

CEVA GLOBAL REFERENCE IN IBD CONTROL

270 BILLION BIRDS PROTECTED AGAINST

GUMBORO DISEASE WITH

CEVA VACCINES





MORE than
1000 HATCHERIES in
91 COUNTRIES using
CEVA VACCINES

Supported by.



Ceva A nimal Health m akes available the C.H.I.C.K ProgramQ uality Code of Practice to their affiliates for the control of good hatche ry v accination practices in poultry.

FOREWORD

Ceva Santé Animale has been contributing to the control of **Gumboro disease (IBD)** since its foundation, accumulating expertise and developing a portfolio for the poultry industry, including vaccines such as **CEVAC IBDL**®, **TRANSMUNE®** and recent new developments like **NEXTMUNE®**.

Ceva has protected, since 2006, over **270 billion broilers** against Gumboro Disease, acquiring a large expertise and knowledge.

In this book you will find a technical & economical summary of **NEXTMUNE®**, containing all the concepts required to understand IBD control.

In a recent IBD Survey conducted during 2023-2024, independent researchers and Ceva Santé Animale Veterinary Specialists have gained a deeper understanding of the evolution of IBDV around the world.

We have analyzed these complex situations using real world evidence tools in regions like South & Central America, Europe, Middle East, Europe, Africa, and Asia. Our experiences include an updated explanation of the dynamics of IBDV and **NEXTMUNE®**, economic calculations based on a specific and updated scale, suggesting the value of using **NEXTMUNE®**. These field cases compare **NEXTMUNE®** (administered either subcutaneously or in-ovo) versus different vaccination programs.

Ceva Santé Animale is committed to sharing information and updated scientific data with partners and customers.

Please contact us should you require further information or explanation.

Enjoy your reading.

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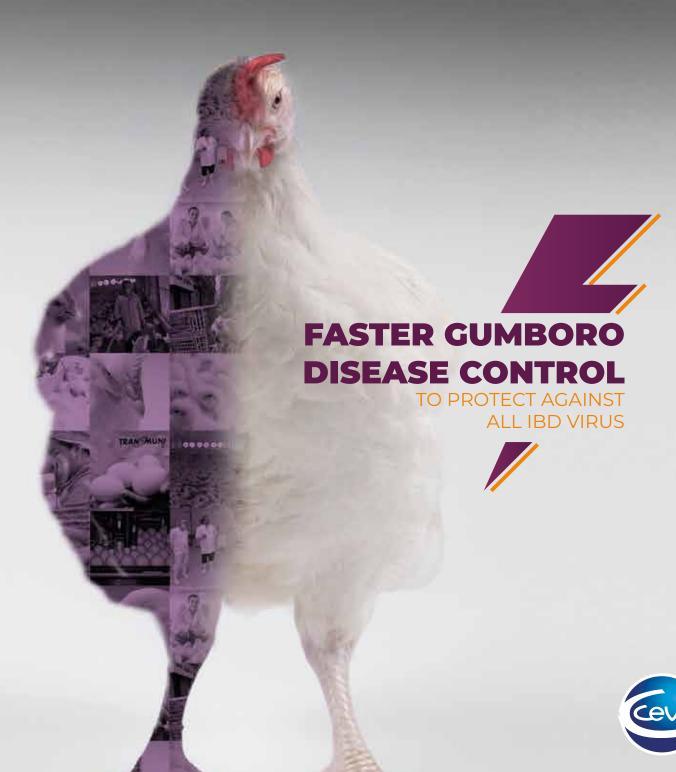






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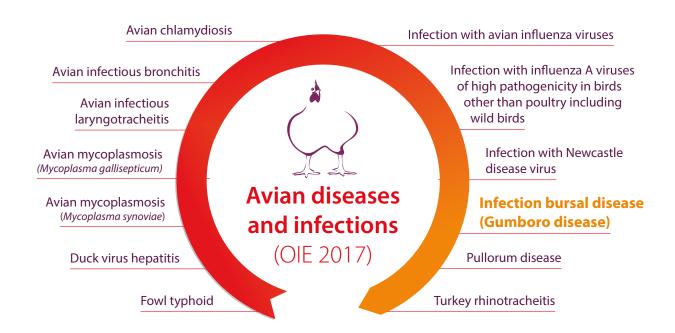




INFECTIOUS BURSAL DISEASE:

a threat to the poultry industry

Infectious Bursal Disease (IBD), (mostly known as Gumboro disease), is still very much present and ranks among the top five diseases in almost all countries globally. The World Organisation for Animal Health (OIE) listed IBD as a notifiable disease with importance in 2017.



One of the reasons for the worldwide distribution of the IBD virus is that it is very persistent, surviving in poultry houses in the absence of chickens during downtime periods. The industry is concerned about the immunosuppressive effects of IBD, and the development of safe and effective vaccines is of utmost importance to overcome its detrimental effects



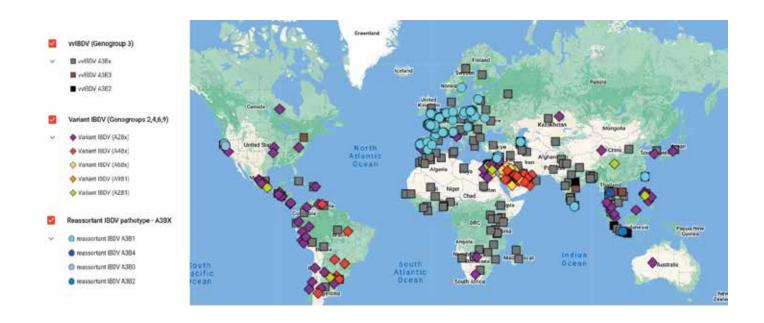


Figure 1. Worldwide IBDV presence (Ceva data 2024)

After the initial outbreak in the US, clinical IBD was reported in many other countries in the 1960s and 1970s. More virulent forms were later reported around the same time with a very immunosuppressive form of IBD in the USA (starting around 1985), which later spread to Central and South America (Figure 1). The very virulent (vvIBDV) form of IBD spread around the same time to Western Europe, Northern Africa, the Middle East, and Asia. In the late 1990's and early 2000's, the very virulent form spread to Central, South America and California. Today, the very virulent form is predominantly present worldwide, although variant IBDV strains which lead to a subclinical form of the disease are also very present regionally, especially the Americas and the Middle East.

The cost of IBD has been very well described in different publications in the last decade. It has a direct impact on mortality, ranging from 5% to 30%, depending on the degree of protection of the birds and the pathotype of the disease (Rosenberger et al., 1986; Van den Berg, 1991). In subclinical cases, it can reduce the income per flock up to 14%, with a 11% reduction in yield and 10% profit reduction due to weight loss and increased FCR (McIlroy, 1992). In layers, it can even reach mortality rates of 60% (Nunoya, 1992).

More recently, interesting calculations have been performed in North America with focus on the subclinical IBD variant strains.



Figure 2. Rejected livers with hepatitis in the processing plant (Ceva Animal Health)





Infectious bursal disease (IBD) is a viral disease that affects chickens worldwide and it has a major economic impact on the modern poultry industry

• It is caused by an **Avibirnavirus**, called infectious bursal disease virus (IBDV), which is a bi-segmented, double stranded RNA virus, highly resistant in the environment. The RNA encodes for five viral proteins (VP1 to VP5) of which VP2 contains hypervariable portions that enable classifying strains into various antigenic and genetic groups. In addition, VP2 contains most neutralizing sites. Depending on pathotype, IBDV strains are classified as very virulent, virulent, and subclinical.

Antigenically, two serotypes (serotype 1 and 2) of IBDV exist, although antigenic variants are described within serotype 1. Serotype 1 strains are the ones that affect domestic poultry of the Gallus gallus species. There have been, however, description of non-clinical serotype 1 passages in turkeys, for example, making accidental carriers an important factor for the spreading of the disease.

 Only the serotype 1 virus is pathogenic for chickens, causing depression, diarrhea, hemorrhages in muscles and proventriculus, and inflammation, necrosis, and atrophy of the bursa of Fabricius, depending on the virulence of the strain. Variant IBDV strains generally do not cause clinical signs or mortality but can cause severe atrophy of the bursa without marked external pathological signs.



Depending on the age of the birds at infection and their immune status, IBDV can also induce immunosuppression. Recent reports confirmed the presence of new IBD variants in various parts of the world, namely South America, North America, Africa, China, the Middle East, and Europe. Therefore, IBD control is a global concern.



