Gumboro disease special

Effectively vaccinating your flock against Gumboro disease

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WORLD POULTRY
Our objective: 100% well vaccinated birds

Our Ceva Hatchery Immunisation Control Keys Program is a 5 point program to improve the quality of vaccination:

- Vaccine Care
- Vaccination Techniques
- Equipment Care
- Audit and Monitoring
- Expertise and Education
Disease control in the evolving poultry Industry

Over the last 50 years the poultry industry has developed rapidly, driven by a strong increased demand for higher volumes, improved quality and more economic need for animal protein. This development has led to the industrialisation and concentration of production. The drive for improved feed efficiency, higher yields and improved liveability in poultry production has put pressure on genetic potential and increased the risk of infectious diseases like Infectious Bursal Disease (IBD). From a consumer perspective, the reduction of antibiotic usage and welfare has limited the options to treat animals leading to usage of more preventative methods such as vaccines.

In the area of vaccines a true revolution has taken place with the availability of new technology vaccines such as immune-complex and vector vaccines, allowing vaccination to be done at the hatchery. Vaccination at the hatchery can be better automated, reducing the risk of mistakes in the vaccination process and stress on the birds. Automation in vaccination has also gone through major developments with the availability in many hatcheries of in-ovo equipment allowing more advance methods of vaccine application, while increasing the number of vaccinated eggs per hour compared to day old injection. These new developments in the area of vaccines and vaccination equipment, requires skilled labourers which need continuous training in order to perform the vaccination correctly.

Ceva Animal Health is highly committed to researching, developing, marketing and supporting poultry vaccines, vaccine application, such as in-ovo and day old vaccination and hatchery automation. Ceva Animal Health’s offerings consist of a wide range of vaccines, based on classical, immune complex and the widest range of vector vaccines. It is also a primary goal of the company to continuously look into selecting, researching and testing possible new vaccine candidates. The objective of this Gumboro Special is to bring more information on IBD (Gumboro disease) and answer questions regarding what these new vaccine technologies can bring to protecting birds from the disease, but also how they work in reducing the field pressure (shedding). Reduction of field virus shedding is of crucial importance to reduce viral load on farms. This magazine will also highlight the ability of these vaccines when applied in-ovo to provide strong immunity and ways to check this immunity by serology and other methods.
Gumboro disease was first described in the 1960’s in Gumboro, Delaware, USA. Many scientific authors also refer to it as Infectious Bursal Disease (IBD) as the virus invades and replicates in the Bursa of Fabricius.

Soon after the first discovery of Gumboro disease it became clear that biosecurity and cleaning and disinfection were not sufficient to control the disease, and vaccines were quickly developed and widely used. These vaccines proved to be efficacious as the clinical signs of the disease disappeared. Over the last 50 years the economic development in many countries has increased the number of birds placed per farm and had led to stricter biosecurity and surveillance programs. Gumboro disease, despite the wide use of vaccines and increased biosecurity, is however still very much present and ranks among the top five diseases in almost all countries globally. One of the reasons for this dominance is that the Gumboro disease virus is a very persistent virus surviving in poultry houses in the absence of chickens during downtime periods.

Past, present, future
After the initial outbreak in the US, Gumboro clinical disease was reported in many other countries in the 1960s and 1970s. The clinical form later disappeared and the condition became mainly sub-clinical between 1970s and 1985. More virulent forms were later reported around the same time with a very immunodepressive form of Gumboro disease in the USA.
(starting around 1985), which then later spread to Latin America. The very virulent form of the Gumboro disease spread around the same time to Western Europe, North Africa, the Middle East and Asia, see map 'Isolation of Gumboro disease virus on a global level'. In the late 1990’s and the late 2000’s the very virulent form of Gumboro disease spread to Latin America and California. Today the very virulent form of Gumboro disease is still present in many countries, and variant strains of Gumboro disease are present in several countries, leading to sub-clinical forms of Gumboro disease.

Stop the Gumboro cycle
As the Gumboro virus is a very persistent virus, in many cases it is already present inside the farm, which can lead to a field challenge when new birds are placed. The characteristics of this challenge in chickens (age of birds, severity, consequences, etc.), will vary from house to house but the challenge will definitely occur. In this case vaccination should aim at both protecting the chickens and preventing the Gumboro challenge from getting out of control.

When considering a sound Gumboro vaccination program the main objectives must be:

- Ensure continuous protection of the chickens against farm infection of Gumboro disease, or 'Prevention of Infection';
- Protect against the clinical signs of infection or 'Clinical Protection';
- Prevent or significantly reduce the amount of virus shed after challenge or 'Reduction of shedding of the Gumboro field virus';
- Prevent the build up of a higher virus pressure, production cycle after production cycle,
- Prevent the evolution of the farm Gumboro disease towards a virus that could escape the protection program.

