

Gumboro Disease *Special*

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The rational use of vaccines
and vaccinations for real
control of Gumboro disease

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Gumboro disease; also known as Infectious bursal disease (IBD), Infectious Bursitis and Infectious Avian Nephrosis, is a highly contagious disease of young chickens caused by infectious bursal disease virus (IBDV).



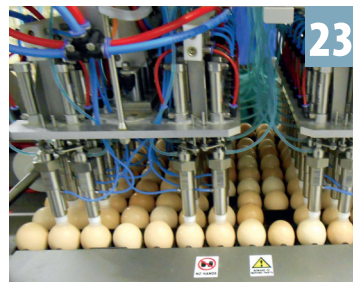
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Optimal disease control through knowledge



The poultry industry has dramatically evolved over the last decades. An increased demand for higher volumes, better quality and competitive prices has led to industrialisation, intensification and concentration of production. This has put a lot of pressure on animals that have the genetic potential for higher yield and better efficacy, resulting in an increased risk of contagious infectious diseases. In addition to controlling these diseases, the pressures to reduce the use of antibiotics and other chemicals, and eliminate food borne as well as zoonotic agents have grown tremendously, forcing the producers to better and deeper understand and control diseases. In this context, biosecurity and vaccination have strongly improved and become essential tools. Additionally and probably not independently, the concepts of vaccines and vaccinations have undergone such a revolution that the personnel involved in the veterinary part of the industry need to stay well informed.

Ceva Animal Health is deeply committed to researching, developing, marketing and supporting poultry vaccines as well as poultry vaccination processes. The offer from Ceva Animal Health is broad and includes many vaccines based on new technologies like Immune Complex as well as the largest range of recombinant vector vaccines for poultry. It is also a primary objective of Ceva not to stop at producing vaccines, but also to actively investigate how to select, combine and use them in order to reach an optimal control of infectious diseases. For this reason significant investment is made each year in internal and external dedicated research.

The objective of this special magazine is to bring answers to questions about vaccines and vaccinations that are not necessarily found in the registration dossiers or the leaflets accompanying the products. Key questions like efficacy against the variations of a particular microorganism that can be found in various parts of the world; compatibilities between vaccines; capacity to increase resistance or prevent (or reduce) shedding after challenge, which is critical to lower the risk of persistence or spreading of a disease; the influence or interference of passive immunity, the interest of using one category of vaccine preferably to another one, etc.

The information is based on data that we have produced, data available as scientific publications and information gathered as part of our experience. We trust that it will be useful to all poultry professionals.

Yannick Gardin, Director Biology Innovation Strategy, Ceva Animal Health

Gumboro, a worldwide problem

The first description of 'Gumboro Disease' was made by Albert Cosgrove in the early 1960s from a clinical case that occurred on a farm located in the small town of Gumboro, Delaware, USA. This is why many people refer to the Infectious Bursal Disease Virus as 'Gumboro Disease'.

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It is likely that the geographical extension of the causative virus ('Gumboro virus') was already larger at the time of first description by Cosgrove, but Gumboro is where the corresponding condition was initially recognised as something new, or where the expression of the disease changed from being undetectable, or sub-clinical, to clinical, probably because of a change in the pathogenicity of the virus. From this little town and within a few decades, the 'Gumboro' virus, or the corresponding new pathotype of an older virus, spread to almost all chicken producing countries on earth, and because of its high resistance, it is nowadays virtually present, with variations, on almost every chicken farm and is recognised as a worldwide concern for the poultry industry.

Very soon after this 'discovery', it was made clear to everyone that cleaning, disinfection and biosecurity were not sufficient to protect chickens, and that vaccination was unavoidable. Within a short period of time, specific live attenuated vaccines were developed and marketed almost everywhere and widely used. They also proved to be very efficacious, and this efficacy, evidenced by the disappearance of the clinical signs, was obvious to all.

50 years of vaccine protection, and the problem is still there!

In many countries, where economic development has reduced the number of backyard flocks and live bird markets and where the poultry industry has become more organised, many poultry diseases have been eradicated or reduced down to a low level thanks to National Eradication Plans, stricter biosecurity and adapted vaccination programs. But in similar conditions, and contrary to other diseases, Gumboro Disease is still there and still very present.

Although it has been more than 50 years since Gumboro Disease joined the list of poultry diseases and vaccines have been used, the most striking fact regarding this condition is that it is still widely present, and still ranks among the top five infectious problems in almost all countries.

This can be explained by the incredibly high resistance of the Gumboro virus, allowing it to survive in the poultry house in the absence of chickens during

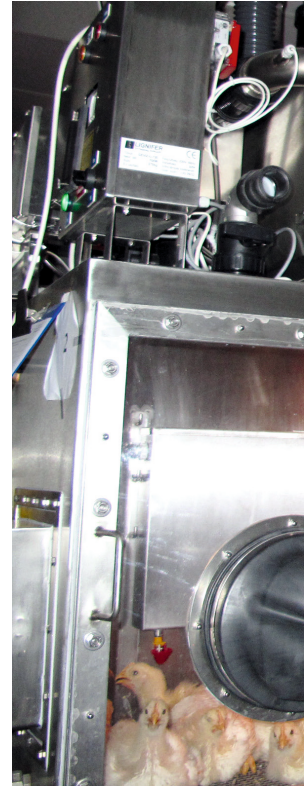
down periods, despite cleaning and disinfection, as well as by its capacity to escape post infection and/or post vaccination passive and active immunities, by selection of antigenic mutants.

Understanding the past, explain the future...

Over the past few decades, intensive public and private research, with more and more powerful laboratory and diagnostic tools, have been dedicated to poultry viruses, poultry diseases and vaccinology, so that we now have better knowledge of the factors of virulence, the factors of protection, the variability attached to the micro-organisms, and the corresponding mechanisms of pathogenicity.

It has also been, and is still, the focus of Ceva Animal Health to conduct internally or in collaboration with external research structures, extensive investigation studies to better and more thoroughly understand vaccine potentialities and performances and substantiate what recommendations should be given to users to better control infectious diseases when using vaccines.

The study of how Gumboro Disease has



The town of Gumboro, Delaware in the United States was the first place where Gumboro was found.



Isolators at Scientific Support and Investigation Unit (SSIU) Ceva-Phylaxia, Hungary.

clinically appeared over the past 50 years, and how vaccines have been selected and used, is certainly a source of useful information. Clinical at the time of its first description in the USA, this is also how it was initially reported in many countries (around the 1960s and 1970s). The clinical expression then disappeared and the condition became mostly sub-clinical (approx. between 1970 and 1985). More virulent forms were later reported, almost all at the same time, with a very immunodepressive form in the USA (starting at around 1985), and very virulent clinical form in Africa and Western Europe (starting between 1985 and 1990). The highly immunodepressive form spread from the USA to Central and Northern parts of South America, while at the same time the very virulent form spread from Western Europe to Eastern Europe, North Africa, the Middle East and Asia. Between the late 1990s and the late 2000s, the very virulent form of Gumboro Disease spread to Latin America and even to California. Today, even if the very virulent form is still strongly present in some countries or regions, there is a global tendency for

Gumboro Disease to go back to a more dominant 'subclinical' form, which does not mean that the virus has gone or has lost its pathogenicity. On the contrary, past experience is telling us that this is probably the most critical time to select and implement an effective control program. The heart of the problem is the Gumboro virus, not necessarily the Gumboro Disease.

The ups and downs of Gumboro Disease

It is easy to summarise the history of Gumboro Disease by a waving curve alternating between subclinical periods and clinical periods. The efficacy of a vaccination program in the presence of the sub-clinical form of the disease is obviously more difficult to monitor than when the clinical form is present. A vaccination failure in the face of a subclinical Gumboro outbreak can easily pass unnoticed, which is not true if the disease is clinical.

When the subclinical form of Gumboro Disease is present, a vaccination failure means infection of chickens by the field virus. This may not necessarily cause an immediately perceptible negative effect,

but it will create the opportunity for the field virus to multiply, naturally produce variants and spread, with two very important consequences that will impair future sanitary and/or economical performances of the poultry house or farm:

- The virus pressure (the amount of virus to which the chickens of the next cycle will be exposed) will increase
- An opportunity will be created for a variant virus to colonise the farm. The Gumboro viruses that multiply are then the best adapted to get around passive protection (from the breeders program) and active protection (from vaccination).

Each time the Gumboro Disease has become more clinical, a heavier, generally more demanding and more expensive vaccination program has been implemented, including the use of less attenuated, more effective vaccines, which was followed by clearly good efficiency results. On the other hand, the presence of the sub-clinical (non visible) form of the disease has step by step encouraged users to simplify and lighten the vaccination program, and doing so to favour the return of vaccine breaks.

Hence, a really successful vaccination program against Gumboro Disease, whether clinical or sub-clinical, should not only ensure the 'protection' of the chickens against clinical signs following infection, but should also ensure the 'prevention' of the disease by decreasing the population of Gumboro virus shed, so that the virus pressure in the poultry house does not increase, and by significantly reducing the risk of emergence of a variant virus. 'Protection' and 'Prevention' are the two objectives assigned to a Gumboro vaccination program aiming for a real 'Control' of the disease. This should be understood as part of a long term action plan. The objective of this special magazine is to explain the key points that need to be considered and understood to actually implement a real 'control' of Gumboro Disease, and how to achieve this in practical terms considering the constraints of the different productions (broilers, commercial egg layers, breeders), as well as the characteristics of the different Gumboro Disease vaccines and vaccination programs that are available.

Controlling Gumboro disease

Controlling a disease includes both prevention of the disease, that is, reduction of the probability of challenge and, protection of animals against negative consequences of infection in case of challenge.

For most of the diseases, especially potentially epizootic infectious diseases like Newcastle Disease, Avian Influenza, Infectious Bronchitis, Laryngotracheitis, etc., prevention comes mainly from biosecurity, management, sanitary policy, national eradication plans, etc., while protection comes mainly from vaccination. This is essentially because the causative agent comes from outside the farm and the probability of exposure is highly variable. Most of the time it is low, except during epizootics. Vaccinations are then generally given for protection, just in case. There are even countries where vaccinations against certain highly contagious diseases (like ND) are not given at all, since the probability of exposure is almost nil because of low prevalence and reliable

prevention measures.

This clear separation regarding the setting of the tasks (prevention to biosecurity and eradication plans, protection to vaccination) is also due to the fact that most of the vaccines do not significantly contribute to prevention. This is because they have no or little action on the resistance of vaccinated animals to infection and no or little action on the re-excretion of the challenging agent. Most of the time, vaccination does not help significantly in reducing the spread of a disease or the amount of virus or bacteria shed, i.e. in reducing the probability of a challenge to neighbouring pen mates, or houses or farms. The most typical example of this is vaccination against Marek's Disease, which protects chickens well against the expression of the clinical signs and corresponding

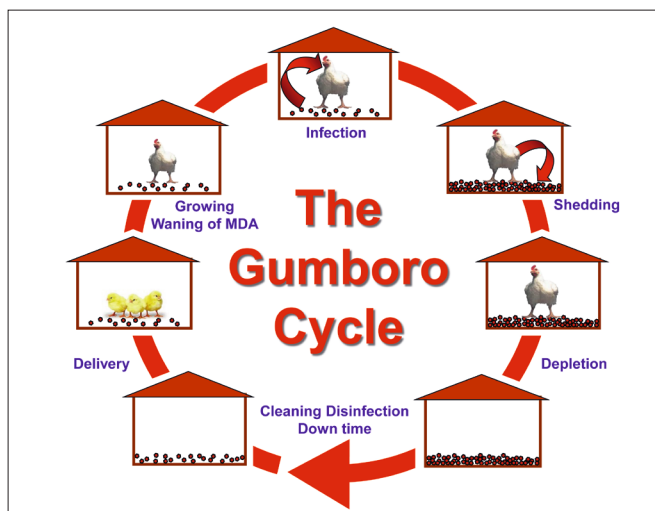
economic losses, but does not prevent infection or shedding of the virus.

Situation is different with Gumboro Disease

Depending on the countries, but very frequently, the causative virus is already present inside the farm, in the litter, or before the day-old chicks are released onto the floor, so that the probability of challenge for most farms is exactly 100%. The characteristics of this challenge (age, severity, consequences, etc.) will vary from poultry house to poultry house and according to factors that we will discuss later, but challenge will definitely occur. In this special situation, it is understandable that vaccination should aim at both: protecting the chickens and preventing the challenge from getting out of control, that is, at 'controlling' the Gumboro Disease.

The objectives of a sound Gumboro vaccination program must be:

- to ensure continuous protection of the chickens against infection by the Farm IBDV, from delivery of the day-old chicks until departure to the slaughtering plant or laying house ('viral protection').
- if this prevention of infection is not possible, then the chickens should at least be protected against the clinical consequences of infection ('clinical protection').
- to prevent or significantly reduce the amount of virus shed after challenge ('protection against shedding').
- to prevent the build-up of a higher virus pressure, cycle after cycle
- to prevent the evolution of the Farm IBDV toward a virus that could escape the protection program. These last two points are the consequences of the 'protection against shedding'. In other words, the objectives of a sound Gumboro vaccination program should aim at stopping the Gumboro cycle.



Only a sound vaccination and Gumboro prevention scheme can break the disease cycle and stop the buildup of virus pressure.

A highly variable, widely prevalent, very costly and damaging disease

Gumboro Disease or Infectious Bursal Disease (IBD) is a disease in chickens caused by an Avi-birnavirus called Gumboro Virus or Infectious Bursal Disease Virus (IBDV). Clinical signs differ, but in all cases it is very damaging.