Figure 3. Clinical signs of depression are not always visible, and diagnosis might be complicated.



Avibirnavirus replicates and damages the bursa of Fabricius and other organs in the domestic chicken fowl. It is a disease which basically presents: **immunosuppressive/subclinical** and clinical.

The immunosuppressive-subclinical form is the consequence of the infection of chickens aged less than 2 weeks of age. 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. There is occurrence of typical clinical signs (hence the term 'sub-clinical') or direct mortality. The bursa of Fabricius might show signs of variable intensity and persistence. Most of the time, only poor or sub-optimal performances are detected.

The clinical form is the consequence of infection of chickens with an IBD virus that replicates very rapidly and at a high level increasing the mortality rate. Clinical signs may or may not be present. Post-mortem examinations describe a strong edema of the bursa and, sometimes hemorrhages of variable intensity that can also be seen in the form of petechiae or suffusions in the thighs and breast muscles. The 'very virulent' or 'hypervirulent' (vvIBDV) cases of Gumboro disease that were reported in Western Europe in the late 1980s and still present in other parts of the world, show this clinical form as well. The mortality rate a lot but it is generally higher in slow growing chickens like layer pullets, layer/broiler breeder pullets or organic chickens (generally more than varies 25%) than in broilers (in general less than 15%).

The sub-clinical (or economical) form of the disease corresponds to infection of chickens after 2-3 weeks of age, by an IBD virus without occurrence of typical clinical signs (hence the term 'sub-clinical') or direct mortality. The bursa of Fabricius might show signs of variable intensity and persistence. Most of the time, only poor or sub-optimal performances are detected.





Biosecurity is key in preventing IBD. However, vaccination is regarded as the key tool to complement biosecurity efforts.

Parent stock vaccination is useful to elicit humoral immunity that will be transmitted to the progeny (passive immunity); however, it will only protect the young chicks for the first few weeks of life. Live vaccination is applied in the progeny because passive immunity does not last long enough to ensure adequate protection for the whole broilers' lifespan (level of MDA will impact on passive immunity life). By doing so, chickens develop an active immunity.

Historically, vaccination has been applied in the farm via drinking water using live attenuated vaccines of various residual pathogenicity (mild, intermediate, intermediate plus, hot). Hatchery vaccination is becoming a growing trend because of the increasing capacity of poultry producing companies, a higher degree of integration, and the willingness to better master the vaccine application using automated equipment, either by day-old injection via subcutaneous route, or by in-ovo route. High-throughput automated equipment is now available (in-ovo or day-old injection) enabling a higher quality, consistency, and cost-effective vaccination. In addition, hatchery vaccination removes the risk of vaccination failure in the field (due to poor water quality, inappropriate timing, and insufficient flock coverage); it also prevents the stress of water withdrawal in the farm when using the classical drinking water method. Consequently, applying vaccination in the hatchery improves the welfare of the birds in the farm. Altogether, it offers a significant improvement in terms of immunization success. Advances in technology have enabled the development of new vaccines that are able to escape the neutralizing effect of maternally derived antibodies (MDA) and hence are eligible for hatchery application.



Three types of IBD vaccines are currently available for hatchery application in several parts of the globe:

- An immune-complex vaccine in which a live attenuated IBD vaccine strain has been in-vitro complexed with a high titre polyclonal serum in specific quantities.
- A vaccine using the herpesvirus of turkeys (HVT) vector technology as a carrier expressing IBDV's VP2 antigen.
- A third type of less attenuated, naked viruses or «hot strains», used as vaccines.







As the Gumboro virus is a very persistent virus, in many cases it is already present in the farm, leading to a field challenge when new birds are placed.

Although the characteristics of a field challenge in chickens (age of birds, severity, consequences, etc), vary from house to house, challenge finally occurs. In these cases, vaccination aim at both protection of the chickens and IBDV spreading control.

When considering a proper IBDV vaccination program, the main objectives must be:

- Ensure continuous protection of the chickens against infection, or "Prevention of Infection"
- Protect against the clinical signs of infection or "Clinical Protection"
- Prevent or significantly reduce the amount of virus shed after challenge or "Reduction of shedding of IBDV"
- Prevent the build-up of a higher virus pressure, production cycle after production cycle,
- Prevent the evolution of the disease towards a nastier virus which could escape the preventative program.

The last two points are important for the reduction of the shedding of IBDV since the goal of a strong Gumboro vaccination program should be to stop the Gumboro cycle.

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Molecular characterization and pathogenicity of very virulent infectious bursal disease virus isolated from naturally infected turkey poults in Egypt.

Samah M Mosad, Abdelfattah H Eladl, Mohamed El-Tholoth, Hanaa S Ali, Mohamed F Hamed. Trop Anim Health Prod. 2020 Nov;52(6):3819-3831. doi: 10.1007/s11250-020-02420-5. Epub 2020 Oct 1.





INTRODUCTION TO INFECTIOUS BURSAL DISEASE:

Why controlling IBDV?

Despite the wide use of vaccines and increased biosecurity, IBDV is still very much present and ranks among the top five diseases in almost all countries globally. One of the reasons for this dominance is that the Gumboro disease virus is a very persistent virus, surviving in poultry houses during downtime periods for a long time. IBDV is ubiquitous and resident in the farm and, once infection takes place, it rapidly overtakes the immune system in less than 12-14 hours post-infection, targeting especially the Bursa of Fabricius and other organs from the lumen (namely BALT, GALT, thymus, harderian glands, spleen, heart, blood, ceca tonsils, for example). Some immune organs are colonized by IBDV, but the main infection occurs in the bursa of Fabricius. After the acute IBD phase, the bursa is repopulated with lymphocytes B, migrating from other immune organs in the bird. This phase is called "regeneration".

As a result, the difference between the detected IBDV pathotypes is remarkable, especially regarding age and mechanism of infection, viral dynamics and spread within the farm.

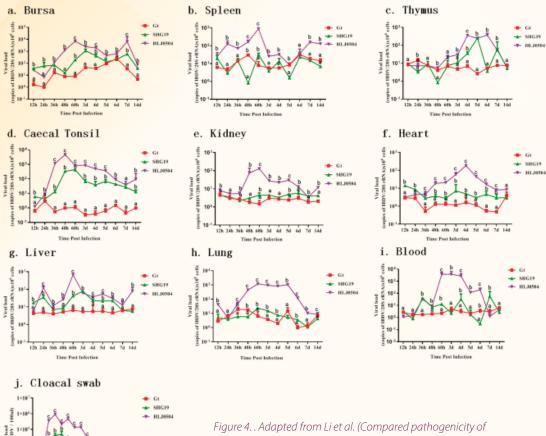


Figure 4. . Adapted from Li et al. (Compared pathogenicity of three strains of infectious bursal disease virus closely related to poultry industry)



There are different classifications for IBDV, by genotype, pathotype and serotype, as explained before in the chapter 1. To understand vaccination, we need first to understand the mechanism of action of the IBDV, depending on the pathotype.

We have identified 3 main pathotypes of IBDVs globally (see map in chapter 1):

Very virulent IBDV. This pathotype creates explosive, fast-evolving outbreaks with high mortality peaks around 20-35 days of age. The bursa becomes edematous, congested, and bloody. Fibrine can also be present during and after the acute phase.

Variant IBDV. This pathotype is a variation of the so-called "classic IBDV strain" and affects the birds during the first 2-3 weeks of life, especially if they are not protected. So it requires a proper "variant" IBD vaccination of the corresponding breeder flock to protect the progeny (chicks) with enough maternally derived antibodies, at least until vaccination elicits an adaptative immune response.

Reassortant nvIBDV (or "new variants-reassortants"). Generally, less visible for the diagnostic and less pathogenic than vvIBDV, these are strains are generated by events including exchange of segments A and B of a wild and vaccinal IBDV, creating sometimes new features, characteristics and therefore helping the virus to adapt to their host and environment. These strains are difficult to distinguish clinically but drop the performance of the flocks remarkably, a situation where protection becomes especially important.

The main objective of a proper vaccination program is to stop the Gumboro cycle and avoid the colonization, replication and spread of a field IBDV within the same farm and surrounding premises.







Worldwide IBD surveys

Ceva Santé Animale Veterinary Service Specialists, in cooperation with independent researchers and external laboratories have conducted IBD studies since 2019 with the objective of updating the IBDV situation around the world.