The last two points are important for the reduction of the shedding of the Gumboro field virus, since the goal of a strong Gumboro vaccination program should be to stop the Gumboro cycle.
One of the major contributors to controlling Gumboro disease is vaccination. Vaccines to control the clinical signs of Gumboro have been used successfully since the initial outbreak of Gumboro disease in the 1960s.

By Rick van Oort, Christophe Cazaban and Yannick Gardin, Ceva Santé Animale (Ceva)

As the Gumboro virus is a very persistent virus and can easily survive in the environment, complete Gumboro control is only possible with a strong focus on cleaning and disinfection, in addition to a solid breeder vaccination program to provide high and prolonged Maternally Derived Antibodies (MDA) in order to prevent early infection of the field Gumboro virus. Also, an efficacious and well applied Gumboro vaccine in offspring (broilers or layers) is essential.

Vaccine choices
In order to control Gumboro disease several vaccine options are widely available and used. Some have been available for quite some time already, whereas others are more innovative and more recently introduced.

- **Inactivated (or killed) Gumboro vaccines** contain a high amount of inactivated whole or subunit IBD virus presented in a mineral oil emulsion. These vaccines are given to boost antibodies for the bird and/or to boost MDA for breeders.
- **Conventional live attenuated Gumboro vaccines** are live attenuated Gumboro viruses that replicate in the bursa of Fabricius, resulting in immunity generated by the replication of the whole virus. There are different Gumboro disease virus strains used in the vaccines and there are different levels of attenuation: Vaccine types are categorised in 'Mild' which are highly attenuated, 'Intermediate' which are very attenuated, 'Intermediate Plus' which are moderately attenuated and 'Hot' which are poorly attenuated.
- **Immune-complex IBD vaccines** are prepared from live attenuated IBDV strains of the intermediate plus type, mixed in with specific anti IBDV serum to regulate safety and release of the vaccine once the MDA levels of the bird are reduced. A correct balance between the IBD virus and the anti IBDV antibodies is of crucial importance for the efficacy and safety of these vaccines. These vaccines have the ability to fully colonise the bursa and to protect against all field IBD viruses.
- **Vector IBD vaccines** are constructed from a genetically engineered virus (the vector) whose genome contains a gene from a specific IBDV (the donor) encoding for the VP2 capsid protein. As of today the Herpes Virus of
Turkey (HVT) is mainly used as a vector. Although these vaccines provide proper protection against clinical signs of IBDV, they are not fully colonising the bursa, leading to field IBD viruses being able to enter and replicate in the bursa.

**Immune complex Gumboro vaccines**

Immune complex Gumboro vaccines are a suspension of a live attenuated Gumboro virus which is then mixed in with antiserum against IBD. The suspension needs to be in well-defined proportions and in strict procedures with antisera prepared in SPF chickens in order to contain a relevant balance between virus and antibodies. The vaccine virus is in this way covered and consequently protected from recognition by the immune system of the chickens by specific immunoglobulins (Virus Protecting Immunoglobulins, or VPI). After injection, VPI are stored in the same way as MDA are stored in the dendritic cells. After decay of the MDA, the vaccine virus is released. The take of the vaccine (which is demonstrated by the replication of the vaccine virus in the bursa) occurs when the MDA level has reached a sufficient level that allows the vaccine virus to leave the immune complex.

The benefits of this technology are that the quality and strength of the protection comes from replication of a complete intermediate plus type live IBD vaccine, resulting in full protection against clinical signs, complete resistance against infection, high reduction of shedding and no selection of farm IBDV population. The vaccine adapts to the immune status of each individual chicken and replicates at the optimum time. Due to the VPI, the vaccine does not get neutralised by MDA allowing it to be applied in the presence of passive immunity. Also, the vaccine has to be injected in the hatchery, improving reliability, quality and consistency of the vaccine application compared to drinking water vaccination. Finally, the vaccine fully colonises the bursa, blocking the entry of field IBD viruses. The safety of the immune complex vaccine is similar to the safety of intermediate plus type Gumboro vaccines, with the additional advantage that every individual chicken is immunised with the same, well controlled dose of vaccine.

When considering the various elements of efficacy of Gumboro vaccines and the capacity to not only protect against clinical signs, but also to control Gumboro disease, immune complex Gumboro vaccines are very attractive compared to other vaccines. Provided passive immunity is adapted to the challenging farm Gumboro virus and cleaning and disinfection procedures have been well respected, active immunity can be induced before challenge occurs and will successfully resist whatever level of Gumboro challenge there is. Chickens will be highly resistant to infection and consequently reduce shedding of the challenge virus. As a result, cycle after cycle, virus pressure will decrease, and no selection pressure on the farm is induced.