The Gumboro virus penetrates the chickens through the oral route and within a few hours is detected in the macrophages and lymphoid cells of the digestive tract, including the caeca, the duodenum, the jejunum, and the liver. Soon afterwards, it enters a first viraemia phase which allows it to reach its target organ, which is the bursa of Fabricius where replication takes place. This replication is responsible for various morphological changes of the organ corresponding to various and highly variable gross and microscopic lesions. This includes a massive destruction of the B-lymphocytes, explaining depletion of the lymphoid follicles of the bursa. After replication in the bursa, a second massive viraemia is observed. The intensity and extension of the lesions explain the clinical consequences of infection. Although this is not fully understood, these consequences depend on several factors including:

- the type of Gumboro virus infecting the chickens,
- the virulence of this virus,
- the genetic type of the chickens,



A post mortem of a very virulent case of IBD. The damage done is enormous.

- their passive and active immunity status,
 - the age at infection,
 - concomitant infection with other pathogens,
 - some environmental factors like the season, the quality of feed, comfort, etc.
- All these possible factors of variation associated with the extreme variability of the virus and the often indirect pathogenic process (consequences of depletion of lymphocyte population and not directly due to the virus) easily explain the variability of the clinical picture, and the difficulty of describing Gumboro Disease in a simple and unequivocal manner. It is however practical to recognise three main theoretical forms of Gumboro Disease.

The immunodepressive form

The immunodepressive form is the

consequence of infection of chickens aged less than 2-3 weeks by any pathogenic Gumboro virus. During this time, the integrity of the bursa of Fabricius is critical since it is the organ where B-lymphocytes need to mature to become functional and provide the chickens with effective humoral immune response capabilities. This early infection is responsible for higher susceptibility to many diseases and poor responses to vaccinations, leaving the flocks more likely to suffer from common infections and epizootic diseases. The severity of immunodepression (please see *note 1* at the end of this document) varies according to age at infection (the earlier, the worse) as well as the type of virus.

Some IBDV provided with pathogenic features similar to the so-called 'variant E' isolated in the USA in the 1980s ➔

are known to be strongly immunodepressive. When compared to more 'classical' strains, they have the capacity to create extensive and persistent depletion of the follicles and consequently to dramatically reduce the size of the bursa.

The clinical form

The clinical form is the consequence of infection of chickens with a Gumboro virus that replicates very rapidly and at a high level and creates mortality. Clinical signs may or may not be expressed by the affected chickens. Post mortem examination generally shows a strong oedema of the bursa with (or without) haemorrhages of variable intensity that can also be seen in the form of petechias or suffusions in the thighs and breast muscles. The clinical case from Gumboro town reported by Albert Cosgrove was in this clinical form, and it is also in this form that the disease was initially recognised and described in most of the countries. The 'very virulent' or 'hypervirulent' cases of Gumboro Disease that were reported in Western Europe in the late 1980s, and then in other parts of the world where they can still be observed, are in this clinical form as well. The mortality rate varies a lot but is generally higher in slow growing chickens like layer pullets, layer/broiler breeder pullets or organic chickens (generally more than 25%) than in broilers (in general less than 15%).

The sub-clinical (also called economical) form

The sub-clinical form of the disease corresponds to infection of chickens after 2-3 weeks of age, by a Gumboro virus



Post mortem examination generally shows a strong oedema of the bursa with (or without) haemorrhages of variable intensity.

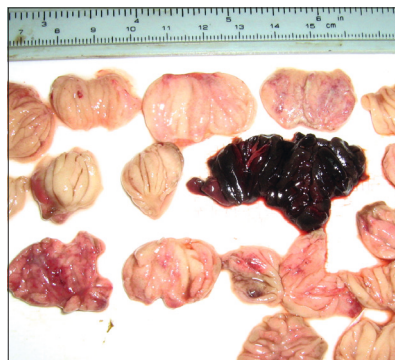


Clinical signs of deep depression are not always clear and present.

without occurrence of typical clinical signs (hence the term 'sub-clinical') or direct mortality. The bursa of Fabricius again shows lesions but of variable intensity and variable persistence. Consequences can vary from nil to serious, depending on the pathogenicity of the Gumboro virus strain infecting the chickens, but close observation and thorough field and laboratory investigations may be necessary to pinpoint the causative agent. Most of the time, only poor or sub-optimal performances are detected and this explains why people often refer to it as the 'economical form' of Gumboro Disease.

Past knowledge can be misleading

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It is important to bear in mind that the Gumboro virus is highly variable in its pathogenicity, virulence and antigenicity features, as shown in these BF's.

Gumboro virus is highly variable in its pathogenicity, virulence and antigenicity features, so that it is too simplistic to associate one type of virus to one clinical form. The frequently presented classical scheme, 'classical IBDV/subclinical form, very virulent IBDV/clinical form, variant IBDV/immunodepression' is simply misleading and prevents us from understanding properly what the real situation is. Very virulent or classical virulent IBDV strains can also be responsible for sub-clinical Gumboro Disease.

Similarly, the use of an artificial separation between 'classical' and 'variant' IBDVs that dates back to the 1980s when the first 'US variant IBDVs' were described and found to be different from the 'classical' reference Gumboro viruses using virus neutralisation is also misleading. These 'variants' were antigenic variants that could be detected by serology. This dichotomy has become unsuitable since we now know that variations between IBDVs are much more frequent, deeper and more complex thanks to more studies and use of new characterisations tools, including molecular. The fact is that when compared to others, almost any IBDV is a 'variant', at least from the genetic perspective. Whether this detected variation is relevant or not for the chickens or the Gumboro disease control program is then the most important question.

Key points to consider for controlling Gumboro disease

Preventing chickens to come into contact with the Gumboro virus is almost impossible. The virus is everywhere, unique in its appearance and chickens are susceptible all their life until sexual maturity takes place. That makes controlling Gumboro disease instead of only protecting against clinical signs advisable. Five key points to consider.

IBDV is a highly resistant virus, so that it easily escapes well conducted routine cleaning and disinfection procedures. Most of the time, once contaminated, a poultry house tends to remain contaminated. This is particularly true in countries (e.g. USA, Brazil) where the litter is not systematically removed after the chickens are sent to the slaughtering plant. Instead, most of the time, the litter stays in place and is sometimes, but not always, simply

covered by another layer of fresh litter on top of which another grow out is produced. The old litter can also be accumulated on one side of the house for composting while cleaning is done in the house, and will be spread again only when day-old chicks are housed. This is called the built up litter system and the real cleaning and disinfection procedures are only applied after five to 10 rounds of chickens have been produced. Even when the 'all in – all out' produc-

tion system is used, elimination of any IBDV from a contaminated farm is a very ambitious objective, but it is reasonable to aim at reducing the amount of virus present in the farm and consequently the amount of virus that will challenge the chickens. This is what is routinely called 'lowering the virus pressure'.

The challenge is unavoidable and always specific

This outstanding resistance of IBDV makes prevention of IBD quite unique since it is almost always present before the chickens have been delivered to the poultry house, and not after as is commonly seen with other diseases like Newcastle Disease, Infectious Bronchitis, Infectious Laryngotracheitis, Avian Influenza, etc.

The IBDV present at the farm will infect the young chickens as soon as they are susceptible, that is, after their level of passively transferred Maternally Derived Antibodies (MDA) has reached a non-protective level.

Challenge at each farm is always unique and will depend on the type of IBDV present, the level of virus pressure, the quantity and nature of MDA, the type and potency of vaccine applied as well as the quality of vaccine application. The presence of other possible contaminants and challenging pathogenic agents as well as the general health status of



With young chicks, at the start of the Gumboro cycle everything looks fine clinically.

the chickens and their capacity of resistance are also important factors in understanding the specificity and consequences of an IBDV challenge on a given farm.

Vaccination has an impact on the evolution of the situation

Chickens are susceptible to infection by IBDV all their life. Early clinical outbreaks of very virulent Gumboro Disease have been reported in one week-old broilers, and late outbreaks in 16-20 week-old layer pullets with the bursa of Fabricius reaching the size of a walnut, and showing typical haemorrhagic lesions (e.g. Morocco, Nigeria). Because of this continuous potential challenge by IBDV, there is a need for continuous protection by passive immunity for the first weeks of life, and then by active immunity induced by vaccination for the rest. Production procedures as well as cleaning and disinfection will play an important role in lowering the severity of the challenge, but no real control of IBD can be expected if action does not also involve careful consideration and management of passive and active immunisation programs. It is critical to understand that because of its high prevalence, high resistance, susceptibility to mutations as well as the widespread presence of vaccination induced selective pressures, IBDV is likely to evolve and because of these possible changes in its antigenic and biologic properties, control of IBD must also take the time into account. What is done today will impact on tomorrow's situation and what might appear to be working well for a few or even several rounds, might be detrimental in the longer term.

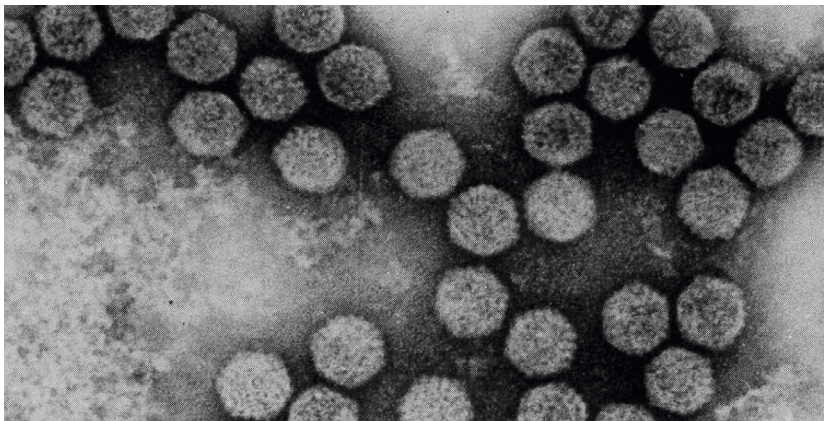
MDA are of critical importance

Chickens are susceptible to IBDV infection as soon as the first day of age, and the consequences of early infection are by far the most detrimental since they have a direct and definite negative effect on immune functions with special regards to the humoral part.

Early infection of the chicken will result in severe depletion of the follicles of the bursa of Fabricius, extensive destruction of the B-lymphocytes population, and consequently, a severe reduction in antibody production. Severity and duration of this immunodepression will depend on the characteristics of the IBDV strain, as well as, more importantly, the age at infection. After three weeks of age, the consequences regarding immunodepression are not relevant.

Because of the time necessary for the chicken to develop immunity and the possible consequences of residual pathogenicity of live attenuated vaccine if given at a young age, it is impossible to protect the young chick against early infection by direct vaccination. The only way to ensure this extremely important early protection of the chickens is to provide them with adapted passive immunity. Passive immunity is transmitted from the breeders to the progeny in the form of antibodies reflecting both the amount and the nature of circulating antibodies present in the breeders at the time the embryonated egg is laid.

As a general rule, the higher the amount of antibody present in the breeders, the higher the MDA level in the chickens. Antibodies also vary according to the antigenic profile of the virus that has infected the breeder, or has been used



The Gumboro virus infects and destroys, actively dividing B cells in the bursa of Fabricius.

for vaccination, so that MDA are also specific to certain antigenic type or types of IBDV. This is why in the USA, where variant IBDV are largely spread, and infection with IBDV is generally early because of the widespread use of built-up litter, inactivated IBD vaccines of the commercial or autogenous types also contain one or several 'variant' IBDV as antigens together with IBDV antigen of the 'classical' type. Without vaccination, breeders naturally transmit MDA, but this transmission is usually low, variable and might not be well adapted to the field IBDV the chickens are going to face, especially when breeders are reared in a well-protected environment, geographically far from the farms where their progeny will be delivered. This naturally transmitted passive immunity usually turns out to be too short-lived to cover the first three weeks of age (i.e. the first three weeks of high risk) and may also turn out to be antigenically poorly adapted. These are the main reasons why the vast



majority of vaccination programs for breeders applied world-wide include injection of a killed adjuvanted vaccine as a booster to a live vaccination, so that circulating antibodies reaching very high levels are passed on to the progeny. This active immunity of breeders may also qualitatively benefit from extra immune responses to field infections. These uncontrolled exposures occurring when the animals are still pullets will enlarge the spectrum of protection of MDA provided to the progeny.

In the USA as well as other countries, the identification of variant Gumboro virus strains has generated the use of multivalent IBD killed vaccines in the breeders and even (for the USA only) the use of specific autogenous IBD vaccines. This will be further discussed at the time when vaccination guidelines for optimal control of Gumboro Disease are given.

Establishment of a vaccination program for the progeny is difficult

A well designed vaccination program for

The drinking water method of vaccination is usually recommended, however this vaccination needs to be done at the farm, by the farmer or the farm workers, and as such has proven to be unreliable.

broilers should take into account, and respond to, several constraints that make the exercise difficult. The two most important points to consider are:

- The need to determine the optimal time for vaccination; Day-old chicks are provided with MDA that can partially or totally neutralise classical live attenuated IBD vaccines and leave the chickens unimmunised. For this reason, it is essential when using this category of vaccines to determine the optimal time for vaccination: not too early so that the vaccine is not neutralised and not too late so that no opportunity is left for the field IBDV to infect the chickens. The use of ELISA serology to quantify the amount of MDA transmitted to the day-old chicks, combined with mathematical formulas to determine the optimal time for administration of IBD vaccine(s), have clearly demonstrated their useful-

ness, with special regard to live attenuated IBD vaccines of the intermediate plus type (please see *note 2* at the end of this document). The interest of this approach for vaccines of the intermediate type is not so strongly substantiated, most probably because of their weaker capacity to overcome interference with MDA as well as their limited spreading capacities that prevent horizontal transmission to compensate for uncertainty in determination of the optimal time for vaccination and heterogeneity of the levels of MDA.

- The difficulty of correctly applying live attenuated IBD vaccines; Live attenuated vaccine IBDVs need to reach lymphoid cells of the digestive tract before entering the bloodstream and then be spread to various organs including the bursa. For this reason, the drinking water method of vaccination has turned out to be the most efficacious and is usually recommended. This vaccination needs to be done at the farm, by the farmer or the farm workers, and as such has proven to be unreliable. When checked at the level of an organisation using laboratory testing methods like serology, histopathology or PCR, it is common to detect as many as 30-50% of the flocks not immunised at all, although all were given an IBD vaccine. When conducted according to state-of-the-art recommendations, drinking water vaccination is very time consuming, cumbersome and to some extent not considered as important if the motivation that comes with an immediate return-on-investment is not there.