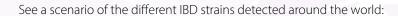


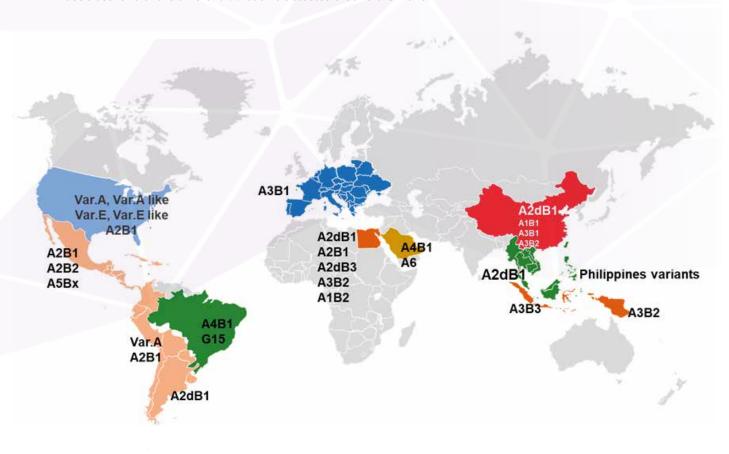
Figure 1. Worldwide IBDV presence (Ceva data 2024)

In the evolution of the knowledge a new IBD strains classification was suggested by Islam et al, 2021. The classification takes in account the segment A (VP2) and segment B (VP1) to determine the genotype:

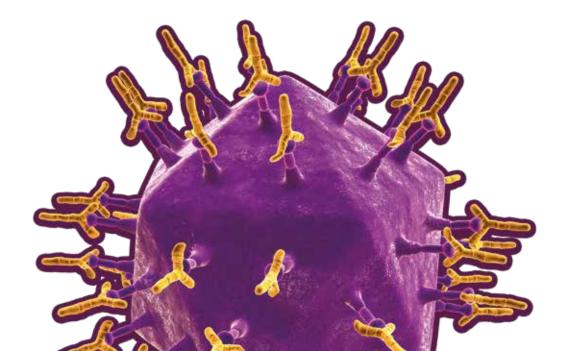
VP2 GENOGROUP	VP1 GENOGROUP	GENOTYPE	
	B1 CLASSICAL-LIKE	A1B1	
A1 CLASSICAL	B2 VERY VIRULENT-LIKE	A1B2	
	B3 EARLY AUSTRALIAN-LIKE	A1B3	
A2 US ANTIGENIC VARIANT	B1 CLASSICAL-LIKE	A2B1	
	B1 CLASSICAL-LIKE	A3B1	
	B2 VERY VIRULENT-LIKE	A3B2	
A3 VERY VIRULENT	B3 EARLY AUSTRALIAN-LIKE	A3B3	
	B4 POLISH & TANZANIAN	A3B4	
	B5 NIGERIAN	A3B5	
A4 DISTINCT IBDV	B1 CLASSICAL-LIKE	A4B1	
A5 MEXICAN	N.A.	A5BX	
A6 ITALIAN	B3 CLASSIC-LIKE	A6B1	
A7 EARLY AUSTRALIAN	B3 EARLY AUSTRALIAN-LIKE	A7B3	
A8 AUSTRALIAN VARIANT	B3 EARLY AUSTRALIAN-LIKE	A8B3	
A9 PORTUGUESE	B1 CLASSICAL-LIKE	A9B3	
A0 SEROTYPE 2	B1 CLASSICAL-LIKE	A0B1	







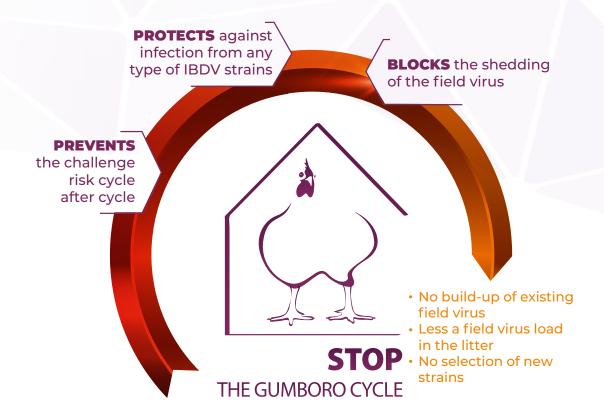
In the next pages, you will access the results of several studies which demonstrate the presence of different field strains in Europe, Middle East, Asia, America and Africa.



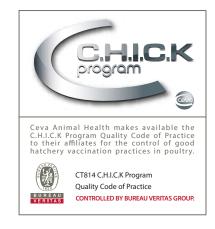


The strategy designed by Ceva focuses on the 3 following steps. If these 3 points are not well addressed, the virus will accumulate in the farm and will escape from the immune response. These 3 steps are:

Vaccination and proper immune response development: choosing the right vaccine and demonstrating that the vaccine protects the immune system of the birds is the most important factor to protect against IBDV.



C.H.I.C.K. program: Ensuring an appropriate vaccination quality administration is key for a vaccine to be effective. The preparation and application of the vaccine in the hatchery are definitely the most important factors to immunize the target bird population.





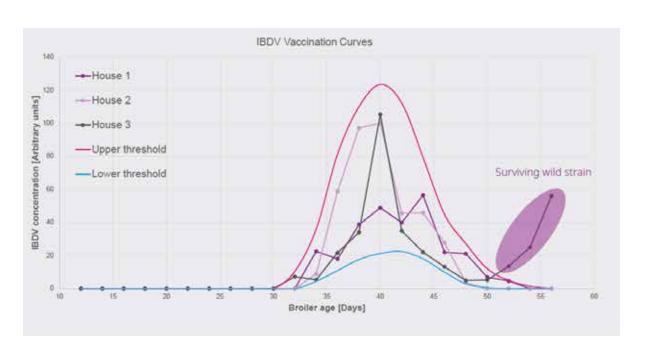
Investigate the farm and surrounding environment correctly: IBDV accumulates in the farm and environment, where it can remain for months. It can accumulate in organic material, cracks, surrounding areas, dust, etc. Therefore, reducing the amount of virus in the farm will remarkably help to win the "race" against a foreign IBDV strain present in the environment and the risk of infection. The Ceva Teams have been trained in defining an adapting a cleaning and disinfection (C&D) protocol.

How can we monitor the environment and define if our C&D protocol is effective against IBD?

- 1. It is recommended to sample the litter and environment **after C&D** to calculate the amount of IBD viruses present and the effectiveness of the C&D process:
- 2. Use sampling techniques which could detect the virus during down-time (after 1st cycle). These techniques include sampling techniques such as: swabs, dry cloth with disinfectant's neutraliser, ATP-meters.
- **3.** Define a protocol for IBDV sampling after cleaning and disinfection, right before 1st day old chick placement (1st cycle). Number of samples & places to check.
- 4. Repeat the same process in the next cycle.

Results (after 3-4 cycles): W2512 shall be the most prevalent strain if your vaccination if C&D was properly applied.

If this is not achieved, wild IBDV presence would escalate and produce what is known as "elevator effect", infecting the next batch of birds as soon as they arrive in the "clean house".







Monitoring IBD (GPS – Global Protection Services)

Ceva perform three main activities in the field to support our customers, called "GPS – Global Protection Services".

GPS includes all investigation and diagnostic activities in the frame of troubleshooting, disease analysis in the field ("awareness of the disease"), monitoring vaccine application and the immune response of the birds.

In this chapter will be described how to monitor the different vaccination programs that can be applied against IBD, monitor vaccination quality and the immune response of the birds to the vaccine.

The use of NEXTMUNE® and the outstanding Ceva services team up for a perfect combination to stop the Gumboro cycle.

Thanks to our long experience in collaborating with our customers, Ceva is proud to offer an outstanding range of services from the hatchery to the further processing, namely: Ceva Hatchery Immunization Control Keys (C.H.I.C.K.) program®



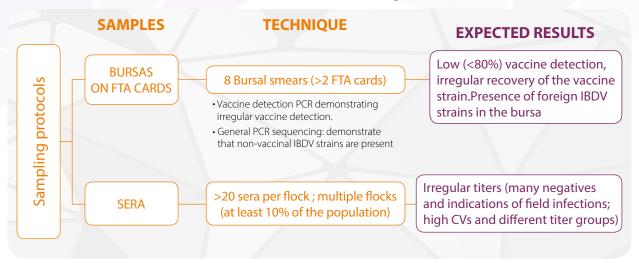




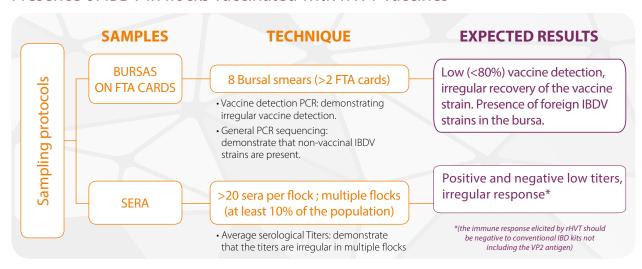
Conducting IBD surveys is key to understand the extension and disease challenge in poultry production.