**Transmune vaccine solution**

Transmune is a Gumboro disease immune complex vaccine consisting of the original Winterfield 2512 strain which is blended with specific antibodies called Virus Protecting Immunoglobulins, see Figure 1. The product was developed in the 90’s, after which it was registered in many countries in Asia, Latin America and later in Europe. Currently the vaccine is marketed in over 75 countries worldwide. As Figure 2 shows, it has been used in over 53 billion broilers worldwide since 2006.

As the vaccine is registered in Europe a unique QC procedure had to be developed safeguarding the efficacy and safety of the vaccine. Every single production batch is thoroughly tested using a CID (Chick Infective Dose) 50 test. This test is used with live birds and with the final blended vaccine to guarantee the potency and safety of the vaccine. 

As the formulation between attenuated vaccine virus and specific antibodies needs to be extensively tested, to have the optimum balance for the safety and efficacy of the product, more than 100 different formulations were tested before introduction on the market. In order to monitor the correct application of the vaccine, Ceva developed and implemented several years ago the C.H.I.C.K program. More recently Ceva has introduced the GPS-IBD services to screen IBD pressure on farms and to monitor the replication of Transmune in the bursa. These service programs are services by local and fully dedicated vaccination services managers and veterinary services experts. Several scientific papers and publications are available which demonstrate the efficacy, safety and compatibility of the vaccine. A good example is the compatibility between Transmune and Vectormune ND providing protection against Gumboro, Newcastle Disease and Marek’s Disease in one application.

**Figure 1 - Transmune vaccine.**

- Specific Antibodies (Virus Protecting Immunoglobulins, VPI)
- Vaccine Virus Strain (Winterfield 2512)

VPI protect the vaccine virus from maternal antibodies.
VPI avoid the early contact between the embryo and the live virus.
VPI delay the virus release by 7 - 10 days.

**Figure 2 - A 10-year perspective of Transmune usage.**

(In billion doses used.)
Reduction of shedding: Laboratory studies

Reduction of shedding, study 1
Two different laboratory studies compared the protection induced by Transmune, an immune complex live Gumboro vaccine, and a vector IBD product in front of different Gumboro disease virus challenge strains and the capacity these two vaccine technologies have to reduce their shedding. One day-old commercial broilers were divided into three groups. Two groups were vaccinated subcutaneously either with Transmune or with a vectored IBD product (rHVT-VP2) constructed with a VP2 gene insert donated by a classical type IBDV (Faragher 52/70). The third group was left unvaccinated to serve as control. Prior to the challenge, the replication of both vaccines was confirmed by histology and antibody response for Transmune and by PCR and antibody response for the rHVT-VP2 vaccine. On day 28, subgroups from the three groups were orally challenged with 104 EID50 per chicken of various Gumboro disease virus strains from different countries and different pathotypes or genotypes:
- a very virulent IBDV from Turkey (D407/02/04 TR) – abbreviated as TR
- a variant IBDV from USA (Delaware E) – abbreviated as Del.E

Gumboro Disease virus is extremely resistant and even after strict cleaning and disinfecting procedures, it is very likely to persist in the environment and therefore already be present inside the house even before the day-old chicks are released onto the floor. As a consequence, if nothing is done, the likelihood of a Gumboro challenge for most of the flocks is very high. In this situation, it is desirable that a vaccination should not only aim at protecting the chickens from the negative consequences of the infection with a field Gumboro disease virus but also at preventing chickens from the infection itself.

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• a subclinical IBDV from Mexico (D1256/56/09 MX) – abbreviated as MX
• a subclinical IBDV from South Africa (D430/3 ZA) – abbreviated as ZA
• a subclinical IBDV from Brazil (D1311/7/09 BR) – abbreviated as BR
• a variant IBDV from USA (AVS-EL) – abbreviated as AVS-EL

Protection against infection or against bursa lesions was evaluated 4 days post challenge by histology of the bursa (acute lesions and percentage of affected follicles) and virology (PCR). A chicken was considered as ‘fully protected’ if less than 10% of the follicles of its bursa were showing acute lesions. The results are summarised in Figure 3.

Following the IBDV challenges, all bursas from the Transmune vaccinated chickens showed chronic signs of bursitis caused by the replication of vaccine virus, but no signs of acute lesions that would indicate replication of the challenge virus were detected. The molecular identification of the IBDV in the bursa of Fabricius confirmed the presence of the W2512 live attenuated vaccine strain only.

Alternatively, after challenge, acute lesions were observed in the bursa of a variable number of rHVT-VP2 vaccinated chickens, whatever the challenging virus was, indicating infection, replication and consequently shedding of the challenge virus.