The recent introduction of live vaccines that can be injected systematically and mechanically to all chicks using the in-ovo or the subcutaneous routes of vaccination, with a high degree of reliability when it comes to actual injection as well as vaccine take, has greatly changed the picture. These vaccines based on the Immune Complex or Vector vaccines technologies are now largely applied in the hatcheries as 'broiler or pullet IBD vaccines', and have dramatically improved the flocks' IBD vaccines coverage. However, they are more than just different types of IBD vaccines, and an understanding of their mechanisms of action and respective advantages also allows users to make the right choices and get the most out of them.

Strategic factors of a Gumboro challenge

As already stated, many factors explain the nature of an IBD challenge, but when it comes to the farm situation four of them are the most critical. Farm IBDV, virus pressure, passive immunity (MDA) and active immunity.

It is important to realise that these four factors are not independent from one another, and can combine and lead the IBD challenge toward an earlier or later IBDV infection, as well as in the direction of a more severe or lighter disease. *Figure 1* summarises the interactions between the four strategic factors.

Farm IBDV

The Farm IBDV (also called resident IBDV) is the Gumboro virus that is already present when the day-old chicks are delivered, and which is very likely to infect them once they become susceptible, after their level of MDA has

decreased to non-protective level. If infection occurs, the severity and consequences of the disease will depend largely on the pathogenic properties of this virus as well as on the amount of virus that has challenged the chickens.

Virus pressure

The virus pressure (also called challenge pressure) indicates the amount of virus that is challenging the chickens. It is related to the management system applied at the farm ('all-in, all-out', accumulated litter, multiage, cages, etc.) as well as to the quality of cleaning and disinfection and the situation of the farm relative to other neighbouring

farms. For farms (with concrete floors) where the 'all-in, all-out' system is used and disinfection is perfect, pressure is (usually) low.

Virus pressure also varies in the course of the growing period. If IBDV is successful in infecting some chickens, then these chickens will multiply the virus, shed it, and in doing so, contribute to an increase in the virus pressure. The higher the virus pressure, the higher the risk of infection and the stronger the clinical consequences.

Passive immunity

Passive immunity provided by Maternally Derived Antibodies (or MDA) plays a critical role in containing virus pressure and preventing infection as long as it is present in the chicken in a sufficient concentration (= at a sufficient level). After an increase observed during the first days after hatching, and due to the release of immunoglobulins still present in the yolk sac into the blood stream, the MDA level declines according to the time and the growth rate of the chickens, until it reaches a non-protective level corresponding to age at susceptibility of the chickens.

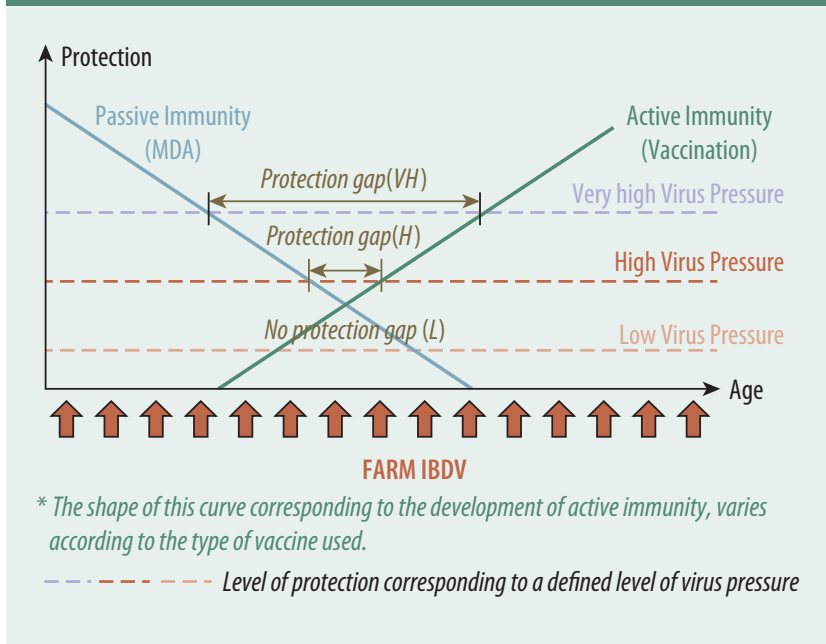
Age at susceptibility (i.e. at potential infection) depends on:

- the initial level of MDA: the higher this level, the stronger and the longer the protection,
 - the level of virus pressure, simplified in *Figure 1* as very high (VH), high (H), or low (L): the higher the pressure, the shorter the MDA protection,
 - the virulence of the virus causing the infection: the more virulent the virus, the higher the level of MDA it can break through, and consequently, the shorter the protection
 - the specificity of MDA relatively to Farm IBDV: homologous MDA are more protective than heterologous.
- In summary, the challenge is expected to



The virus pressure (also called challenge pressure) indicates the amount of virus that is challenging the chickens. It is related to the management system applied at the farm.

Figure 1 - Principles of protection against IBD.



be earlier if the MDA level is low, if virus pressure is high, if the challenging virus is more virulent or is antigenically different from the one that has (the ones that have) been used to vaccinate or challenge the breeders.

Active Immunity

Active immunity (or vaccine immunity) induced by administration of a vaccine will develop according to the vaccine(s) employed, the quality of application and immune status of the chickens at the time the vaccine(s) is (are) given.

All Gumboro vaccines employed to induce immunity against Gumboro Disease are live vaccines, either of the attenuated type or of the immune complex type or of the recombinant vector type. Consequently, they all need to replicate (i.e. to 'take') to 'work'.

The 'take' of a live Gumboro vaccine depends on the right timing of application because of interference with MDA. If given too early, in the presence of an excessively high level of MDA, the vaccine virus is neutralised or its replication is delayed. If it is given too late, a window of opportunity (also called protection gap) is offered to the Farm virus to infect the flock. This optimal timing depends on the level of MDA and the invasiveness of the vaccine, that is, its capacity to overcome a given titer of

MDA. This problem of timing has been solved by the development of Gumboro hatchery vaccines (Immune Complex or Vector vaccines) that have the capacity to overcome interference from passive immunity whatever the level.

The 'take' depends as well on the quality of application, which is extremely critical, and can be unreliable in case of farm application. The objective of a proper vaccine administration is not only to reach every chicken, but also to ensure that the full dose is received by each of them. The capacity of a vaccine strain to overcome MDA is also linked to the dose of vaccine administered. Although they are based on different scientific concepts and work according to different immunological mechanisms, both Immune Complex and Vector vaccines require a perfect injection process at the hatchery, either in-ovo or subcutaneous to day-old chicks.

If a Gumboro vaccine is properly applied (and at the right time for classical live attenuated), then the vaccination is successful, which does not necessarily mean that the chickens are protected and the disease is under control. Other factors also need to be taken into consideration. In case of high virus pressure, field infection may also occur before vaccination has induced a sufficiently high level of protection. In this case, the type of

In case of successful vaccination, active immunity develops:

- If passive immunity is adapted and virus pressure is low, active immunity reaches protective level before passive immunity reaches non-protective level. In this situation, at the level of the flock, no protection gap is left and a continuous protection of the chickens against Green Square infection by IBDV is achieved.
 - If passive immunity is not adapted and/or virus pressure is high, active immunity cannot reach protective level in due time, and a protection gap offers an opportunity for the Farm IBDV to infect the chickens before vaccine protection is established. This infection by Farm IBDV can affect all or only part of the chickens.
 - If passive immunity is not adapted and/or virus pressure is very high, then the protection gap is wider and, what is worse, susceptibility to infection by the Farm virus appears earlier, so all chickens are affected. Since infection occurs at a younger age, consequences are more severe.
- One can easily understand that the lack of adapted passive immunity and the presence of high virus pressure are the key explanatory factors of an early infection, which is the most serious for chickens since it has the capacity to deeply and permanently compromise their immune system. Quality of vaccine application and optimal timing are not the only variables to be taken into account for a successful Gumboro control program.

In case of unsuccessful vaccination, active immunity does not develop, or develops much later:

- If passive immunity is adapted, when the virus pressure is low and farm virus poorly pathogenic, this can be of no or little consequence to the health of the chickens or growth performance.
 - If passive immunity is adapted but virus pressure is high and farm virus pathogenic, then sub-clinical or clinical consequences will be observed.
- One can then also easily understand why a successful vaccination is critical to prevent infections with pathogenic Farm IBDV that can occur after three weeks of age and are responsible for mortality and/or poor performances.

vaccine that is selected is critical since depending on the vaccine strain, IBD vaccines can break through different levels of MDA and consequently do not have the same onset of immunity. Taking into account these elements of a strategy for controlling Gumboro Disease, it is now important to investigate the features, benefits and drawbacks of the commercially available Gumboro vaccines, and what recommendations should be made on how to use them.

Gumboro vaccines and vaccinations

Because of the characteristics of the Gumboro virus and the unique pathogeny of the disease, Gumboro vaccines and vaccinations must fulfil specific requirements that are different from what is generally required for other diseases. The search for the ideal Gumboro vaccine is ongoing.

The Gumboro virus is so widespread and so resistant in the environment that almost every chicken will be challenged during the course of its life. For this reason, vaccine-induced immunity must constantly be at a high level and the vaccination coverage rate must reach 100%. On top of this, vaccination is also supposed to lower the challenge pressure for the following cycles of production, so Gumboro vaccination is not only a protection tool but must also accomplish the mission of controlling the disease.

Protection during the first weeks of life comes from passive immunity transmitted by the breeders to their progeny, and is then ensured by active immunity induced by vaccination. Passive protection must be high, homogeneous and adapted, so that age at susceptibility to challenge is delayed at least after 2 to 3 weeks. Active protection must be able to start in the presence of this still-effective passive protection and take over in all chickens when it has come down to an insufficient level.

A successful vaccination program means a successful combination of both a careful selection of an adapted vaccine and the use of an effective procedure of administration. These two components are not independent because every vaccine is more or less linked to one or more recommended routes of administration.

An ideal vaccine against Gumboro

Disease needs to fulfil the following five basic requirements:

- be safe, so that no immunodepression or any other side effect is induced,
- be efficacious in protecting chickens against the negative consequences of infection by the specific Farm IBDV, so that growth potential and capacity of resistance to diseases remain untouched,
- make broilers or commercial layer pullets resistant to infection so that viral pressure will be progressively reduced, and no antigenic selection will be exerted on the resident population of IBDVs. This way, no help will be available for a new variant to pop up, escape passive protection, and create the detrimental early infections.
- be able to take in the presence of passive immunity, whatever it is, so that the timing of vaccination is not an issue and active immunity can actually take over from passive immunity.
- be able to compensate for (limited) vaccination misses by spreading or other mechanisms.

The available Gumboro vaccines

At present, Gumboro vaccines from the following four categories are available:

- Inactivated (or Killed) IBD vaccines containing a high amount of inactivated whole IBDV or subunits of IBDV presented in an emulsion made of mineral oils that plays the role of adjuvant. Most of the time, available emulsions are of the 'water-in-oil' type.
- Classical Live attenuated IBD vaccines

are prepared from live attenuated IBDV strains and presented as freeze dried (lyophilized) products. The IBDV strains used for these vaccines have been naturally or artificially attenuated so that we can recognise different types depending on their degree of attenuation: 'Mild' (highly attenuated), 'Intermediate' (very attenuated), 'Intermediate Plus' (moderately attenuated) and 'Hot' (poorly attenuated).

- Immune Complex IBD vaccines are prepared from live attenuated IBDV strains of the Intermediate Plus type mixed in well-defined proportions together with specific anti-IBDV serum. The final product is freeze dried.
- Commercially available Recombinant Vector IBD vaccines are made from a genetically engineered virus ('the vector') whose genome contains a gene from a specific IBDV ('the donor') encoding for the VP2 capsid protein of IBDV. As of today, the Herpes Virus of Turkey (HVT) is used as a vector and is presented in the nucleus of chicken embryo fibroblasts (CEF). For this reason, the vaccine is a suspension of infected cells kept frozen in liquid nitrogen.

Many investigations and experiments as well as years of use have greatly increased our knowledge regarding these various categories of vaccines, so that today we know much better what can, and should be, expected from them, beyond the simple criteria of 'protection'. The points to take into consideration are safety under real field conditions, efficacy under various conditions of challenge, and considering protection against the clinical (or sub-clinical) disease and against infection ('viral protection'), as well as short and long term consequences on the evolution of the disease and dynamic of the immune response, with special regard to the onset of immunity, which needs to fit the characteristics of the challenge.

Inactivated Gumboro vaccines

Inactivated (also called killed) Gumboro vaccines are intended to stimulate the production of very high levels of antibody in the vaccinated chicken. This is due to the combination of a high antigenic mass with an adjuvant made from mineral oils.

The antigen can be the whole Gumboro virus, grown in embryonated eggs, cell cultures or bursa tissue. The antigen can also be of the sub-unit type with selected viral proteins (like the Virus Protein 2 or VP2) or the overall structure of Gumboro virus particles (so-called Virus Like Particles or VLP), produced in yeast or insect cells or other expression systems. Inactivated IBD vaccines may contain only one (monovalent) or more (polyvalent or multivalent) IBDV strains. They can be produced industrially in large numbers ('commercial') or as 'custom' (or 'autogenous') vaccines in much smaller size serials. Inactivated IBD vaccines are totally safe and no side-effect is attached to them, except with some particular commercial preparations of poor quality where local tissue reaction can occur at the site of injection.

Efficacy and dynamic of the immune response

Following injection of inactivated IBD vaccines, antibody response increases and reaches a peak at around 3 to 5 weeks after vaccination. This immune response is somewhat faster, much higher and also for a much longer duration if the chickens have previously been in contact with IBDV. This 'priming' can be of vaccine and/or natural field challenge origin. A vaccination program including a vaccination with a live attenuated IBD vaccine (so-called priming in a vaccina-

tion program) followed by injection of a killed IBD vaccine ('the booster') is inducing the so-called 'hyper-immunisation' process and is used worldwide as the reference for vaccinating parent stocks.