In Ceva, we always conduct epidemiological IBD surveys to recognize the potential impact of the disease in the operations of our customers. First, we focus on the real problems of our customers in a specific setting and, once that they are correctly identified, we discuss different solutions to address them. This is how we believe that our solutions should be implemented. To accomplish this task, we propose the following diagrams that explain the different approach according to each real situation on the field

Presence of IBDV in flocks vaccinated with drinking water vaccines

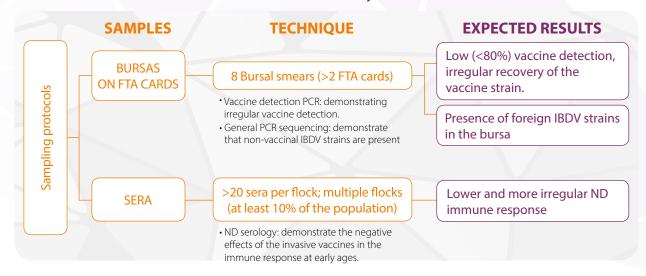


Presence of IBDV in flocks vaccinated with rHVT vaccines





Presence of IBDV in flocks vaccinated with very invasive vaccine strains



Presence of IBDV in flocks vaccinated with other immune-complex vaccines







Monitoring NEXTMUNE®

After monitoring millions of broilers vaccinated with **Nextmune®** arounde the world, CEVA has developed the following sampling guidelines.

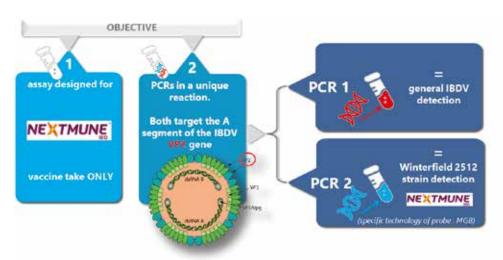
Summary of sampling guidelines:

DISEASE	TARGET	SAMPLES		# SAMPLES/FLOCK					
DISEASE		AGE	ТҮРЕ	<3 HOUSES	BETWEEN 4-10 HOUSES	MORE THAN 10 HOUSES	POOLING?	КІТ	EXPECTED RESULTS
	VACCINATION QUALITY	28-35 DAYS	BURSA OF FABRICIUS	2 FTA CARDS (8 BIRDS)	3 FTA CARDS (12 BIRDS)	5 FTA CARDS (20 BIRDS)	NOT ALLOWED ONLY INDIVIDUAL SAMPLES	PCR DUPLEX	POSITIVITY
IBD	MONITORING IMMUNE RESPONSE	32-35 DAYS	SERUM	20 SERA	20 SERA (4-5 FLOCKS)	20 SERA	NOT ALLOWED ONLY INDIVIDUAL SAMPLES	• IBD CONVENTIONAL ELISA (IDEXX, BIOCHEK) • IBDV ELISA IDVET • VP2 IBD ELISA (IDVET, BIOCHEK)	POSITIVITY AT 32-35 DAYS OF AGE

Assessing vaccination quality of **NEXTMUNE®** becomes easier now with the new **DUPLEX PCR** assay designed by our Scientific Support and Investigation Units, which is highly sensitive to samples containing W2512.

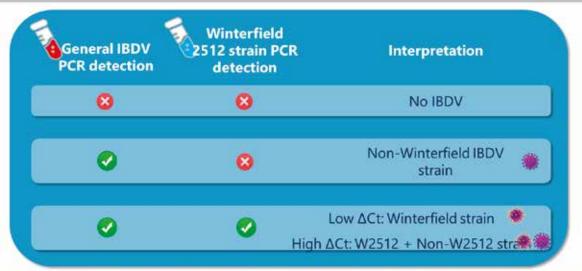
In general assays, the method used is known as "nested PCR", which requires more time and effort to conclude if a sample is positive or negative to W2512. As a result, this requires more time to analyze if a flock has been correctly vaccinated or not.

Today, for the first time, we can quickly conclude in our SSIU's and associated external labs if a sample is positive or negative to **NEXTMUNE®** and if there is a co-infection at bursal level, running only one assay. Using the **DUPLEX PCR**, in 3 to 4 cycles, we can demonstrate that the W2512 strain (**NEXTMUNE®**) controlled a field IBDV successfully.





INTERPRETATION OF RESULTS



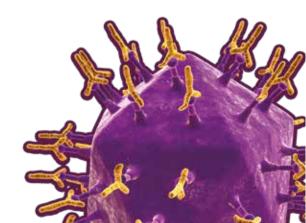
Caution message: Δ Ct value should be adjusted in each lab after analysis of a larger number of field samples.



Serological results:

Determining the level of immunization of a flock can be achieved by measuring individual sera of birds. Serological baselines can help us interpret the immune response elicited in the birds (table below) by **Nextmune***.

SITUATION EXPECTED TITRES	BIOCHEK ®	IDEXX ®	IDVET ®
FIELD CHALLENGE	>14000	NOT DISCLOSED	>7000
SERONEGATIVE (CUT-OFF)	<391	<396	<875







The Netherlands

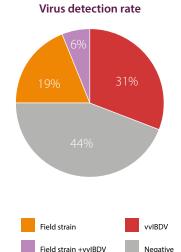


MATERIAL AND METHODS

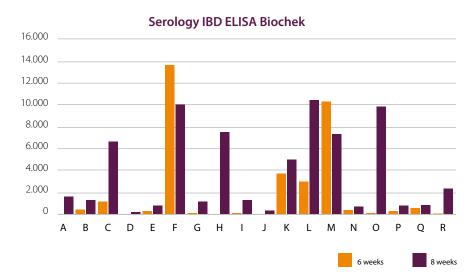
SAMPLING PERIOD	2019-2020
TARGET	Layers
SAMPLING AGE	6 and 8 weeks
NUMBER OF SAMPLES	Bursas from all 18 flocks
TECHNIQUE	Serology (Elisa Biochek) and RT-PCR (bursas)







Negative





CONCLUSION

Field and vvIBDV strains were detected in several rHVT-IBD vaccinated flocks. rHVT-IBD vaccine could not stop the Gumboro cycle.



Portugal



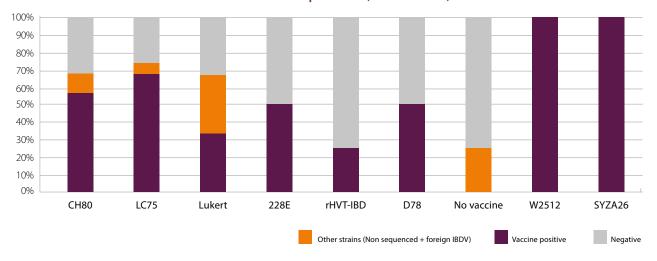
MATERIAL AND METHODS

SAMPLING PERIOD	November 2020 - February 2021
TARGET	Broilers and Layers
SAMPLING AGE	> 35 days of age
NUMBER OF SAMPLES	104
TECHNIQUE	RT-PCR
LABORATORY USED	MAPS, Italy





% vaccine detection per strain (N=104 farms)





CONCLUSION

A potential new IBDV genogroup was discovered in Portugal.
Farm vaccination with other types of vaccines (not immune complex) could not protect the bursa of Fabricius.



China



MATERIAL AND METHODS

SAMPLING PERIOD	March 2019 - August 2019
TARGET	Broilers
SAMPLING AGE	35 days of age
NUMBER OF SAMPLES	249 BURSAS: Fresh (174); FTA Cards (75)
TECHNIQUE	Serology; RT-PCR;
LABORATORY USED	LAB – IND, Malaysia

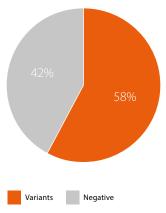


VACCINATION PROGRAM

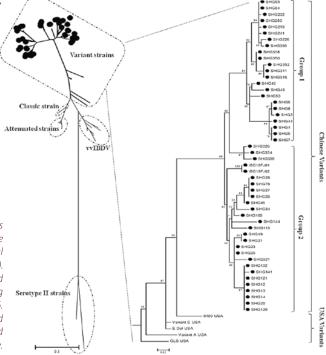
- **GROUP 1** (2 farms):
 - Cevac® IBD L + Cevac TRANSMUNE®
- **GROUP 2** (2 farms):
 - Chinese subunit ND-H9-IBD Inactivated



RESULTS



Phylogenetic tree analysis
of the amino acid sequence
of the representative partial
fragments of VP2 (aa 183-441) (A).
The trees were generated
by the neighbor-joining
method using MEGA6.
The variant strains detected
in this study are highlighted
with a solid circle.