Under the conditions of this trial, Transmune was able to induce full protection against a wide range of Gumboro disease virus strains. In fact, the protection against clinical signs as well as replication of the challenge virus and shedding make it a tool not only to protect the chickens, but to really control the disease (the spread of the field virus). Conversely, the rHVT-VP2 product showed poor to very poor protection for the shedding of the Gumboro disease challenge virus.

Reduction of shedding, study 2

In another experiment to assess the ability to stop the infection (and by this way the shedding) with field Gumboro Disease virus strains, one day-old commercial broilers were vaccinated subcutaneously either with Transmune or with the vector HVT-VP2 product. The third group was left unvaccinated to serve as control.

At 28 days of age, after confirming the vaccine take, subgroups were challenged with one of two field viruses: vvIBDV and Del E. Four days after challenge, non-vaccinated hatch mates (contact birds) were co-mingled with challenge groups. At 42 days of age, ten days after the introduction of the contact birds, detection and quantification of challenge virus replication in the bursa was carried out. The results are summarised in Tables 1 and 2.

The results show that, at 14 days’ post-challenge (10 days after the introduction of the sentinel birds), no presence of challenge virus (vvIBDV or Variant Del E Gumboro disease virus) was detected either in the Transmune vaccinated broilers or in the contact birds. On the other hand, in the groups vaccinated with the vector IBD vaccine and challenged either with vvIBDV or Variant Del E disease virus isolates, 40% and 80% of the bursa of Fabricius had moderate to severe lesions, respectively, caused by the replication of the challenge strains.

Furthermore, the severe gross-lesions of bursas in all contact birds indicated unrestricted spread of the challenge virus.

Conclusions

Under field conditions, these two vaccine technologies have a different impact on disease control. As the vector HVT-VP2 product is not able to prevent the bursa colonisation by the challenge virus, a possible consequence is the relative increase of the pathogenic Gumboro disease virus for the following flocks. With Transmune, the attenuated vaccine virus W2512 will colonise the bursa stopping the re-excretion of pathogenic virus therefore preventing the field challenge from getting out of control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Challenge strains</th>
<th>IBDV strains at 14 dpch (D42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W 2512 (vaccine strain)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Challenge virus</td>
</tr>
<tr>
<td>Transmune vaccinated, challenged</td>
<td>vvIBDV</td>
<td>100%</td>
</tr>
<tr>
<td>Contact controls (not vaccinated)</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Transmune vaccinated, challenged</td>
<td>Del E</td>
<td>100%</td>
</tr>
<tr>
<td>Contact controls (not vaccinated)</td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Challenge strains</th>
<th>IBDV specific histological lesions in the bursa (%) at 14 dpch (D42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No lesions (full protection)</td>
</tr>
<tr>
<td>rHVT-VP2 vaccinated, challenged</td>
<td>vvIBDV</td>
<td>60%</td>
</tr>
<tr>
<td>Contact controls (not vaccinated)</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>rHVT-VP2 vaccinated, challenged</td>
<td>Del E</td>
<td>20%</td>
</tr>
<tr>
<td>Contact controls (not vaccinated)</td>
<td></td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 1 – Transmune results as assessed by the detection of different Gumboro disease strains with real-time PCR (%) at 14 dpch (D42).

Table 2 – rHVT-VP2 results as assessed by the characterisation of Gumboro disease specific histological lesions in the bursa (%) at 14 dpch (D42).

Figure 3 - Protection of the bursa elicited by an immune-complex IBD or a rHVT-VP2 vaccine according to histopathology lesions 4 days post-challenge with different Gumboro Disease virus strains.
Reduction of shedding: Field experiences

Infectious bursal disease virus (IBDV) is a pathogen that is able to persist in poultry houses and to withstand usual disinfectants. Therefore, it represents a permanent threat to flock performance, because of its immunosuppressive properties. Field trials were carried out in three locations to determine the efficiency of vaccination on farms.

Due to its unique features, Transmune is able to quickly and fully colonise the main target organ, namely the bursa of Fabricius, in susceptible chickens. In this way, the infection by the field IBDV is impaired flock after flock and the overall performances are improved. This can be monitored in several simple ways, like serology analysis (e.g., ELISA) on a fixed schedule (e.g., before slaughter), or molecular analysis. By sampling the bursa of Fabricius in chickens and by submitting them to a PCR analysis and sequencing it helps in identifying which IBD virus is present in the flock. It also enables comparisons of different vaccination protocols.

Spain
A large broiler integrated company in Spain tested two different Gumboro vaccination programs over a 3-year period (2012-2015). The vaccination programs were as follows:

- Drinking water: intermediate vaccine (37 farms);
- Hatchery, in ovo route: Transmune (34 farms).