Inactivated vaccines have sometimes been used in the past to protect young chickens with variable success, but the interference of MDA with the vaccine as well as the costs associated with the vaccine and individual injection explain why this has been abandoned.

Today, inactivated vaccines are used in almost all countries to hyper-immunise the breeders and ensure the transmission of a high and homogenous passive immunity to their progeny. This is probably the most critical part of a vaccination program aiming at a real control of Gumboro Disease, considering that passive immunity is the only source of protection for the young chicken during the first weeks of life, i.e. the most critical period regarding susceptibility to IBDV-caused immunodepression.

Three important points related to inactivated IBD vaccines need to be mentioned:

- Inactivated vaccines do not spread, so a breeder not properly injected will never produce a high amount of antibody and consequently will never properly protect its progeny against early challenge. Injection of the breeders with inactivated vaccines needs to be closely monitored.

- Immunity induced by inactivated vaccines is almost exclusively of the humoral type, i.e. composed of antibodies. Part of these antibodies is transferred to the progeny through the egg yolk. They are called Maternally Derived Antibodies (MDA).

Antibodies are very much specific to the antigen that has induced their production, so the corresponding protection has a narrow spectrum. If 'classical' IBDV is (are) used in the vaccines, then MDA will be efficacious against field virus of the 'classical' type. If the field virus changes significantly, then the only way to keep a high level of protection and prevent early infection is to include this new IBDV in the inactivated vaccine. This is why some commercial vaccines contain more than one strain of IBDV, and this is also why, in the USA (which is the only country where this is allowed), custom IBD vaccines are made and used, and from time to time updated.

- Humoral immunity induced by good quality inactivated IBD vaccines and according to a hyperimmunisation process is not likely to decline significantly. However, if this happens, it is possible to re-vaccinate the breeders during production with an extra injection. This is frequently done in some countries and is a way of ensuring passive immunity is kept at a high and steady level, and of mixing day-old chicks originating from flocks of different ages without heterogeneity problems.

Classical Live attenuated Gumboro vaccines

Live vaccines contain live attenuated Gumboro viruses that replicate in the bursa of Fabricius. As a result, the induced immunity generated by the replication of a 'whole' virus is complete and includes humoral as well as cellular components.

This immunity starts developing within a few hours after the vaccine has reached the bursa and is well established 2-3 days after vaccination. It is a very fast process, so the so-called 'Onset of Immunity' (or OOI) is very short. The vaccine virus is a 'free virus', fully susceptible to neutralisation by MDA, so administration needs to be done at the farm when the level of MDA has reached a moderate level. For this reason and as already highlighted, the optimal timing of vaccination and quality of administration are extremely critical.

Replication of live attenuated IBD vaccines in the bursa always implies destruction of some lymphocytes. In theory, this can damage the health of the chickens and possibly compromise their immune functions. But in reality, in field conditions these theoretically negative effects are not relevant, and these are the reasons why:

- the IBDV strains that are employed are attenuated so their pathogenicity is significantly reduced. This is required and checked by registration authorities before granting marketing authorisation.
- they are applied (and consequently replicate) after two weeks of age, an age when the maturation of the immune system of the chickens has almost finished and migration of the B-lymphocytes to

secondary lymphoid organs has been completed. This is why the damage created in the bursa and the corresponding destruction of B-lymphocytes, even if macroscopically visible, has no relevant effect on the immune system.

- they are applied in the presence of moderate to residual levels of MDA that are limiting replication of the vaccine virus and buffering corresponding consequences.

Efficacy

Besides the effect on the safety, the attenuation process also has other important consequences on the efficacy of the vaccine. The more attenuated the strain, the lower the level of MDA the vaccine can break through, the slower the bursa colonisation ability, and the weaker the capacity to spread within the flock from vaccinated to non-vaccinated chickens. These are at least three of the reasons why 'Mild' type IBD vaccines, which were unable to overcome even very low levels of MDA, have been abandoned, and why 'Intermediate' type IBD vaccines, which are unable to establish protection early enough, have often failed to work properly in high virus pressure farms or to reliably protect against very virulent Gumboro virus strains. 'Intermediate Plus' as well as 'Hot' type IBD vaccines have the capacity to take early enough, in the presence

of moderate levels of MDA, so efficacy in high virus pressure conditions and against very virulent IBDV has been clearly proven. This efficacy is explained by:

- the capacity to 'take' (i.e. to infect the chickens) in the presence of moderate levels of MDA so that vaccination could be successful before challenge occurs,
- the spread of the vaccine virus, which compensates for (moderate) vaccination failures and ensures 100% vaccine coverage,
- and, last but not least, the ability to achieve a fast and full colonisation of the bursa.

The attenuation slows down the speed of replication of the vaccine virus, so that in case of very attenuated strains (Intermediate type IBD vaccines), it





Despite useful tools like the blue dye to monitor distribution, vaccination on farms is not reliable.

takes too long (compared to Intermediate Plus type IBD vaccines) to get all follicles of the bursa stimulated and protected before the progression of the vaccine virus is stopped by the immune response. This explains why it is still possible to 're-infect' chickens previously immunised by Intermediate type IBD vaccine, and to have them shedding the challenge virus (See Experiment A, *Page 28*). Conversely, replication of 'Intermediate Plus' type vaccine strains is much faster. All follicles of the bursa are stimulated (and protected) within a few days, and chickens are far more resistant to re-infection. For this reason, chickens actually immunised with 'Intermediate Plus type' IBD vaccines are highly resistant to infection, and no, or very limited, sign

of replication, as well as no, or very limited, sign of shedding, of the challenge virus are detected, whatever the antigenic type of challenge IBDV (See Experiment A and C, *Pages 28 & 30*). For this reason also, and provided field application is perfect, the use of Classical Intermediate Plus type vaccine on successive cycles may potentially reduce the field virus pressure without exerting any selection pressure on the Farm IBDV's population, and hence without favouring the emergence of variant viruses. Unfortunately, when it comes to real control of IBD, these outstanding potentialities of Intermediate Plus IBD vaccines cannot be reliably expressed in full and converted into real progress. This is because all Classical Live IBD vaccines need to be administered on the farm

(most of the time in drinking water), which is not reliable. Although partial immunisation of a flock can be sometimes sufficient to provide clinical 'protection', vaccine administration at the farm has proven to be very often poorly conducted.

In field conditions of use, this category of vaccine in combination with the farm vaccination procedure has clearly shown limitations and cannot be regarded as steadily reliable, and definitely not efficacious in ensuring the 'prevention' part of a full IBD control.

Dynamic of the immune response

When a Live attenuated IBD vaccine is properly administered to a flock of chickens, then all chickens are exposed and their immune system stimulated, provided the quantity (i.e. the dose) of the vaccine virus they receive is sufficient and their level of MDA matches the level the vaccine can overcome.

These chickens are the 'primary vaccinated' animals (directly hit by the vaccination and susceptible to it). Their immune response is then very fast and within a couple of days, the chickens are protected to a level corresponding to the potential of the vaccine used (See Experiment A). In the meantime and in the following days, the vaccine strain is re-excreted so that the chickens not already hit by the initial vaccination, or not susceptible to it, are then stimulated in turn. These chickens are the 'secondary vaccinated' animals (hit by the vaccine virus shed by the primary vaccinated chickens). Secondary vaccination does occur with the Intermediate Plus type of IBD vaccine, but not, or poorly, with the Intermediate type because the spreading capacity of this category of vaccine virus is limited.

It is important to understand that if properly conducted, this vaccination corresponds to the simultaneous application to each chick of a flock of a full dose of vaccine virus, at an age where the mean MDA level is still moderately high. It is a way to 'force the MDA barrier' and induce immunity at an early age (at around two weeks of age with an Intermediate Plus type IBD vaccine). For this reason, Live Intermediate Plus type IBD vaccines can be a genuine tool to get the situation back under control in case the virus pressure has gone too high.

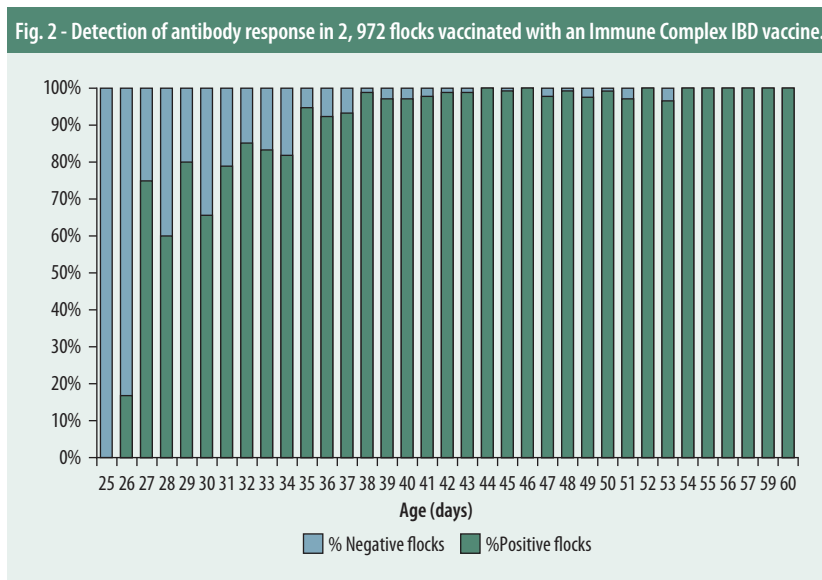
Immune Complex Gumboro vaccines

A suspension of Live attenuated Gumboro virus of the Intermediate Plus type is mixed in well-defined proportions, and according to well-defined procedures, with antiserum of defined avidity prepared in SPF chickens hyper immunised against IBD.

This way, the vaccine virus is covered and consequently protected from recognition by the immune system of the chicken by specific Immunoglobulins ('Virus Protecting Immunoglobulins' or VPI). Following injection, VPI are catabolysed at the same time as the MDA and the vaccine virus is released. The take of the vaccine (that corresponds to replication of the vaccine virus in the bursa) occurs when the MDA level has reached a level that allows the vaccine to take and before the flock has become susceptible to infection. The big advantages of this technology are the following:

- The quality and strength of the protection coming from replication of a complete Intermediate Plus type Live attenuated IBDV are conserved: full protection against clinical signs, high degree of resistance against infection whatever the challenging IBDV strain, high level of prevention against shedding, no selection pressure on the Farm IBDV population.
- The vaccine adapts individually to the immune status of each chicken and always replicates at the 'optimum' time,
- The vaccine can be administered in the presence of passive immunity, so it does not contradict and may even complement the breeders program,

- The vaccine can be injected in the hatchery, so reliability and consistency of application is at a maximum. Every chicken benefits from the properties of the vaccine.
 - After replication, the vaccine virus is shed and spreads to neighbouring chickens, so some vaccination misses can be offset.
- The safety of the Immune Complex Gumboro vaccines is similar to the safety of Intermediate Plus type Gumboro Live vaccines, with the additional advantage that the vaccination process is predominantly of the primary type so that every chicken is immunised with the same, well controlled dose of vaccine. Many years of field experience of use in broilers have demonstrated the perfect safety of this category of vaccine.



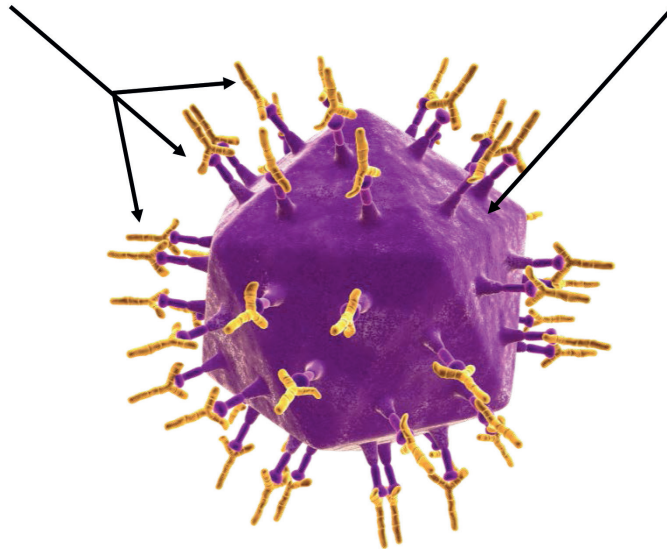
Vaccine take with Immune Complex type vaccines generally occurs between three and four weeks of age. ELISA antibody response is detected at least one week later.

Efficacy

When considering the various aspects of efficacy expected from IBD vaccines and in particular their capacity to not only protect against clinical signs, but also to serve as a tool for the control of the disease, Immune Complex IBD vaccines are very attractive when compared to other existing categories of IBD vaccine. Provided passive protection is adapted to the challenging Farm virus and basic cleaning and disinfection procedures are applied, active immunity can be induced before challenge occurs and will successfully resist whatever this level of challenge, and whatever the type of challenging IBDV strain. Chickens will be highly resistant to infection and consequently highly resistant to replication and

Immune Complex IBD Vaccine

Specific Antibodies VPI
(Virus Protecting Immunoglobulins) + **Vaccine Virus Strain**
(Winterfield 2512)



Compared to live attenuated IBD vaccines of the Intermediate Plus type, the immunity does not come from replication of a complete virus, triggering all the arms of the immune system but essentially from antibody response to the VP2 antigen of the IBD virus expressed by the recombinant HVT vector.