CONCLUSION

New IBDV variants were not perceived as a threat by the producers, despite their impact on performance. Traditional ND-H9-IBD vaccines could not stop the Gumboro Cycle.







MATERIAL AND METHODS

SAMPLING PERIOD	June - October 2018
TARGET	Broilers
SAMPLING AGE	1 day of age and 35-38 days of age
NUMBER OF SAMPLES	22 BURSAS on FTA Cards: 2500 sera
TECHNIQUE	Serology ELISA Biochek IBD; RT-PCR
LABORATORY USED	LAB – IND, Malaysia, Ceva SSIU Phylaxia



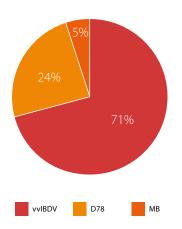
VACCINATION PROGRAM

 Drinking water vaccination (D78 or MB group) at 12-13 days of age

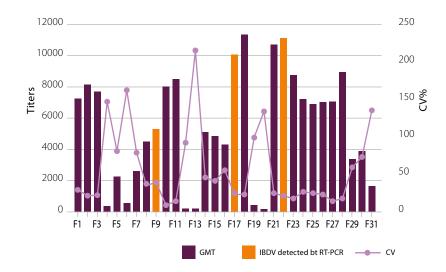


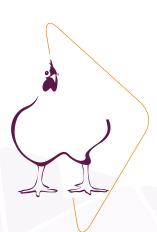
RESULTS

RT-PCR detection rate



Drinking Water IBD titers at slaughter age





CONCLUSION

Conventional Drinking Water IBD vaccines could not protect 100% of the Bursa of Fabricius and could not stop the Gumboro cycle.





MATERIAL AND METHODS

SAMPLING PERIOD	2023 / 2024
TARGET	Broilers farms
SAMPLING AGE	30-35 days of age
TECHNIQUE	IBDV RT-PCR

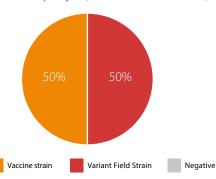


VACCINATION PROGRAM

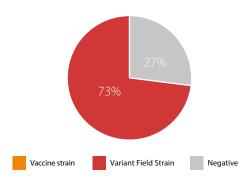
• rHVT-IBD vaccine, In-Ovo application



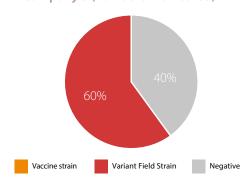
Company 1 (10 flocks monitored)

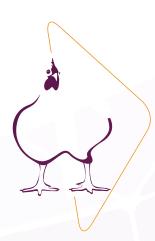






Company 3 (10 flocks monitored)





CONCLUSION

New IBDV variants were not perceived as a threat by the producers, despite their impact on performance. HVT-IBD vaccines could not stop the Gumboro Cycle.







MATERIAL AND METHODS

SAMPLING PERIOD	2021
TARGET	Broilers farms
SAMPLING AGE	35 days
TECHNIQUE	IBDV RT-PCR

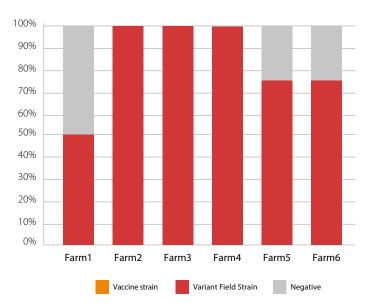


VACCINATION PROGRAM

 Vaccination Program: IBD conventional vaccine by Drink water at 14 days of age



IBDV RT-PCR of Flocks vaccinated by drinking water IBD vaccine





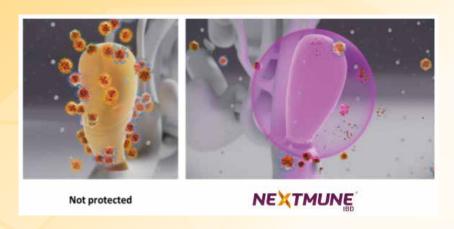
CONCLUSION

In the 6 farms monitored, the flocks vaccinated by drink water conventional vaccine had a high prevalence of variant IBD strain.



3

HOW TO CONTROL GUMBORO VIRUS AND DISEASE?



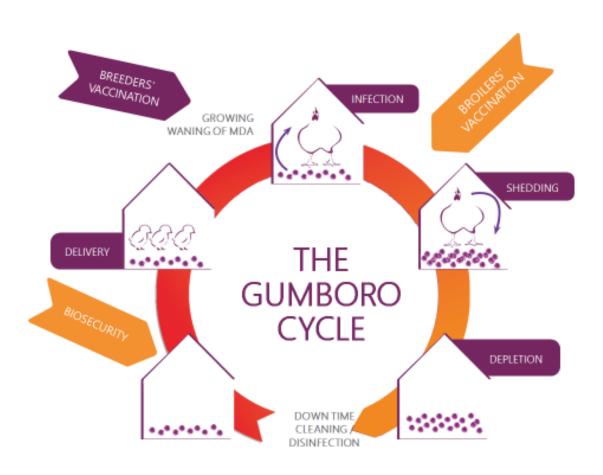
The clinical signs appear when cytokine storm unfolds. No cytokine storm, no disease, that's why variant IBD virus in young chickens show no clinical signs, which are age dependent. After the first IBD virus contact, the second one cannot create again the disease, as the mature BSDC secrete glycoprotein that block the IBDV.

The infectious bursal disease virus (IBDV) infection accelerates the maturation process of BSDC precursors, which results in acute emptying of cortico-medullary epithelial arches (CMEA) and subsequently, numerous immature BSDC emerge. The IBDV infection stops the glycoprotein (gp) discharge, and the gp appears in the newly transformed BSDC, now called virus -containing macrophage-like cell (Mal). The Movat pentachrome staining recognizes the gpin the intracellular spaces of the medulla and after infection in the Mal. The BSDC is the primary target of the IBDV. During IBDV infection, a large number of suddenly formed Mal actively migrate into the cortex initiating cytokine storm and recruiting heterophil granulocytes (Felfoldi et al, 2022).

This way, we understand the immunization of the chick by IBDV W2512 immune-complex vaccine and the biosecurity as the 2 main pillars to control IBD disease.









Biosecurity

Six key biosecurity points need to be successfully managed to control IBD appropriately.

How to create a clean area: Around the farm, walls or wired fences separate external and internal farm areas, thus creating an epidemiological unit. This unit must be isolated and secured. The employees and visitors must take a shower and use farm-specific PPE (boots, overall, gloves, mask, hairnet) to work inside the farm. For IBDV, the higher risk of external contamination are hands, boots, and dust around any house entrance (e.g. gates at the house front for day-old chicks and litter placement and doors at both sides to access the equipment and control room). The area around the cooling pad area is also critical for dust accumulation. The equipment and control room of each house must be divided into 2 zones, each zone using its own pair of boots, identified with different colours (outer area and inner area).



How to avoid vectors entering the farm/house:. Rodents and insects

(darkling beetles, flies) are considered a dynamic vector of IBDV, since they move around different farms and houses and are a potential reservoir of IBDV in the house in turnaround times.

Rodent's control: Rodents can be present anywhere. Set a bait with rodenticide every 10 meters around the house, storage room, office, etc. Record the results(bait concumed or unconsumed) and change the rodenticide bait every month. It should contain differacoum or brodifacum as active ingredient. All feed residues must be thoroughly cleaned, especially under the feed silos.

Flies' control: Flies like the smell of feed and color (red,yellow). To capture adult flies, use insecticides (thiamethoxam, permethrin or neonicotinoid) and sex pheromone baits (tricosene as example) + sugar. Mixing 5 g. of insecticide with 3 water droplets in a recipient is enough to control flies in 50m². To identify wet aera (litter under water pipeline, floor in the corner must be dry.