When using hatchery vaccination by an in ovo route and Transmune, flocks showed a very uniform range of mean titres at slaughter as shown in Figure 4. This demonstrated the consistency and the quality of hatchery vaccination versus farm, and of Transmune versus conventional vaccines. The coefficient variation (CV) proved to be quite good with a few exceptions of 60%. It is the evidence of a strong and consistent control of IBD risk in the field. On the reverse, when vaccinating using a live vaccine by drinking water route very uneven titres were obtained. This means a very uneven quality of vaccination. Some flocks showed a very low mean titre which suggests a poor protection; on the opposite, some flocks showed a very high mean titre which may suggest a field infection. Here, the CV is erratic and sometimes exceeds 100%.

Figure 4 - Monitoring was done by serology on blood samples taken at processing age (ca. D42) using Biochek ELISA.
Russia
In Russia, a large broiler integrated company tested different Gumboro hatchery vaccination programs at two similar sites, in three houses each:
- Cevac Transmune: site 5, houses 8-9-12;
- a vector HVT-VP2 competitor product: site 4, houses 9-10-11.
Monitoring was done by collecting five bursa samples per house at 3, 4, and 5 weeks of age. Bursa were carefully removed and placed in individual containers, kept frozen until processing by RT-PCR/RFLP and sequencing if needed, to identify whether they were colonised by an IBD virus and if yes, which type. These field data show that the Transmune vaccine virus (W2512) is able to colonise the bursa of Fabricius in a quick manner, depending on the residual maternal immunity level (see Table 3). Between the 3rd and 4th week of age, all sampled birds were positive to the vaccine. After the bursa is fully colonised, no other IBD strains are found. This shows a very strong prevention of field infection. Therefore, vaccinated chickens do not pose a risk of amplifying and further shedding the field IBD virus.
On the contrary, the vector based product does not colonise the bursa. As a consequence, vaccinated birds remain susceptible to the infection by any kind of circulating field IBD virus, either of the vaccine type (eg, the very mild D78), or of the pathogenic type (eg, vvIBDV). Following this, infected birds will shed the virus in larger amounts in the environment, hence creating a higher threat for the subsequent flock of chickens.

South Africa
Bursa samples were collected for about ten years in various parts of South Africa in different broiler producing companies. The presence of IBD virus was investigated by RT-PCR and further characterisation was done using either RFLP or sequencing. Figure 5 depicts the summary of IBD virus isolations and types that was collected from more than 2,250 flocks. It can be divided as follows:
- Before 2008: field vaccination;
- 2008-2013: increasing usage of Transmune across the country and the broiler companies;
This survey (especially the interval 2008-2013) shows how Transmune use can progressively and consistently replace the field virus, regardless of its type (very virulent, or variant). This is achieved thanks to its unique property of fast and complete colonisation of the bursa, which prevents infection by another IBD virus and therefore, reduces its prevalence.

Summary of different field experiences
The Spanish experience with Transmune, demonstrates the more consistent titres and better CV value, than flocks vaccinated with drinking water Gumboro vaccines. When Transmune is tested in comparison with a Vector HVT-IBD product, results demonstrate full colonisation of the bursa, blocking the entry of field Gumboro viruses. The Vector IBD vaccinated groups showed presence of other Gumboro strains than the vaccine strain. In South Africa, prolonged use of Transmune in part of the broiler population showed the ability of Transmune to reduce the cases of VV IBDV and number of flocks with a low titre for Gumboro, to increase the percentage of flocks where only the W2512 vaccine strain was found.

Table 3 – Field results Russia – IBD virus isolation.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Site and house</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmune</td>
<td>5-H8</td>
<td>40%</td>
<td>W2512</td>
<td>W2512</td>
</tr>
<tr>
<td>Transmune</td>
<td>5-H9</td>
<td>40%</td>
<td>W2512</td>
<td>W2512</td>
</tr>
<tr>
<td>Transmune</td>
<td>5-H12</td>
<td>20%</td>
<td>W2512</td>
<td>W2512</td>
</tr>
<tr>
<td>rHVT-VP2</td>
<td>4-H9</td>
<td>Negative</td>
<td>20% D78</td>
<td>40% D78</td>
</tr>
<tr>
<td>rHVT-VP2</td>
<td>4-H10</td>
<td>Negative</td>
<td>Negative</td>
<td>20% D78, 20% vvIBDV</td>
</tr>
<tr>
<td>rHVT-VP2</td>
<td>4-H11</td>
<td>Negative</td>
<td>20% D78</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Tools to stop the Gumboro Cycle

The poultry industry has faced enormous technological changes, one of them is the further introduction and expansion of in-ovo vaccination technology at the hatchery and the development of more vaccines that can be applied through the in-ovo route.