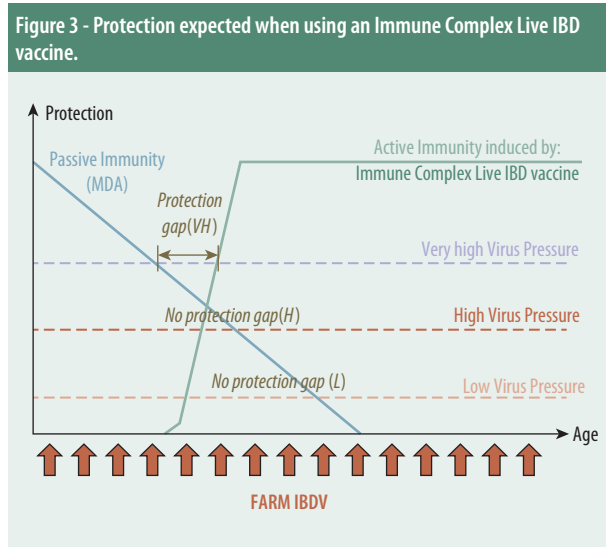
shedding of the challenge virus. As a consequence, cycle after cycle, virus pressure will decrease, no selection pressure will be exerted on the Farm IBDV population and real 'control' of IBD will be achieved.

all the individuals in a flock occurs within a rather short period of time (ELISA Biocheck Kit), usually between 3 and 4 weeks of age (see Figure 2). In almost all farms, this mechanism and corresponding timing works because no chicken hatched with a low

level of MDA (which would make it susceptible to infection earlier than the group in case of field vaccination) is left unprotected. No chicken will have the opportunity to replicate the field virus before the rest of the group is immunised.

Dynamic of the immune response (see Figure 3)

At present, only live attenuated IBD vaccines of the Intermediate Plus type are presented as Immune Complex. Following their injection, either in-ovo or subcutaneously on the first day of age, the vaccine viruses covered by the specific immunoglobulins are protected against neutralisation by MDA. However, these exogenous antibodies are progressively catabolysed during the first weeks of life and vaccine viruses are progressively released. Immunisation will occur when the level of MDA has reached a level that is low enough to permit the vaccine to reach the bursa and start replicating. Many years of field experience and monitoring have shown that immunisation of



Live recombinant rHVT-VP2 vector IBD vaccines

With live recombinant rHVT-VP2 vector IBD, it is necessary for the vector to replicate to have the VP2 expressed to get protection induced.

Compared to live attenuated IBD vaccines of the Intermediate Plus type, this immunity does not come from replication of a complete virus, triggering all the arms of the immune system ('complete' immunity) but essentially from antibody response to the VP2 antigen of the IBD virus expressed by the recombinant HVT vector. That way, when considering protection against the Gumboro virus, the useful part of the immune response is mostly of the humoral type.

HVT is widely used, alone or in combination with the SB-1 or the Rispens virus strains, as a vaccine to protect against Marek's Disease (MD). The corresponding protection mechanism is still far from being fully understood, but it has been established that the onset of immunity against MD comes almost immediately after the viraemia phase, which is to say within less than 10 days after injection. Protection against Gumboro Disease comes from the expression of the VP2 by rHVT-VP2-infected cells, and is a much slower process. Onset of immunity against IBD requires much more time than for MD. One important point to mention when speaking about recombinant vector vaccines in general and rHVT-VP2 vaccines in particular, is that the corresponding properties and potentialities of the final commercial vaccine are very much dependent on the product (the 'construct'). The strain selected to become

the vector, the degree of attenuation of this strain (i.e. its passage level governing its capacity of replication), the gene sequence(s) selected for insertion (which sequences encoding which proteins), the origin of the VP2 insert (which IBDV strain? classical? classical virulent? classical very virulent? which variant?), the selected insertion site, the promoter used to provoke expression of the inserted gene, the way the recombination is conducted, etc. etc. are factors, among many others, that can explain the significant differences observed between vaccines that are provided with similar 'rHVT-VP2' generic names although with different commercial names.

For this reason, it is important to state that the information given in this article regarding rHVT-VP2 vaccines and in particular conclusions regarding their properties and recommendations regarding their use relate to the rHVT-VP2 vaccines currently available on the

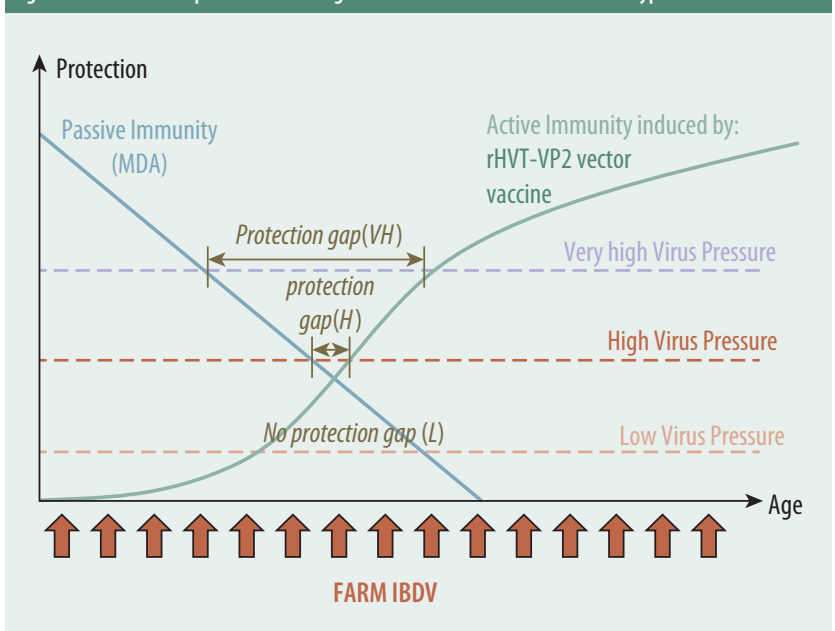
market. These are the ones that we have analysed, tested and challenged. This information could prove to be inaccurate for other constructs based on similar rHVT-VP2 construction scheme or for any other recombinants, vector or not, that are expected to come in the future.

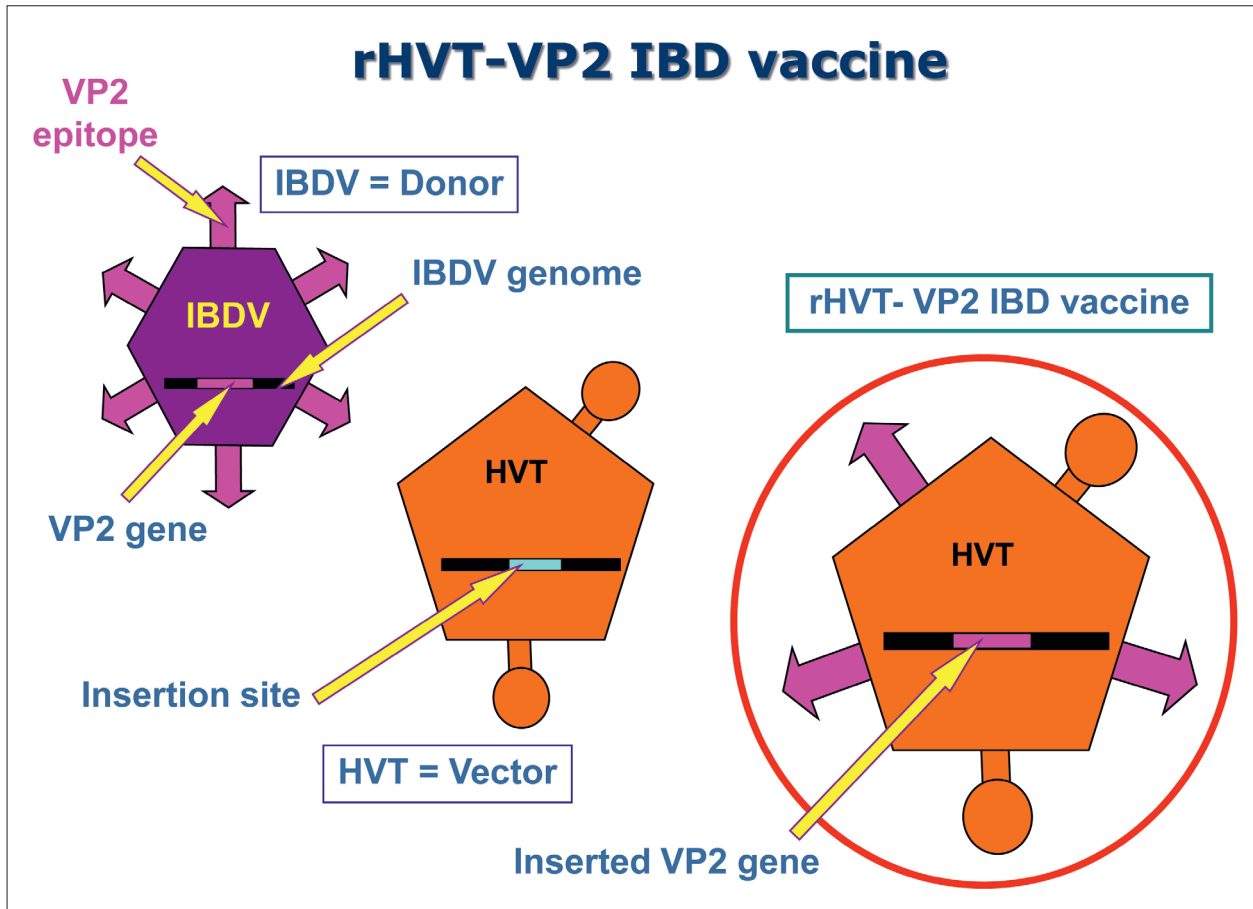
The safety of live rHVT-VP2 vector IBD vaccines is identical to the safety of the HVT vaccine, that is to say excellent. No negative effect has ever been reported in the field regarding this vaccine virus, widely used, and for almost 50 years. This safety is the really attractive feature of this category of vaccines.

Efficacy and dynamics of the immune response (see Figure 4)

Contrary to live attenuated IBD vaccines where complete protection appears within a couple of days following replication of a whole virus, protection against Gumboro Disease induced by a

Figure 4 - Protection expected when using a live Recombinant rHVT-VP2 vector type IBD vaccine.





The immune complex vaccine adapts individually to the immune status of each chicken and always replicates at the optimum time.

rHVT-VP2 vector vaccine comes from the immune response of the chicken to the VP2 antigen expressed by rHVT-VP2 infected cells. It builds up progressively from a few days to several weeks after injection, so the level of protection depends very much on the age at which chickens are challenged. Experimentally, it is easy to show that the protection level against the challenge increases over time and needs some weeks before reaching a significant level (See Experiment B, Page 29).

The other difference, when compared to live attenuated IBD vaccines, is that the development of this active immunity is not hampered by MDA, but, on the contrary, adds to the declining passive immunity, so that, at any time point, protection comes partly from MDA and partly from vaccine-induced immunity. Because of this slow onset of immunity, the presence of high, adapted passive immunity is critical and will prove to be even more important with this category of Gumboro vaccine than with live

attenuated vaccines. A slower decrease of MDA in layers, breeders and free-range chickens (because of a slower growth rate) will make this disappearance of passive immunity less problematic because MDA will ensure protection for a longer period of time and will consequently leave more time for protection from rHVT-VP2 to build up.

This compensation cannot be expected in broilers because MDA decay at a faster rate than in slow-growing chickens. For this reason, when similar live recombinant rHVT vector vaccines are used for prevention of Newcastle Disease (rHVT-F) in broilers, the slow onset of immunity is offset by application of a live attenuated ND vaccine by spray in the hatchery on the first day of age that stimulates the necessary mucosal immunity. If Newcastle Disease pressure is really strong, extra vaccination on the farm at around two weeks of age, with a less attenuated NDV vaccine strain (La Sota) is also recommended. Because of interference between MDA

and live attenuated Gumboro vaccines, a similar live vaccination in the hatchery is not feasible, but in the field, in case of high Gumboro Disease pressure, application of a live attenuated Gumboro vaccine of the Intermediate Plus type at around two weeks of age may be recommended.

Antibody response against VP2 induced by rHVT-VP2 vaccine can be detected using various serological assays, including virus neutralisation and ELISA. Interestingly, all the ELISA kits commercially available and used by the different diagnostic laboratories do not perform in the same way. In particular, the Synbiotics Proflock IBD+ ELISA kit is capable of demonstrating, at an early age, that the rHVT-VP2 vaccine actually replicates and expresses the VP2, and that the chickens produce antibodies directed against this VP2. This is an interesting, easy and cheap tool to monitor vaccination. At around four weeks of age, there is a clear difference between rHVT-VP2 vaccinated and ➔

non-vaccinated chickens.

It is important, however, to understand that positive antibody detection by the Synbiotics Proflock IBD+ ELISA kit does not mean protection and it is easy to show (See Experiments B, E and F, Pages 29, 32 & 33) that chickens that are vaccinated with rHVT-VP2 Gumboro vaccine and are Synbiotics Proflock IBD+ ELISA positive are not protected. There is only protection when the amount of antibodies against VP2 is high. A nice antibody response curve does not replace a true protection curve. Other ELISA kits (Idexx, Biochek) will not respond as quickly and as clearly as the Synbiotics Proflock IBD+ ELISA kit and will require waiting until at least six weeks of age to yield a reliable conclusion. Conversely, these kits will easily and quickly detect field infection so their use is still interesting to investigate if the vaccination with rHVT-VP2 was actually successful (no infection) or not (infection).

Another important feature of the immunity induced by this category of rHVT-VP2 Gumboro vaccine relates to the quality of the protection. Even if there is clinical protection, protection against infection is limited and vaccination does not suppress replication of the field virus in the chickens. As a consequence, virus pressure within the poultry house is maintained or even increased (See Experiments B, E and F, Pages 29, 32 & 33).

