious in the control of Newcastle disease, particularly when caused by velogenic-viscerotropic strains which preferentially target the in-



Velogenic viscerotropic strains of NDV target the gastro-intestinal tract, inducing massive destruction of intestinal lymphoid areas and extensive ulceration of overlying intestinal epithelium.

viruses or mycoplasma infections). These facts clearly state the necessity of vaccines that do not require artificial attenuation before use, suitable for mass inoculation and capable of inducing local and systemic immunity without severe respiratory vaccine reactions.

The Villegas-Glisson/University of Georgia (VG/GA) strain included in the AVINEW vaccine from Merial, represents one of these tools because it has been

strain induces a strong localised mucosal immune response in the intestinal tract, shown by an increased production of NDV-specific IgA. High intestinal IgA production may represent a competitive advantage in the event of a velogenic viscerotropic challenge in which the virus has been reported to target the intestine inducing massive destruction of intestinal lymphoid areas and extensive ulceration of overlying intestinal epithelium, associated with active viral replication.

testinal tract of the birds. The differential replication patterns that diminishes the damage to the respiratory tract and improves local immunity, represented by increased IgA production in the intestinal tract, are unique features of the vaccine.