By Guillermo Gonzalez, Carlos Gonzalez and Miren Arbe, Ceva Veterinary Services and Ceva Vaccination Services & Equipment, Ceva Santé Animale (Ceva)

Egginject Dual Pressure System technology (Ecat-ID) is a clear example of how in-ovo technology can improve protection against different poultry diseases such as Marek’s disease, Gumboro disease, and Newcastle disease, etc. The Egginject patented Dual Pressure Injection System allows automatic and individual adaptation of the injection to each single chicken embryo, and therefore, a better vaccination can be achieved. This ensures a more accurate and safer vaccination process requiring less labour compared to drinking water in the farm or the subcutaneous vaccination route at hatch. Additionally, day old chicks stress and processing time at hatch is greatly reduced allowing faster delivery at the farm and earlier access to feed.

Spanish field example
Since 2009, Ceva’s veterinary services team in Spain has been monitoring vaccination in the field in different locations for their customers. This service, called Global Protection Services or GPS, is a tool to monitor vaccine application and the level of protection in a flock. It also enables detecting epidemiological evolutions, identify risks and propose corrective actions in order to help poultry producers to make quick decisions.

A retrospective study compared and analysed 2,283 broiler flocks delivered from a single hatchery located in Spain, equipped with an Egginject in-ovo machine. The day old chicks (DOC) were delivered from the hatchery to farms located in the nearby regions in the Northeast of Spain. Hatchery and growing farms were set up under the same integration. The age of the birds ranged from 24 to 69 days of age, with a mean age of 41 days. Antibodies against Gumboro disease were tested using an ELISA kit (Biochek), in 10 to 20 birds per flock. Cobb 500 and Ross 308 genetic lines, at an approximate 50/50% rate, were being reared at the time of the study.

Vaccination techniques analysed
The original Gumboro vaccination program for this producer was a commercial live vaccine containing the Lukert infectious bursal disease virus strain (intermediate IBDv strain). The birds were vaccinated twice, once in-ovo and again in drinking water at 15 days of age.

The intermediate Gumboro Lukert strain vaccine is made of conventional technology, therefore it is quickly neutralised by the maternally-derived antibodies (MDA) after injection, contrary to an immune IBD complex vaccine. As a result this group was regarded as actually being vaccinated by drinking water at 15 days of age. In this study 138 broiler flocks were...
Bird responses to vaccination

Within the group of broilers vaccinated twice with the intermediate Gumboro vaccine, a very wide spread of titres from 0 to above 12,000 was found (Figure 6). The results in Table 4 indicate that only 46% of the flocks showed titres between 4000 to 9000 which is regarded as the expected titre range. In addition, 22% of the flocks had very low IBD titres, below 391, which were considered negative or non-vaccinated.

In the group of broilers vaccinated in-ovo with Transmune using Egginject, a much more homogenous spread of the IBD mean titres was observed with 71% of the flocks having titres between 4000 to 9000. Only 3% of the flocks vaccinated with

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Table 4 – Results overview of titres.

<table>
<thead>
<tr>
<th>Vaccination program</th>
<th>Expected titres (4,000-9,000)</th>
<th>Negative titres (&lt; 391)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking Water</td>
<td>46%</td>
<td>20%</td>
</tr>
<tr>
<td>Transmune</td>
<td>71%</td>
<td>3%</td>
</tr>
</tbody>
</table>

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Figure 6 - Titre distribution of the drinking water vaccinated flocks.
Transmune showed a titre below 391 (Figure 7). At the same time, the normal distribution in both groups was very different. The distribution of the titres in the drinking water vaccinated birds is very variable, on the contrary, the titres of the Transmune vaccinated birds using Egginject in-ovo vaccination were showing a much better distribution with 50% of the titres between 5142 and 7886.

When analysing the titres again, it’s important to observe that more than 12% of the drinking water vaccinated birds showed a value >9000 (Figure 8). Interestingly, 37% of the birds vaccinated with the drinking water vaccine showed either a very high titre or a very low titre (quartile 25). When looking at the coefficient of variation (CV) (%) of the titres, the Transmune/Egginject group showed an excellent median value of 31% (Figure 9), whereas the drinking water vaccine showed a value of 56% (Figure 10). A remarkable difference could be noticed, especially around 31 to 33 days in the drinking water vaccinated group, as in this group the titers were below 2000 which is considered low (Figures 10 and 11). No data was available before 31 days of age, but the polynomial curve in the analysis for the drinking water group estimated a probability that between 27-28 days of age the titres were very low. The Transmune and Egginject group did not show any average mean titre lower than 2000 at day 27. Basically, drinking water Gumboro vaccination in these results showed much more erratic figures, including negative titres which suggested a vaccination failure. When using Transmune applied by the Egginject in-ovo equipment, average mean titres were much more even, and strongly positive, regardless of the age of sampling. Antibody titres of flocks using the drinking water vaccination technique were low or even negative in between 31-33 days. On the contrary antibody titres in the Transmune-vaccinated birds were clearly positive at that age, suggesting an earlier and more uniform vaccine take.