Finally, we have recently confirmed again that the level of protection induced by rHVT-VP2 vaccines is

dependent on the IBDV strain challenging the chickens. As has already been said, protection comes from stimulation of the immune system with a specific VP2 protein and not with the whole virus, making this protection more effective against field viruses carrying a similar VP2 (See Experiments E and F, Pages 32& 33). These characteristics can prove to be limiting factors in a number of situations:

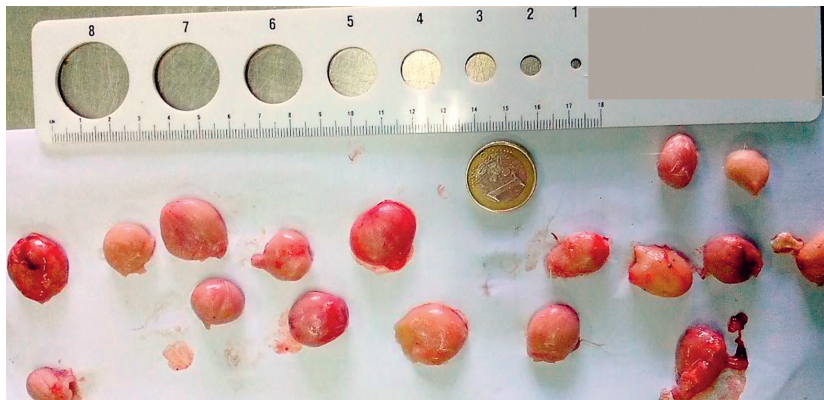
- In the short term, immunity induced by rHVT-VP2 IBD vaccines is significant when fully established against a homologous field virus, but limited due to its too slow rise ('onset of immunity') in the case of an early challenge, as observed in areas where virus pressure is high. This is why it is common to see flocks vaccinated with rHVT-VP2 vaccines showing signs of infection (observation of gross and microscopic lesions with detection of field virus by PCR in the bursa at slaughter time, or why it is commonly advised to complete the vaccination program with a live attenuated vaccine in case of high disease pressure or a risk of very virulent strains.
- In the longer term, the fact that immunity is antigen type specific and does not protect equally against all Gumboro virus strains has a negative impact on the prevention of the disease. rHVT-VP2 protects poorly against infection and shedding, and this protection is even less with some strains, favouring the emergence of new variant IBDV strains. Those strains that are less effectively addressed by the rHVT-VP2 vaccine will then replicate more in the bursa and will be shed in the environ-

ment and consequently become more prevalent over time. This phenomenon could be considered selection of Gumboro virus strains. As protection is partial, the IBDV strains that are different will escape vaccine protection and this will favour the emergence of new variant IBDV strains. These new strains will have the ability to break through MDA earlier than before, meaning that the challenge will come earlier, which will then increase virus pressure and allow Gumboro Disease out of control. The possible solutions to this drawback include the use of live attenuated Gumboro vaccine of the Intermediate Plus type for a number of cycles, or the development of a specific autogenous (or custom) vaccine for breeders that will give the progeny the specific MDA required for control of this new variant IBDV. The first solution is applied in a number of countries outside the USA, while the second is commonly used in the USA.

Conversely, these characteristics are of interest for the development of adapted passive immunity in breeding flocks. These chickens are almost always reared in high-quality environments so that the challenge is low and usually delayed until around five weeks of age or later. This allows time for the rHVT-VP2 vaccine to build up significant immunity and ensure good protection against homologous field virus.

As rHVT-VP2 vaccines do not prevent infection, breeders will also be in contact with the IBDV strains circulating in the region and will therefore produce specific antibodies against this virus and transfer adapted passive immunity to their progeny in the form of specific MDA. This transmission of locally-adapted passive immunity has been demonstrated to be highly beneficial, especially in the USA.

The last point worthy of mention regarding rHVT-VP2 vaccines is that the speed at which the HVT vector replicates after injection and the parallel expression of the VP2, which are both related to the onset of immunity, are dependent on the dose of vaccine injected. A lower dose of vaccine means delayed onset of immunity, if not a lower level. For maximum efficacy against Gumboro Disease, rHVT-VP2 vaccines need to be injected at full dose.



Bursas collected at slaughter time from chickens vaccinated with an rHVT-VP2 vaccine at the hatchery. Heterogeneity and sizes indicate earlier infection with Gumboro virus and subsequent shedding into the environment.

Guidelines for optimal control of Gumboro disease

Critical recommendations for optimal short-term and long-term control of Gumboro Disease can be divided into five sections; Biosecurity and cleaning and disinfection, passive immunity, hatchery vaccination, selection of the best program and monitoring and adaptation.



Vaccination in the hatchery is more and more replacing the classical on farm drinking water vaccination.

The Gumboro virus is extremely resistant and it is often a dream to imagine eradicating it from a farm. However, the strict application of biosecurity measures and thorough cleaning and disinfection will play a critical role in reducing virus pressure and preventing the emergence of new variant viruses. This will facilitate vaccinations and contribute actively to the success of the control program. Conversely, use of the built-up litter management system increases virus pressure (and consequently the risk of early infection and immunodepression) and favours emergence of variant viruses.

Passive Immunity

The most dramatic consequences of Gumboro Disease are observed in cases of very early challenges. Only passive immunity can cover this, highlighting its fundamental role and the need to take it

into account. Passive immunity is only composed of antibodies similar in their spectrum of protection to immunoglobulins circulating in the breeders. If we expect passive immunity present in broilers or pullets to be protective against local field IBDV strains, it is necessary to have the corresponding breeders exposed to these same strains, either by infection or by vaccination. This is why it is important to have the breeders preferably reared in the same region as the broilers or commercial layer operations. This is why it is also important not to block the susceptibility of breeders to re-infection at an early age, and to give preference to use of vaccines that will not prevent infection and shedding. Consequently, for breeder pullets (broiler breeders as well as layer breeders), use of live attenuated IBD vaccines of the Intermediate type or rHVT-VP2 vector vaccines is recommended, while the use of live attenuated

Intermediate Plus type vaccines is not, at least for an early protection.

Breeders will also be injected before the laying period with inactivated IBD vaccines containing one or more IBDV strains so that the passive protection transmitted to the progeny is high and of a broader (or more relevant) spectrum.

In the USA, the risks associated with the built-up litter system are quite well compensated for by the particular emphasis placed on a strong and comprehensive breeder program, and some companies are relying exclusively on passive protection to protect their broilers, with no broiler vaccination being applied in some cases. This is the consequence of some of the particularities of the US poultry industry and USDA regulations.

- built-up litter is the dominant management system for broiler production,
- in most poultry houses, age at infection with IBDV is between two and ➤

three weeks, i.e. an age where post-infection immunodepression would be important,

- since the mid 80's, following the Variant E IBDV, strongly immunodepressive IBDV strains have emerged and spread throughout the country to most poultry houses,
- presence of very virulent Gumboro was reported in 2008 in California and more recently in Washington State, until now, has shown no tendency to spread outside a limited territory, contrary to what has happened in other countries,
- production and use of viral autogenous (custom) vaccines is legal and routine. Because of this situation, inactivated vaccines for breeders in the USA also contain variant IBDV strains (Delaware E, A, GLS, AL-2, etc.) along with classical strains, so that the routinely-used killed IBD vaccines for breeders are multivalent.

On top of that, and in order to get the most locally adapted passive protection, research centres (like Aviserve LLC) are running so-called 'progeny challenge trials' for customers. Under controlled conditions, broilers of a quality representative from the integration are challenged with various IBDVs isolated from the farms of the integration to check the level of resistance. If protection is not satisfactory, specific custom vaccines are produced and added to the regular breeder program of the integration, in order to enlarge the spectrum of passive immunity transmitted to broilers.

Hatchery vaccination

The first and probably most important limiting factor in the success of vaccinations is the quality of their administration. No vaccine will work if not properly applied, and no flock will be protected if a significant percentage of chickens are left unvaccinated. To protect against Gumboro Disease where, most of the time, a challenge cannot be avoided, the percentage of chickens actually vaccinated in a flock must be close to 100%. This is why hatchery vaccination has become a must for genuine control of Gumboro Disease. Many years of experience have confirmed that, on the level of an organisation, vaccination through drinking water is not reliable. Field testing has frequently shown percentages of "theoretically vaccinated but actually not



In-ovo vaccination is convenient for large capacity hatcheries (Egginject - Ecat).

immunised" birds reaching 30% or above. No control program can work with such a low performance. Many poultry producers have understood this and this is why Gumboro vaccination at the hatchery has met with so much success since the introduction of Gumboro vaccines actually designed for hatchery application. Within a bit more than five years since introduction, more than 30% of the broilers produced each year worldwide are vaccinated against Gumboro at the hatchery and this percentage is constantly increasing. If hatchery vaccination is a way to reach very high percentages of immunised chickens, using subcutaneous or in-ovo injections, it does not mean that it is always perfect, and careful training and monitoring of vaccination crews are necessary to achieve optimal results. To date, only two categories of Gumboro vaccines can be used (by injection) in the hatchery, live attenuated, Intermediate Plus Type Immune

Complex vaccines and live recombinant rHVT-VP2 Type Vector vaccines. It is important to keep in mind that injection quality must be perfect for the latter category because, contrary to the former, these vaccines do not spread, meaning that a non-injected chicken will remain totally unprotected.

Careful selection of the vaccination program

Taking into consideration the information mentioned previously, as well as the specificities attached to the production conditions of breeders, layers, and broilers, the recommendations for a vaccination program that would provide optimal control of Gumboro Disease are different, and, to some extent, complementary. We now have the knowledge in our heads and the tools in our hands to decide on an optimal vaccination program to 'control' Gumboro Disease.

- For broiler breeders and layer breeders: As explained, the use of rHVT-VP2



vector vaccines is recommended for broiler breeders as well as layer breeders. For these kinds of production, biosecurity is generally high and virus pressure low, meaning that the risk of severe Gumboro problems is low. Besides, immunity induced by rHVT-VP2 Gumboro vaccines will not prevent reinfection. This will allow extra vaccination with live attenuated Gumboro vaccine of the Intermediate type and/or infection by field virus, with all these stimulations making the passive immunity transferred to progenies broader and, hopefully, better adapted to local challenges.

Late administration (at around 10 to 12 weeks of age) of an Intermediate Plus Live IBD vaccine to increase the level of circulating antibodies in the breeders and consequently the level of MDA in the progeny is advisable and is done frequently before the injection of killed IBD vaccines for a proper hyperimmunisation.

- For commercial egg layers: When it comes to biosecurity, commercial egg layers are reared as pullets in very variable environments, meaning that Gumboro virus pressure can vary a lot, as can the corresponding required levels of protection. All the various categories of Gumboro vaccines can be used for layers.

Because of their slow growth rate, the decline in MDA in these animals is slow, meaning that immunity induced by rHVT-VP2 vector vaccines can develop securely under the protection provided by passive immunity. If the challenge gets out of control and protection proves to be insufficient, it is possible to offset the known slow onset of immunity by application of a live attenuated Gumboro vaccine, preferably of the Intermediate Plus type, at around 3 to 4 weeks of age, through drinking water.

Another argument to support the use of rHVT-VP2 Gumboro vaccine in layers is that when compared to broilers, the production cycle for these animals is much longer and, consequently, the emergence of variant IBDV is less likely to occur.

To summarise, live attenuated vaccines of the Intermediate and Intermediate Plus types, presented under the 'free virus' or 'Immune Complex' forms, can be used as long as they are presented as usable in layers.

- For broilers: Production of broilers is always intensive and the levels of biosecurity, cleaning and disinfection associated with this type of production are often sub-optimal. For these reasons, virus pressure is generally high, due to various antigenic types of IBDV, and always needs to be reduced.

Because of their fast growth rate and the subsequent fast decay of MDA, the use of rHVT-VP2 is not a recommended option for broilers. Even if protection appears to be there for a few cycles, this category of vaccine cannot reliably prevent infection and shedding of the field virus. Because of this, virus pressure tends to go up, and opportunities are offered to the field viruses to mutate and escape both passive and active protection. In the long term, no progress is made towards better control of Gumboro Disease.

In broilers, the best option is to use a live attenuated Gumboro vaccine of the

Intermediate Plus type. Only this category of vaccine can bring a high level of protection against various types of IBDV and effectively prevent shedding so that virus pressure will decrease cycle after cycle, and the likelihood of emergence of variant IBDV will be prevented.

Since vaccination in the hatchery is the only method to ensure 100% vaccine coverage, then this vaccine should be presented under the Immune Complex form.

Monitoring and adaptation

As presented earlier, Gumboro Disease challenge is the consequence of many factors and can sometimes get out of control. It is then important to implement several measures to monitor the situation. Below is the list of what can be done/what can be suggested for each farm:

- Monitoring of growth performances and recording disease problem history.
- Investigation of the age at which the challenge takes place.
- Monitoring of the quality of injection of inactivated vaccines in the breeders.
- Testing of the level and homogeneity of passive immunity in chickens aged one to three days of age.
- Checking the efficacy of the passive immunity using progeny challenge trials.
- Monitoring of vaccination at the hatchery (or at farm if still done).
- Monitoring of vaccine take using histology, serology or virology (RT-PCR) at various time points, depending on the vaccines used.
- Monitoring of vaccine efficacy using histology, serology or virology at slaughter time

Vaccines and/or vaccinations may need to be changed or adapted if the situation deteriorates.

Whatever the program in place, if the virus pressure gets very high, it is then necessary to compensate for the likely protection gap. In this situation, it is always advisable to use a live attenuated Intermediate Plus type IBD vaccine presented as a 'free virus' at the 'optimal vaccination time' (see note 2, *Page 35*) for one to three cycles together with stronger cleaning and disinfection procedures. This will significantly suppress replication of the challenge virus during these cycles and will allow a return to the Immune Complex form.





EXPERIMENT A

Comparison of the protection induced by classical live IBD vaccines of the intermediate or intermediate plus types

Materials and Methods:

Three week-old SPF chickens were divided into three groups. One group was left as control while chickens from the other two were vaccinated individually by oral route with one dose of a live attenuated IBD vaccine of the Intermediate type, or of the Intermediate Plus type.

All chickens were challenged with a very virulent Gumboro virus strain of Turkish origin (D407/2/04/TR) at a dose of 10⁵ EID50 per chicken, on 2, 3, 4 and 5 days post-vaccination.