Darkling beetles' control: once the very last broiler leaves the house, the darkling beetles hide quickly in wall cracks or areas with hard access. You have only a few minutes to spray a concentrated solution of insecticide containing pyrethroids (tetramethrin), pseudo pyrethroids (Etofenprox), Nicotinoids (Acetamiprid) or spinosad on 50 centimeters around the wall-floor junctures. Cracks and holes in the floor and the walls must be filled. Around 80 L. of solution is enough to pray a floor perimeter of 250 m2 (house of floor surface 1000 m2 for example.

Manure management: 1 gr. of used litter might contain up 106 bacteria and 107 virus. At clean-out, all dust resulting from litter removal must be cleaned up to greatly decrease viral challenge. Ideally, use conveyors tunnels made of plexiglass directed to trucks with a trailer cover. Be careful with the wind currents. The floor must be cleaned with automatic sweeper (if available) and manually. For dirt floor, Quicklime powder (add water to create quicklime activation) from the previous cycle would help while cleaning up litter residues.

Cleaning and disinfection protocols (all surfaces, including minor equipment): The objective of a cleaning compound is to reduce the amount of biofilm on the surface. Acid cleaners are used against mineral materials and enzymatic cleaners against organic material; however, basic cleaners are most effectively sprayed with a foam-gun. After 30 minutes of contact time, the contact surfaces must be rinsed with high pressure water.

Applying the disinfectant: One must calculate the developable surface by adding up all the elements inside the space we need to disinfect.

E.g.: Developable surface (m2) = floor + wall + roof + equipment + cooling pad + fan + pipeline + feed line + plates. This calculation might vary, depending on which material the house is made of or contains (concrete, slate floors, or cages). The developable surface would then vary accordingly, between 3-44 times more than a regular concrete floor house (this coefficient calculation is available, ask our local team).

Dosage: Disinfectant suppliers generally describe the % of disinfectant needed to inactivate a virus (using specific virucidal efficacy tests during conformance processes). If the IBD virus activity is 1%, we should spray 3 ml. of disinfectant per m². Multiply this by the amount of developable surface (calculated above) and you will obtain the required amount of disinfectant needed. Depending on the surface's liquid retention capacity, you might need to estimate the quantity of water required to humidify all surfaces, to not get short of solution.

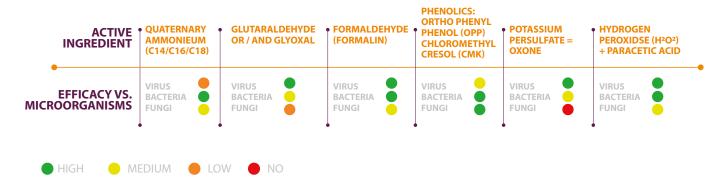


Disinfectant choice:

Glutaraldehyde-quaternary ammonia; oxidizers, phenolics are effective if the contact time is enough and an appropriate quantity of disinfectant is sprayed on all surfaces. It is important to make a proper cleaning and disinfection of the wall-base because this part of the wall is in direct contact with day-old chicks and litter residues might remain uncleaned. Disinfectant activity reduces 5 logs of IBDV each 30 minutes.

Minor equipment could be cleaned and disinfected in a specific (outside the house) using a specific protocol. Firstly, remove the organic material attached by brushing it and place it in a cleaner tank. After 30 minutes of contact time, rinse it and add the disinfectant solution. Let it dry out on a specific area.

The cleaning application (Cleaner+Foam Gun) produce nice cleaning foam



Floor disinfection (e.g. house 1000 m²) A sodium hydroxide solution (100 kg. / 500 L. water) can be applied on the floor, expecting a 2-15 mm. of penetration within the material. After 6 hours, apply 250 kg. of quicklime powder (doubling the dose for earthen floor) and spray water for activation (200-600 L.)

Down time period definition: Time spent between cleaning and disinfection of all surfaces and the new litter and the equipment is placed again. An effective downtime period could be maximally reduced to a few days (2-4 days, to be sure that all surfaces are dry).

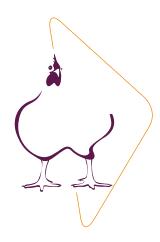
Specific case of the slat: To optimize efficiency of slat disinfection, the the slats must be disinfected outside in a specific area (as small equipment).

Protocol:

- 1. Immersion in water tank
- 2. Brush
- 3. Mix with detergent. Contact time: 30'
- 4. Rinse
- 5. Disinfect
- 6. Remove from tank, dry out in specific area and store safety.



Litter placement (or slat positioning). The litter (with good quality raw material: no dust) reduces the risk of contact between the DOC's and the floor (considered the major virus reservoir). A thickness of 10 cm. of litter is recommended for good protection, liquid retention, and to avoid fermentation. Litter turning could be dangerous because the virus might then be exposed to the surface and come in close contact with the birds.



CONCLUSION

A proper cleaning and disinfection protocol is essential to decrease the IBDV load during the downtime period and just before new placement of day-old chicks.









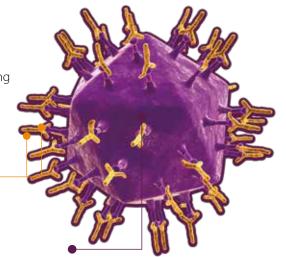
WHAT IS NEXTMUNE®

Nextmune® is a frozen IBD immune complex vaccine consisting of the Winterfield 2512 strain linked to specific antibodies called Virus Protecting immunoglobulins.

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SPECIFIC ANTIBODIES

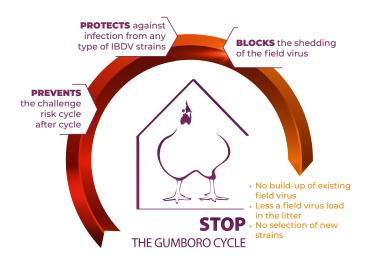
(Virus Protecting Immunoglobulins **VPI**)



VACCINE VIRUS STRAIN (Winterfield 2512)

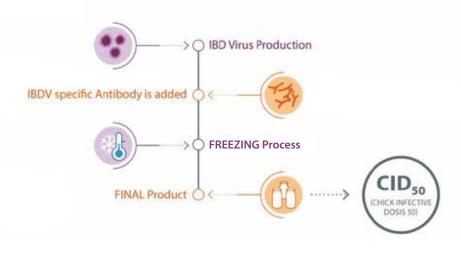
It has been designed to be applied in the hatchery (in-ovo or subcutaneously) and it has been used in broilers and breeders worldwide.





Since the vaccine is registered in Europe, a unique QC procedure was developed to safeguard the efficacy and safety of the vaccine. Every single production batch is thoroughly tested using a CID (Chick Infective Dose) 50 test. This test is performed with the aid of live birds and with the final blend of vaccine-antibodies, to guarantee the potency and safety of the vaccine.





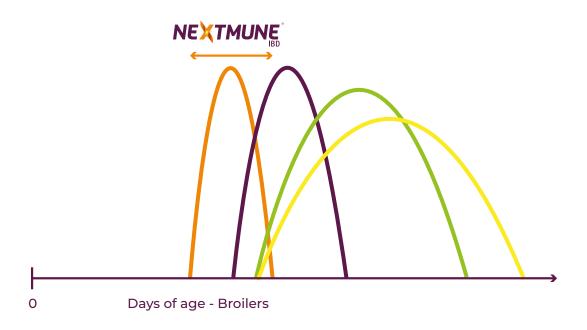




Nextmune® provides earlier protection

For Nextmune®, Ceva brings a new formulation on the IBD immune-complex vaccine, using the Winterfield 2512, to provide an earlier protection, and face all the different Gumboro strains challenges in the field.

New technology, new formulation, new balance of virus & antibodies, allows Nextmune a faster onset of immunity.