Conclusions of the field trial
This retrospective field trial compared Transmune vaccinated flocks using the in-ovo Egginject equipment and previous flocks which were vaccinated using a drinking water vaccine. The main points could be summarised as stated below:

- Clear differences in mean titres and CV’s between the drinking water vaccination group and the in-ovo group using Transmune and Egginject were observed. The Transmune vaccinated flocks demonstrated more uniform vaccination titres and a lower CV value.
- In many cases the Maternally Derived Antibody titre (MDA) is not homogenous in a given flock of day old chicks. Therefore, it’s is practically impossible to find a vaccination date when almost 100% of the birds are able to build up an immune response to vaccination. This makes Transmune, with its ability to adapt to different MDA levels per chick, the best option.
- With drinking water vaccination, a high proportion of
birds non- or partially-protected, amounted up to approximately 37% or the flock. This percentage of birds is at high risk of facing a field IBD infection with a potential loss of 10% of net income per bird (McIlroy et al. 1992).

Advanced technology like the Egginject in-ovo equipment and new technology vaccines, like Transmune, are becoming more popular among hatcheries, since they enable fast, safe and welfare friendly vaccination of chicken embryos. The time between hatch and delivery to the farms is reduced, and less handling is needed in the field due to extra vaccinations. This vaccination application needs specific high quality equipment, close support, maintenance and monitoring of correct vaccine application. It also needs specific vaccines which are able to overcome the presence of MDA, while inducing an active immunity. Transmune is able to do this.
GUMBORO SPECIAL

**IBD experiences and approach in the US**

Since the initial occurrence of Infectious Bursal Disease (IBD) in the US, the disease has spread globally. By 1976, mortality caused by IBD was under control following extensive vaccination and increased biosecurity. The US poultry industry currently follows several approaches to combat the impact of the disease on their flocks.

By Marshall Putnam, Director of Veterinary Services, Ceva Animal Health US and Rick van Oort, Global Product Manager IBD Vaccines, Ceva Santé Animale (Ceva)

The US poultry industry is to a large extent fully integrated, in which many efforts are made to build a strong immunity in the breeder stock to prevent disease in both the breeder production cycle and in the broiler offspring. As IBD is a resident disease, the virus can easily survive in litter and other organic materials. A common practice is to immunise breeders by including one or two inactivated IBD strains in the vaccines. Generally, these vaccines contain both classic and variant IBD strains and are available in combination with Newcastle Disease (ND) and IB strains.

**IBD control program**

In order to address the changing IBD virus situation in the US poultry industry, Ceva developed the IBD Control Program. This program consists of the following parts:

1. To prevent the occurrence of new variants, continuous monitoring and diagnosis of the field IBD challenge is needed. When a new variant is found, tests need to be done to determine if the current vaccine solutions are still efficacious.
2. If the current vaccine solutions are not fully protecting, the disease strain is isolated and a customised solution is developed to further improve the Maternally Derived Antibodies (MDA) levels of different IBD strains to provide strong passive immunity.
3. Vector IBD provides efficient active immunity after the MDA levels in the birds have declined.
4. Ensure optimal vaccination, by providing hatchery services and field services to ensure correct preparation and application of the vaccines.
5. Breeder immunity with commercial vaccines provides strong protection against classical and variant IBD vaccines.

**Vector IBD vaccines**

The broiler industry in the US has largely adopted the use of Vector IBD vaccines. As litter is re-used, the Marek’s protection coming from these HVT Vector based vaccines, helps to reduce Marek’s pressure.

**Custom vaccines**

As IBD and reoviruses drift antigenically, the commercially available vaccines may provide a strong basis of protection. However, when new variants develop, they will ‘break through’ maternally derived antibodies. In this case, custom vaccines offer the best option to widen the protection against these new variants. Custom vaccines, scientifically referred to as autogenous biologics, are made and utilised when no commercial vaccine is available. They contain flock specific (homologous) antigens but must have an established and supported epidemiological link between flocks for non-adjacent usage. Custom vaccines are regulated by the United States Department of Agriculture (USDA) Centre for Veterinary Biologics.

Over the past few years, Ceva has worked to establish themselves as the industry leader in custom vaccines. This is primarily due to the companies approach to production and marketing of custom vaccines. Another key component has been Ceva’s Veterinary Service Team which has extensive experience in isolation samples for laboratory submission. Moreover, they collect and identify the disease causing strain of IBDV or other disease challenge through the use of internal and external lab resources to determine the best possible isolate for protection from the sample. The (custom) vaccine is then produced in a dedicated facility using extensive experience in the manufacturing of custom vaccines. Prior to being released and shipped, vaccines must meet USDA and Ceva quality standards.
The trend in vaccination technology has lead to more vaccines being used in the hatchery. In order to have the full efficacy of these new technology vaccines like Transmune, a correct preparation, application with usage of accurate equipment is necessary. Follow up in the field in the replication of Transmune is needed to demonstrate the take of the vaccine.