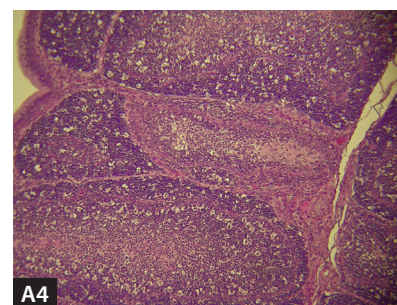
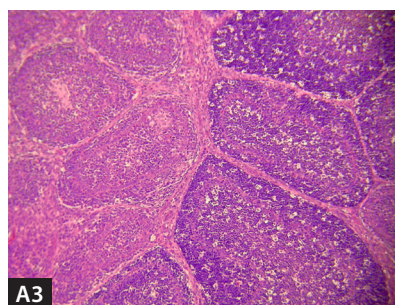
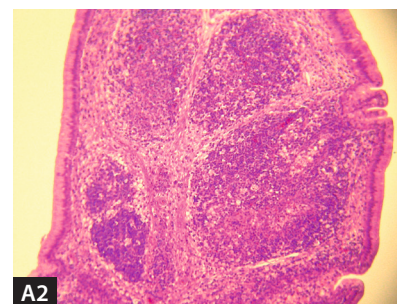
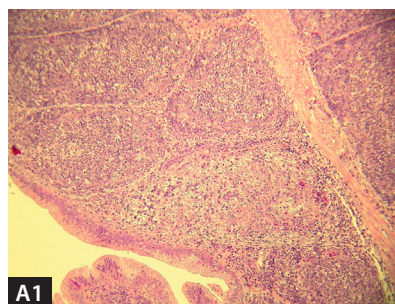
Vaccine take was assessed by histology (lesions) of the bursa of Fabricius (BF) and serology. Protection was assessed by histology of the BF, serology and virology (RT-PCR / RFLP).

Results:

100% morbidity and 30% mortality were observed in the controls but no clinical sign was recorded in any of the vaccinated chickens, whatever the vaccine used. Histology, serology and virology results are summarised in *Table A1*.

Conclusions:

A few days after vaccination of susceptible chickens with a live attenuated Intermediate Plus type IBD vaccine, col-



onisation of the BF is complete (100% of the follicles in 100% of the chickens) and protection is also complete, not only against clinical signs but also against replication of the challenge virus, which also means against shedding.

Conversely, with the Intermediate type vaccine, the vaccine virus is not detected in all bursae and even if no clinical sign

is observed, almost all bursae have lesions due to challenge, and show presence of the challenge virus. There is no protection against infection and shedding. Protection observed with an Immune Complex Live IBD vaccine would be identical to the one observed with a Classical Intermediate Plus type Live IBD vaccine because both contain a Live Intermediate Plus type IBDV.

Table A1 - Histology, serology and virology results.

Vaccine type	Days post vaccination	Vaccine take before challenge		Protection after challenge		
		histology	serology	histology	Virology	
		BF Pos./test*	Pos./test	Protected / tested	Positive to Vaccine virus	Positive to Challenge virus
Interm. Plus	2	5/5	1/5	5/5	5/5	0/5
	3	5/5	3/5	5/5	5/5	0/5
	4	4/5	4/5	5/5	5/5	0/5
	5	NT*	NT	NT	NT	NT
Interm.	2	NT	NT	NT	NT	NT
	3	4/5	0/5	0/5	1/5	4/5
	4	3/5	0/5	0/5	1/4	3/4
	5	4/5	1/5	0/5	2/5	3/5

*Pos./test = number of positive chickens / number of tested – NT = Not Tested.

(SSIU, R&D CEVA-PHYLAXIA Results)

Histological pictures:

Pictures A1 and *A2*: BF of SPF chickens vaccinated with Intermediate type Live attenuated IBD vaccine at three weeks of age (pictures taken five days post-vaccination): colonisation (i.e. protection) varies from follicle to follicle. *Picture A1*: partial colonisation. *Picture A2*: full colonisation. *Pictures A3* and *A4*: BF of SPF chickens vaccinated with Intermediate type Live attenuated IBD vaccine at three weeks of age and challenged five days after. Pictures were taken 13 days after challenge. As can be seen, the challenge virus replicated in unprotected follicles.

EXPERIMENT B

Investigation of the protection induced by an rHVT-VP2 vaccine

Materials and Methods:

Day-old broiler chickens of US origin were vaccinated in-ovo, either with a HVT+SB-1 Marek's disease vaccine combination or with rHVT-VP2 + SB-1 vaccines.

Chickens from the two groups were challenged or not (controls) with a sub-clinical Gumboro virus (US variant E) at a dose of 10^{3.5} EID50 per chicken on 2, 3, 4 and 5 weeks after vaccination. Protection was assessed by clinical observations as well as measurement of the B:BW ratios (see abbreviations and definitions on page 35), histology and virology (re-isolation of the challenge virus) of the bursae, seven days post challenge.

Results:

Results of protection regarding bursa lesions (B:BW ratios and lesions) and resistance to infection (virus re-isolation) are summarised in Figures B1 and B2.

Conclusions:

Following administration of an rHVT-VP2 vaccine, protection against challenge starts building up very soon after administration and keeps increasing for several weeks. Some protection against lesions and replication of the challenge virus can already be evidenced three weeks post-vaccination and keeps on

increasing but is still less than 100% six weeks after vaccination, which means that shedding of the challenge virus is not prevented.

Complementary investigation on the protection of the bursa:

Extra investigation has revealed that lesions of the bursa induced by challenge are better evaluated 10 days after challenge instead of seven, because intensity of the lesions keeps on increasing. Evaluation of the lesions seven days post challenge tends to give an optimistic conclusion (but this may vary according to the challenge strain used).

When looking at protection at that time and at that level, histology reveals that some follicles are protected when others are not, so that the overall picture is of the 'black and white' type corresponding to a 'plus or minus' reality for the protection (see Picture B1).

Picture B1: histological picture of BF of broiler chickens vaccinated with an rHVT-VP2 Live vector vaccine on the first day of age and challenged at four weeks of age with a vvIBDV strain. The picture has been taken 10 days post-challenge. Green circle: protected follicle - Red circle: unprotected follicle: depletion of lymphocytes.



B1

Figure B1 - Results of protection regarding bursa lesions (B:BW ratios and lesions) and resistance to infection (virus re-isolation).

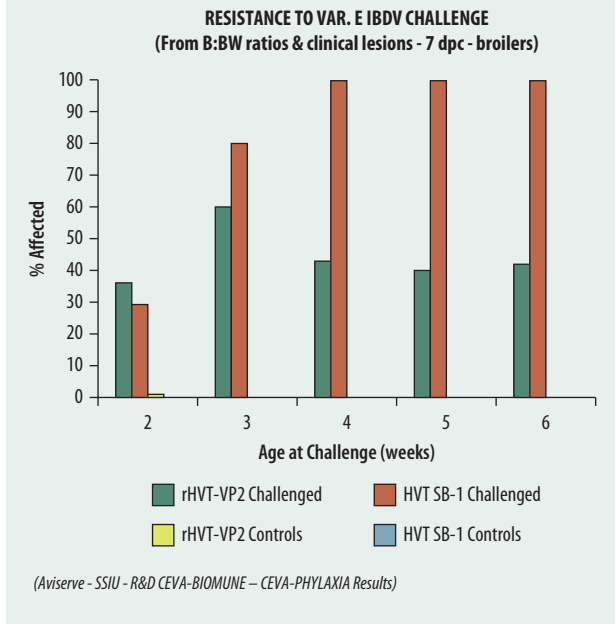
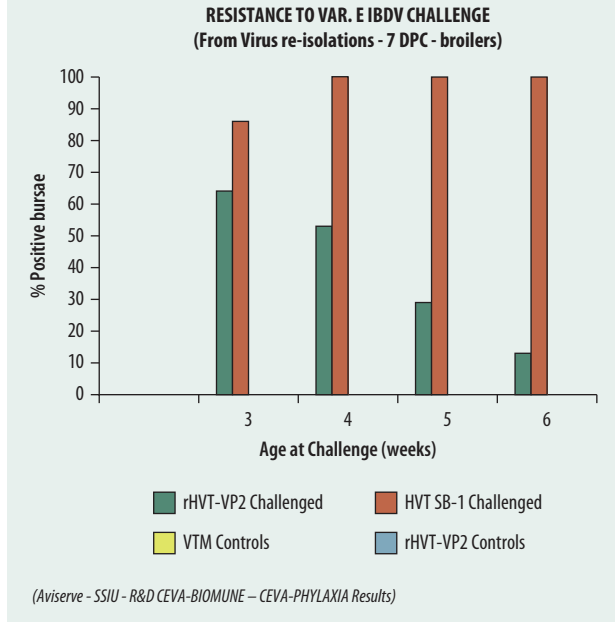


Figure B2 - Results of protection regarding bursa lesions (B:BW ratios and lesions) and resistance to infection (virus re-isolation).



EXPERIMENT C

Comparison of the protection induced by an immune complex live IBD vaccine versus an rHVT-VP2 live vector IBD vaccine

Materials and Methods:

Broiler chickens provided with passive immunity (mean titers: VN = 11.85 Log₂ ELISA = 7026) were vaccinated subcutaneously at day-old with either an Immune-complex Live IBD vaccine, or with an rHVT-VP2 Live vector IBD vaccine.

All chickens were challenged with a very virulent Gumboro virus strain of Turkish origin (D407/2/04/TR) at a dose of 10⁴ EID₅₀ per chicken at 2, 3, 4 and 5 weeks of age.

Protection was assessed by clinical observation, measurement of the B:B index (see abbreviations and definitions on page 35), histology of the BF and virology (RT-PCR/RFLP).

Results:

Results of protection against clinical signs and bursa lesions as well as against shedding are summarised in *Figures C1* and *C2*.

Conclusions:

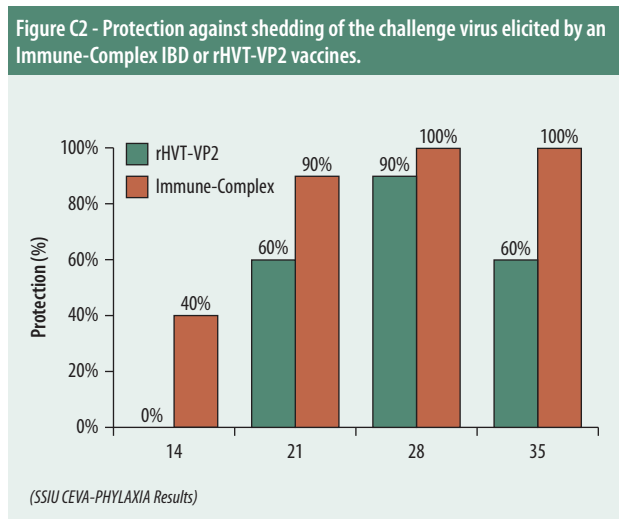
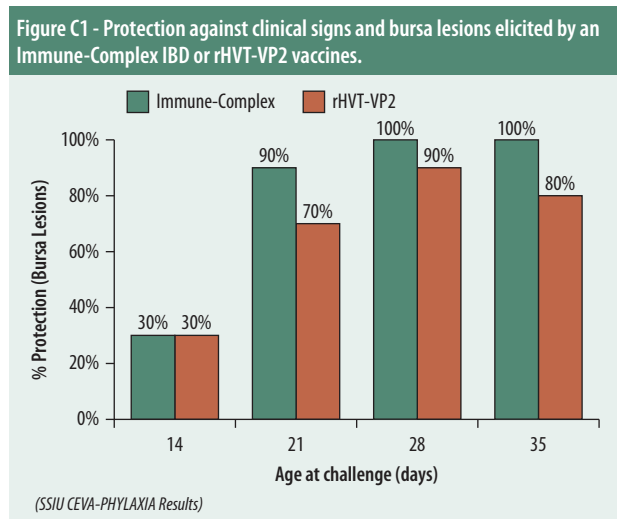
Immune Complex Live attenuated IBD as well as rHVT-VP2 Live vector IBD vaccines can 'take' in the presence of



Inside of a colony house (SSIU Ceva -Biomune, USA).

passive immunity and induce significant protection against challenge with very virulent IBDV at a high challenge dose,

but only Immune-Complex vaccine strongly prevents infection with challenge IBDV and shedding.



EXPERIMENT D

Investigation of the protection induced by intermediate plus type live attenuated IBD vaccine or an complex live IBD vaccine against challenge with different variant IBDVs

Note: this report is a brief summary of various experiments conducted to investigate this point.

Materials and Methods:

SPF or broiler chickens were vaccinated with an Intermediate Plus type Live IBD vaccine through drinking water at 14 days of age, or with an Immune Complex Live IBD vaccine by subcutaneous injection on the first day of age, or not vaccinated.

Chickens were challenged or not at six weeks of age using a Variant E (J. Rosenberger strain USA) or a variant A (Peruvian strain) at a dose of 10^4 EID₅₀ per chicken. Protection against clinical signs, bursa lesions, infection and shedding was assessed through clinical observations, histology of the BF, serology and virology (RT-PCR & RFLP) on days 4, 7 and 14 post-challenge.

Results:

Whatever the IBD vaccine used

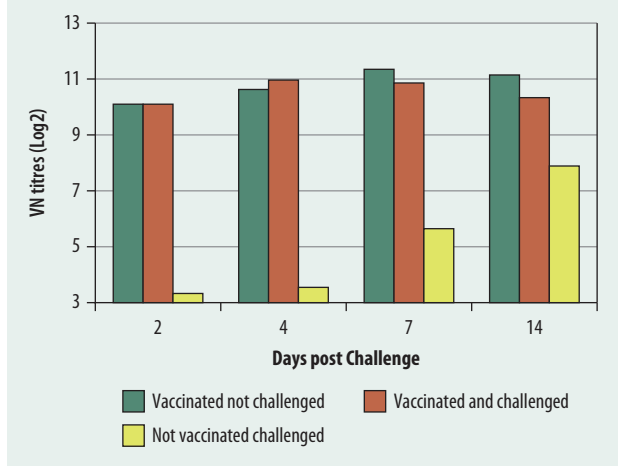
(Classical Intermediate Plus type Live or Immune-Complex) and whatever the challenge strain (US variant E strain or Peruvian variant A), no clinical sign, no lesion of the bursa indicating replication of the challenge virus, no sero-conversion after challenge, and no re-isolation of the challenge virus was detected.