Benefits:

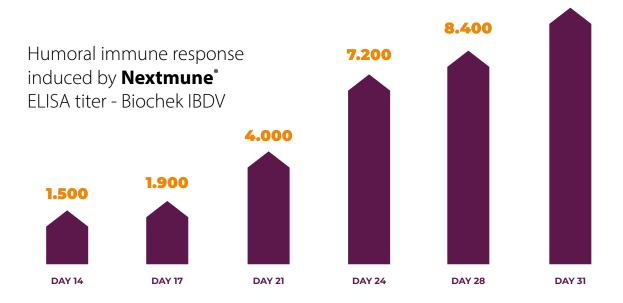
- ✓ Early protection of broilers
- ✓ Stop the Gumboro cycle

Stop the Gumboro cycle earlier than ever

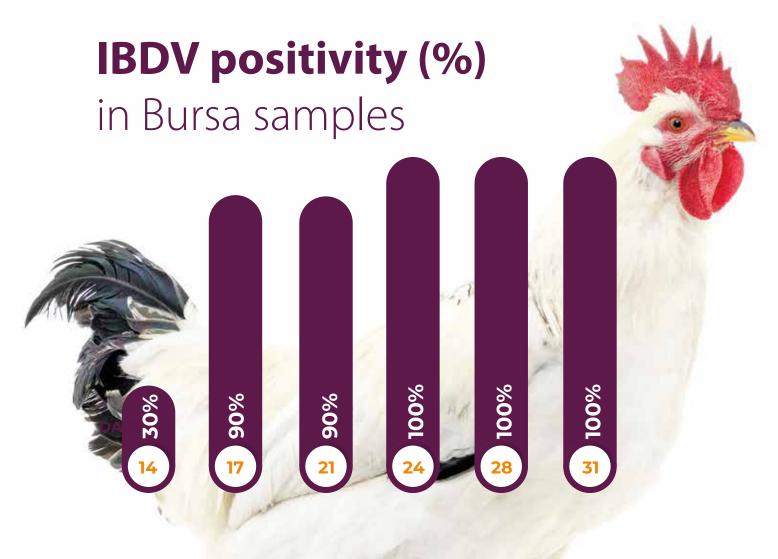




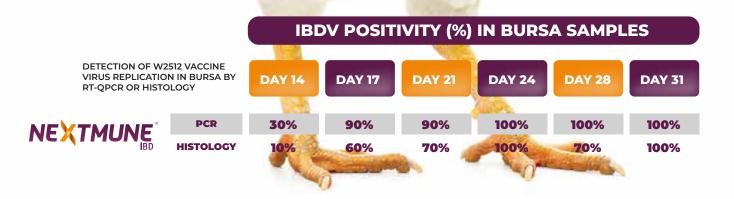
Nextmune°, faster Gumboro disease control to protect against all IBD virus.







Test animals: commercial broiler chickens; breed: Ross308 MDA at day old: 4043 ±2316 (CV%: 57%) GMT: 3287



Source: SID / ssiu Ceva Phylaxia, P149, 2022





Demonstration of earlier vaccine take of **Nextmune®** in the field

For the demonstration of earlier vaccine take CEVA used a monitoring called as "kinetic protocol" using in the methodology analyses as IBD serology and bursa PCR.

The protocol was applied in different countries by Ceva Veterinary Services team. See the protocol applied:

	D1	D18	D21	D24	D28	D35
VACCINATION	X					
BLOOD SAMPLING (N=20)	X	Х	Х	Х	Х	Х
BURSA SAMPLING (N=20)		Х	X	Х	X	X







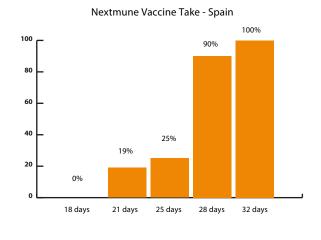


SAMPLING PERIOD	2022
TARGET	Broiler farm
SAMPLING AGE	Serology IBDV: Idexx & Biochek at 01, 14, 18, 21, 25, 28, 32, 35, 42 doa; IBDV PCR (+sequencing): 18, 21, 25, 28, 32 doa; 20 samples per age

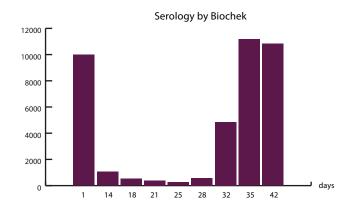
VACCINATION PROGRAM

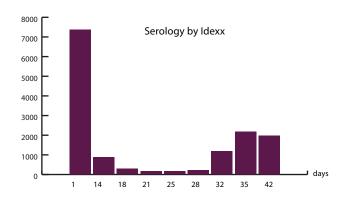
• Nextmune Subcutaneous at 1 DOA













CONCLUSION

The bursa colonization started at 21 days of age, and around 28 to 32 days of age all the broilers were protected.







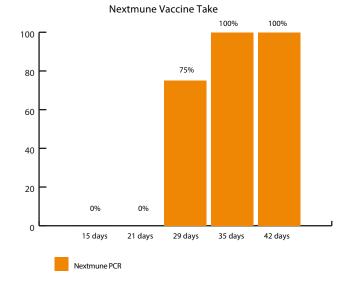
SAMPLING PERIOD	2022
TARGET	Breeder farm
SAMPLING AGE	Serology IBDV: Idexx & Biochek at 01, 07, 15, 21, 29, 35, 42 doa; IBDV PCR (+sequencing): 15, 21, 29, 35, 42 doa; 20 samples per age

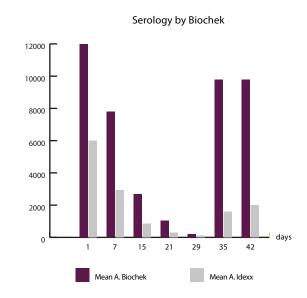
VACCINATION PROGRAM

• Nextmune Subcutaneous at 1 DOA



RESULTS







CONCLUSION

The colonization started before 29 days of age, and between 29 to 35 days of age all the broilers were protected.





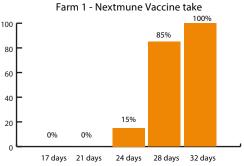


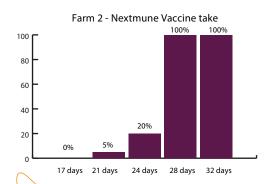
SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	Serology IBDV: Idexx & Biochek at 01, 17, 21, 24, 28, 32 doa; IBDV PCR (+sequencing): 17, 21, 24, 28, 32 doa;

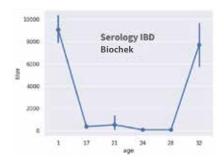
VACCINATION PROGRAM

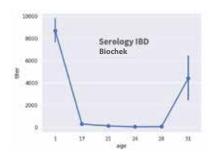
• Nextmune In-Ovo application



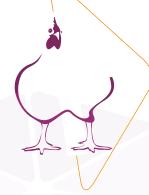












Both farms were **100%** protected, and the bursas were colonized by the W2512 strain.





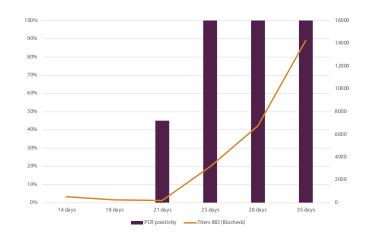


SAMPLING PERIOD	2022
TARGET	Broiler farm
SAMPLING AGE	Serology IBDV: Idexx at 01, 14, 18, 21, 25, 28, 35 doa; IBDV PCR (+sequencing): 14, 18, 21, 25, 28, 35 doa; 20 samples per age

VACCINATION PROGRAM

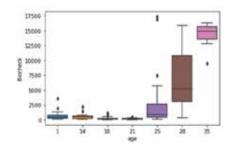
• Nextmune Subcutaneous at 1 DOA







IBD Titers range:





CONCLUSION

The bursa colonization started at 21 days of age, and at 25 days of age all the broilers were protected.





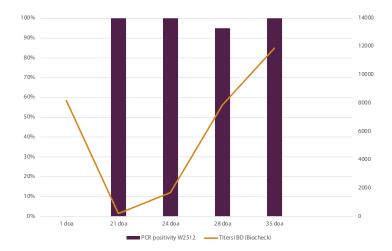


SAMPLING PERIOD	2022
TARGET	Broiler farm
SAMPLING AGE	Serology IBDV: Biochek at 01, 21, 24, 28, 35 doa; IBDV PCR (+sequencing): 21, 24, 28, 35 doa; 20 samples per age

VACCINATION PROGRAM

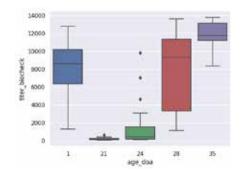
• Nextmune Subcutaneous at 1 DOA







IBD Titers range:





CONCLUSION

100% of the bursas were by at 21 days.



Indonesia



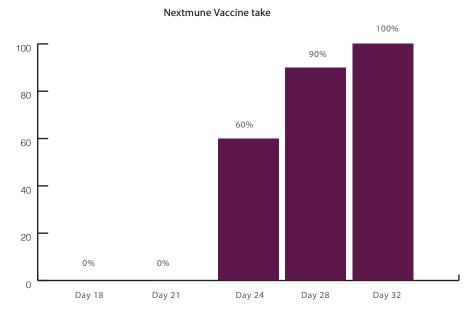
MATERIAL AND METHODS

SAMPLING PERIOD TARGET	2023 Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 18, 21, 24, 28, 35 doa; 20 samples per age

VACCINATION PROGRAM

• Nextmune subcutaneous vaccination







CONCLUSION

The bursa colonization started at 24 days of age, therefore, all the broilers were **100% protected**.