By Paola Cruz and Miren Arbe, Vaccination services and equipment, Ceva Santé Animale (Ceva)

The evolution of vaccine technology, especially for Gumboro disease protection, has given rise to a trend of more and more frequent vaccinations done at hatcheries to take advantage of a highly accurate massive vaccination process reducing field drinking water vaccination and possible limitations when vaccinating on the farm. Controlling and mastering hatchery vaccination is more achievable than dealing with hundreds of farms. However, it does not mean it is an easy task and the consequences of any mistake during the process might have a high impact on the production.

The C.H.I.C.K program
A hatchery vaccination monitoring service program, named the C.H.I.C.K (Ceva Hatchery Immunisation Control Keys) program was created and made available by Ceva for poultry producers. When being part of the C.H.I.C.K program services, a qualified Ceva team visits the customer’s operation on a pre-agreed time basis and conducts a number of tests and measures according to standard operating procedures. This team monitors the vaccination from the storage until the vaccines are administered to the birds. Furthermore, there is an equipment preventive maintenance program along with continuous staff training.

For any vaccination process, a complete service package is defined to control and monitor the critical factors affecting the process involving vaccine preparation control, equipment care about daily operation, cleaning and disinfection, monitoring of the quality of administration, dosing accuracy control and vaccination speed control. Also regular training of hatchery staff is imperative.

During the vaccination process, the data is collected by an innovative electronic application exclusively available for Ceva
specialists, the C.H.I.C.K App. By using this tool, the team shows to hatchery managers in real time the gaps on the vaccination process indicators, they interpret those and will set, in the hatchery, the corrective actions. Additionally, every visit is followed by a report. The C.H.I.C.K program helps a commercial operation to take maximum advantage of new technology vaccines. The joint objective is to have 100% of the birds well vaccinated. This can be achieved by:

1. Monitoring the vaccination process and results through regular pre-agreed hatchery visits.
2. Giving customers real time indicators about their vaccination process; Real time indicators about the quality of the vaccination process are shared with hatchery managers to make informed decisions. The faster hatchery managers get the results of an audit on the vaccination process, the faster the corrective actions can be implemented.
3. Sharing Information easily; The access to the most recent information as well as to the historical data help companies to improve their results. By having a dedicated database for that purpose, the information can easily be retrieved and shared with the teams at their convenience for the preparation of meetings, technical reviews, awarding programs or others.

Worldwide experience
The C.H.I.C.K program is performed by a dedicated Ceva team of 130 specialists worldwide, the largest team in the world dedicated specifically to vaccination services and equipment at hatcheries. More than 800 hatcheries are visited each year as part of the service program in over 40 countries. Over 4,500 audits are performed each year to set corrective actions and an improvement plan. In the more than 15,000 Ceva vaccination equipment units operating worldwide an advanced data management system allows to monitor the overall hatchery vaccination efficiency and evolution.

Global Protection Services (GPS)
The key success factor for a profitable performance of the birds starts when the birds are delivered to the farms. Ceva’s veterinary services staff support customers by monitoring the vaccination of Transmune and other vaccines and checking successful immunisation of the birds. This includes collecting blood samples to run end point serology analyses (eg, Gumboro disease) and regular reporting to customers and updates on monitoring are part of this activity. Ceva also runs epidemiological surveys to identify any change in the infective pressure in a timely manner; which also helps to substantiate the successful displacement of the field virus strain by the attenuated vaccine strain (eg, infectious bronchitis). The company remains at the customer’s disposal for any troubleshooting, diagnosis support (both locally by running necropsy, and by submitting carefully selected samples to a trustful laboratory). In addition, Ceva’s SSIU (scientific investigation units) provide laboratory services in order to investigate epidemiology surveys, check vaccination take and to answer questions with respect to Ceva vaccines and presence of new or variant disease strains. All these data are eventually collected in a database per company to set up relevant baselines, and to help decision makers to implement appropriate changes, if needed. Value added services, called GPS (Global Protection Services) include regular reports with an overview of disease situation, take of the vaccines or other veterinary services.

References indicated in this special are available on request
Egginject®
For modern hatcheries, the Egginject® full line allows safe In-Ovo Vaccination thanks to its Dual Pressure Injection System.
www.egginject.com

Engineered by EcatID
Transmune® stops reinfection and protects against all IBD virus strains

Stop the Gumboro Cycle