Conclusions:

Immunity induced by a Classical Intermediate Plus type live attenuated or an Immune Complex IBD vaccines protects against the clinical signs and lesions associated with Gumboro Disease, as well as against replication and re-excretion of the challenge virus, whatever it is.

This protection relates to classical (sub-clinical, virulent, and very virulent) as well as to variant Gumboro Disease virus strains. The protection induced by this category of Gumboro vaccines, containing an Intermediate Plus type IBDV vaccine strain, is a truly anti-viral protection.

Figure D1 - Evolution of VN antibody titres after challenge.



Colony houses of the Scientific Support and Investigation Unit (SSIU, Ceva-Biomune, USA).

EXPERIMENT E

Investigation of the protection induced by two recombinant rHVT-VP2 live vector IBD vaccines against challenges with different variant IBDVs

Materials and Methods:

Broiler chickens (Ross 708 x Ross 708) were kept in isolation units and vaccinated with an rHVT-VP2 “A” + SB-1 or a rHVT-IBD “B” + SB-1 combined Marek’s disease + IBD vaccines, or just with a HVT + SB-1 Marek’s disease



Isolated units (SSIU, Ceva-Phylaxia).

vaccine. Chickens were challenged or not (Controls) at five weeks of age with $10^{3.5}$ EID₅₀ per chicken of either a Delaware Var. E type IBDV or another variant strain isolated by Aviserve LLC and named AVS-EL. Protection was assessed using clinical observations as well as lesions of the BF and measurement of the Bursa: Body Weight (B:BW) ratio 10 days after challenge.

Note: replication of the challenge virus in the BF induces depletion of the follicles and decreasing of the weight of the BF i.e. decrease of the B:BW ratios. In this experiment, the protection limit was set at the value of Mean B:BW ratios of the unchallenged controls minus two standard deviations = 0.93

Results:

Results of measurements of B:BW ratios

are presented in *Figures E1* and *E2* and *Table E1*.

Conclusions:

Vaccination with two Recombinant rHVT-VP2 Live vector IBD vaccines constructed with different insertions sites, different VP2 inserts (donated by different IBDVs: classical IBDV and variant E IBDV) and using different promoters, induced significant and similar levels of protection against challenge with Delaware E variant IBDV administered five weeks after vaccination (74% and 73%), but replication of the challenge virus could not be prevented in all chickens.

Conversely, protection rates against a different variant IBDV (AVS –EL) applied at the same dose and at the same age, were also similar but very low.

Challenge virus	Vaccine		
	rHVT-VP2 “A”	rHVT-VP2 “B”	Controls
Delaware E variant IBDV	74 %	73 %	0 %
AVS-EL variant IBDV	36 %	33 %	0 %

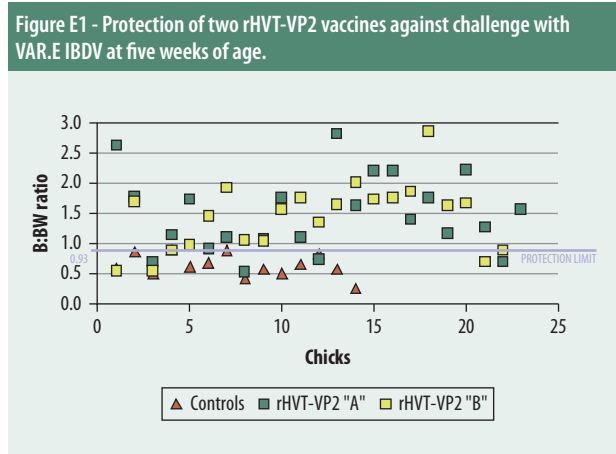


Figure E1 - Protection of two rHVT-VP2 vaccines against challenge with VAR.E IBDV at five weeks of age.

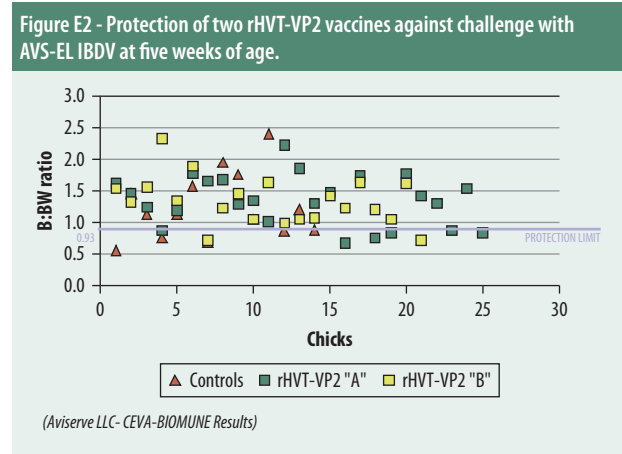


Figure E2 - Protection of two rHVT-VP2 vaccines against challenge with AVS-EL IBDV at five weeks of age.

(Aviserve LLC- CEVA-BIOMUNE Results)

EXPERIMENT F

Comparison of the protections induced by an immune complex or rHVT-VP2 Gumboro vaccines against challenges with various IBDVs

Materials and Methods:

One day-old commercial broilers (Ross 308) with MDA to IBDV (mean VN titer = 3394 – mean Biochek ELISA titer = 5420) were divided into three groups. Two groups were vaccinated subcutaneously on the same day either with an Immune Complex Live IBD vaccine, or with a Recombinant rHVT-VP2 Live vector IBD vaccine constructed with a VP2 gene insert donated by a classical type IBDV (Faragher 52/70). The third group was left unvaccinated (controls). On day 28, subgroups from the three groups were challenged with 10^4 EID50 per chicken of various Gumboro viruses from different countries and different pathotypes or genotypes:

- a very virulent IBDV from Turkey (D407/02/04 TR) – abbreviated as: TR
- a subclinical IBDV from USA (Delaware E) – abbreviated as: Del.E
- 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 subclinical IBDV from USA (AVS-EL) – abbreviated as: AVS-EL

Protection against infection or against bursa lesions were evaluated four and 14 days post challenge from histology of the bursae (acute lesions and percentage of affected follicles), as well as from various other data including B:BW ratios, B:B index, antibody response and virology (PCR).

Serology (VN and ELISA with Biochek, Idexx, Idexx X-R and Synbiotics Proflock IBD+ kits) was used to detect antibody response to vaccination as well as possible booster effect of challenge, indicating no or partial protection



One day-old broilers were vaccinated subcutaneously with either an Immune Complex Live IBD vaccine or with a Recombinant rHVT-VP2 Live vector IBD vaccine.

Table F1 - Mean antibody titers before and after challenge using ELISA (Biochek).

In each cell, Mean titer - Number of ELISA positive samples / number of samples tested.

Challenge groups	Before challenge (day 28)			After challenge (day 42)		
	Im. Cplex	rHVT-VP2	Controls	Im. Cplex	rHVT-VP2	Controls
TR	5 815 - 20/20	419 - 8/20	157 - 1/20	8 116 - 10/10	3 296 - 10/10	8 212 - 10/10
Del. E	5 794 - 20/20	432 - 5/20	167 - 1/20	6 514 - 10/10	7 397 - 10/10	7 825 - 10/10
MX	6 665 - 20/20	357 - 5/20	181 - 1/20	7 064 - 10/10	5 884 - 10/10	6 693 - 10/10
ZA	6 442 - 20/20	619 - 11/20	141 - 1/20	6 444 - 10/10	4 281 - 10/10	6 939 - 10/10
BR	6 354 - 20/20	642 - 11/20	213 - 5/20	7 126 - 10/10	4 811 - 10/10	6 438 - 10/10
AVS-EL	6 360 - 20/20	411 - 4/20	132 - 1/20	6 586 - 10/10	7 023 - 10/10	7 067 - 10/10

(SSIU, CEVA-PHYLAXIA Results)

against infection.

A chicken was considered as 'fully protected' if less than 10% of the follicles of its bursa were showing acute lesions.

Results:

The replication of both vaccines was confirmed by histology and antibody response for the Immune Complex as well as by PCR and antibody response for the rHVT-VP2 vaccines.

Before challenge, using Biochek ELISA, some controls were detected positive with low titers, because of residual MDA. All chickens vaccinated with the

Immune Complex were positive with high titers when only a limited percentage of chickens vaccinated with rHVT-VP2 vaccine were positive with low to moderate titers, see *Table F1*.

After challenge, ELISA antibody titers in Immune Complex vaccinated chickens did not change significantly. Conversely, a clear rise in titers accompanied by a clear increase in the percentages of positive samples was observed in all rHVT-VP2 vaccinated groups as well as in the controls.

Following challenge, all bursas from Immune Complex vaccinated →

Figure F1 - Protection of the bursa elicited by an Immune-Complex IBD or a rHVT-VP2 vaccine according to histopathology lesions four days post challenge with different IBDV strains.

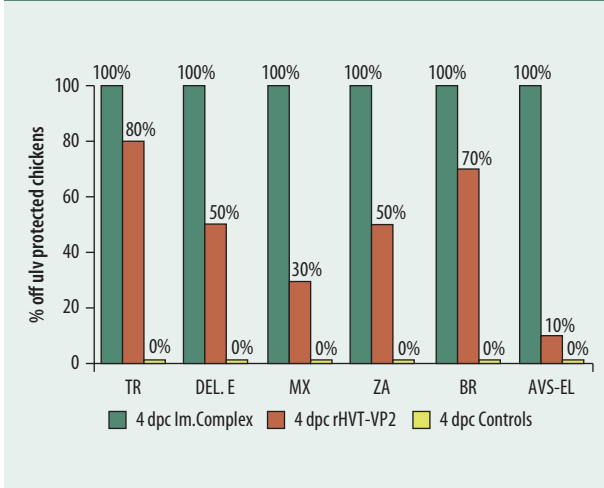
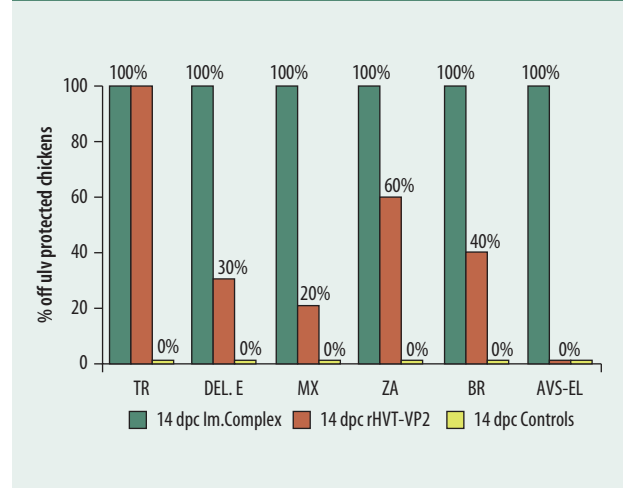


Figure F2 - Protection of the bursa elicited by an Immune-Complex IBD or a rHVT-VP2 vaccine according to histopathology lesions 14 days post challenge with different IBDV strains.



chickens showed chronic signs of bursitis caused by replication of the vaccine virus, but no sign of acute lesion that would indicate replication of the challenge virus was detected. Conversely, 14 days after challenge, all bursas showed signs of regeneration with 70% of them showing regeneration qualified as 'remarkable'.

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

The picture became even worse in most of the groups from bursas collected 14 days after challenge. Fully protected chickens were then 50% or less in almost all the groups, with absolutely no protection against the AVS-EL strain originating from the USA.

Interestingly, the best protection was recorded against the very virulent Turkish strain (TR), which is antigenically and genetically close to the Faragher 52/70 IBDV strain which served as donor for the VP2 gene insert used for the construction of this rHVT-VP2 vaccine.

Conclusions:

An Immune Complex Live IBD vaccine is able to induce full protection against a wide range of Gumboro virus strains. The protection is against clinical signs as well as replication of the challenge virus and shedding so that this category of vaccine can really be considered as a tool not only to protect the chickens, but to really control the disease. Conversely, rHVT-VP2 vaccine showed good efficacy against Gumboro virus homologous to the virus strain that donated the VP2 gene, but poor to very poor performance against strains of a different origin.



Variable aspects of the bursas in a flock with sub-clinical IBD.

¹⁾ In this document, and contrary to other authors, we preferred to use the word 'immunodepression' instead of 'immunosuppression', because we believe that the reduction of the immune defence functions and capacities that can be seen in the field following infection by IBDV does not affect all parts of the immune system, either equally or otherwise. Immunosuppression corresponds to the destruction ('suppression') of all immune capacities of the chicken. It is a very drastic condition that can be seen in a limited number of extreme situations (like after using drugs or radiation) and the corresponding word should be kept for these situations.

²⁾ 'Optimal timing for vaccination' can be determined by using the well-known Kouwenhoven or Deventer formula on ELISA testing results from blood samples collected from young chickens on day 1-3.

Abbreviations and Definitions:

BF: Bursa of Fabricius

BB index: BB Index = B:BW ratio of the tested chicken / mean B:BW ratio of the controls.

B:BW ratio: Bursa Body Weight Ratio = bursa weight (mg) / body weight (g)

DPC or dpc: Days Post Challenge

IBD: Infectious Bursal Disease (also named Gumboro Disease)

IBDV: Infectious Bursal Disease Virus

MDA: Maternally Derived Antibodies

rHVT: recombinant vaccine based on the Herpes Virus of Turkey.

rHVT-F: rHVT vector vaccine containing an inserted gene encoding for the "F" (Fusion) protein of NDV (Newcastle Disease Virus), designed to induce protection against the Newcastle Disease (ND).

rHVT-VP2: rHVT vector vaccine containing an inserted gene encoding for the "VP2" (Viral Protein 2) of IBDV, designed to induce protection against Gumboro Disease.

SSIU: Scientific Support and Investigation Unit

VN: Virus Neutralisation

References:

References indicated in this article are available upon request.

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