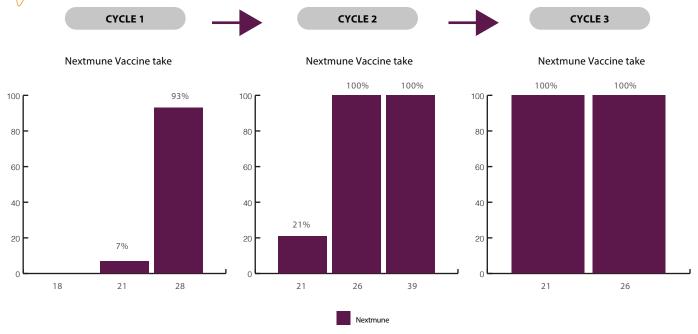


SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 18, 21, 26, 28, 39 doa; 20 samples per age

VACCINATION PROGRAM

• Nextmune In-Ovo application







CONCLUSION

100% of the bursas were colonised by the vaccine strain at 21 days, guaranteeing protection in all the birds.



Ukraine





MATERIAL AND METHODS

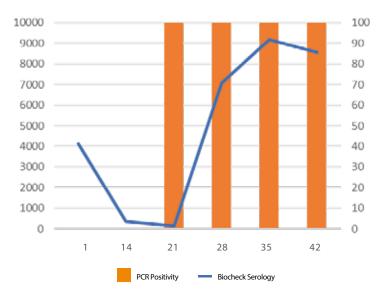
SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	Serology IBDV: Idexx & Biocheck at 01, 14, 21, 28, 35, 42 doa; IBDV PCR (+sequencing): 21, 28, 35, 42 doa; 9 flocks, 20 samples per age each farm

VACCINATION PROGRAM

• Nextmune In-Ovo application



Nextmune Vaccine take & IBD Serology Biocheck





CONCLUSION

100% of the bursas were colonised by the vaccine strain at 21 days, protecting in all the birds.



Vietnam



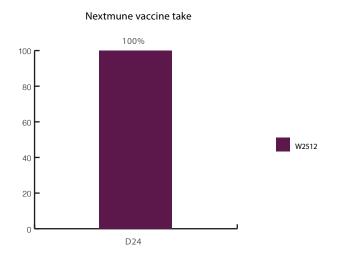
MATERIAL AND METHODS

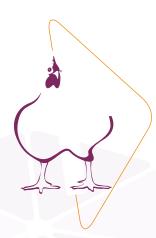
SAMPLING PERIOD	2024
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 24 doa;

VACCINATION PROGRAM

• Nextmune + Vectormune ND, Subcutaneous application







CONCLUSION

100% of Nextmune® (W2512) vaccine in the bursa at 24 days, with just 1 application in the hatchery



Vietnam





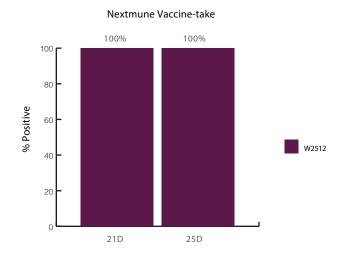
MATERIAL AND METHODS

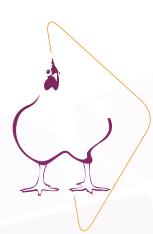
SAMPLING PERIOD	2024
TARGET	Native Broiler
SAMPLING AGE	IBDV PCR (+sequencing): 21, 25 doa;

VACCINATION PROGRAM

 Nextmune + Vectormune AI + Cevac MD Rispens, Subcutaneous application







CONCLUSION

100% of Nextmune® (W2512) vaccine take in the bursa at day 21 with just 1 application in the hatchery.







Benefits of NEXTMUNE®

Together with the clear benefits obtained in the hatchery and immune system, several trials have confirmed the superiority of **NEXTMUNE®** in the field.

In the next pages, you will find a list of studies demonstrating the clear economic benefits obtained throughout years of use of **NEXTMUNE®**.

Based on our experience, the performance results obtained with a good application of a **NEXTMUNE®** vaccination program, compared to different vaccine program types, support clearly the application of **NEXTMUNE®** in the hatchery.

The benefits observed are result of two main aspects:

- Disease control: vvIBDV & variants
- Simplication of the vaccination program



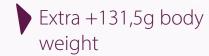


AVERAGE RESULTS:

+76€/1000 birds







The return on investment has been calculated by assuming the following market prices:

By doing so, it was possible to calculate an additional profit related to improvements in the major production performance parameters.

	VALUE / 000 BIRDS	VALUE / 100 M. BIRDS
0.01 FCR	6€/000 BIRDS*	600 K€*
10 g. BW	3.2€/000 BIRDS*	320 K€*
1% MORTALITY	11€/000 BIRDS*	1,100 K€*





Indonesia



MATERIAL AND METHODS

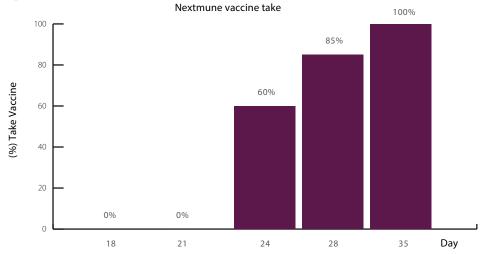
SAMPLING PERIOD	2023
TARGET	Broiler farm (8 flocks)
SAMPLING AGE	IBDV PCR (+sequencing): 18, 21, 24, 28, 35 doa; 20 samples per age

VACCINATION PROGRAM

• Nextmune subcutaneous vaccination



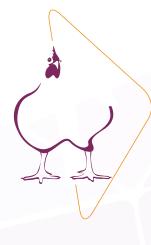
RESULTS





PERFORMANCE

NO	FARM	ватсн	IP	BW	FCR	DEP(%)	IBD VACCINATION PROGRAM
	AVG		316,63	1,98	1,54	4,90	NEXTMUNE
	AVG		260,73	1,71	1,63	5,56	HOT IBD VACCINE







155 EUROS/1000 BIRDS

CONCLUSION

The usage of **NEXTMUNE**® improved the performance, with a benefit of 155 euros per 1.000 broilers, demonstrating the importance of using a safe vaccine.







SAMPLING PERIOD	2022
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 14, 18, 21, 24, 28, 35 doa; 20 samples per age
HISTORICAL	Usage of recycled litter Var. A detected in 100% of the bursas in the pre-sampling Detection of Var. A starting at 14 days of age

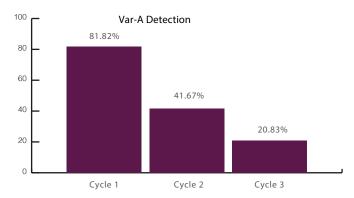


VACCINATION PROGRAM

 Nextmune by subcutaneous application Control group: HVT-ND-IBD vaccine by subcutaneous application

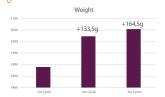


RESULTS: Nextmune protection cycle after cycle



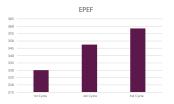


PERFORMANCE













65 EUROS/1000 BIRDS



CONCLUSION

The usage of **NEXTMUNE**® decreased the field strain pressure, stopping the Gumboro Cycle, and improved the performance.



Peru



MATERIAL AND METHODS

SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 21, 28, 35 doa; 20 samples per age
HISTORICAL	Usage of recycled litter Var. A detected in 100% of the bursas in the pre-sampling Detection of Var. A starting at 21 days of age



VACCINATION PROGRAM

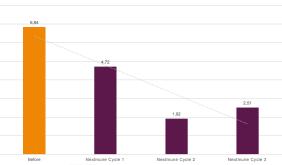
 Nextmune by subcutaneous application Control group: other Immune-complex vaccine by subcutaneous application



RESULTS: Nextmune protection cycle after cycle







Mortality %



CONCLUSION

The usage of **NEXTMUNE®** improved the performance, with a benefit of 67 euros per 1.000 broilers, as it Stop the Gumboro Cycle, and the sub-clinical disease.



France



MATERIAL AND METHODS

SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 10, 24, 31 doa;
HISTORICAL	nvIBDV A3B1 detected in 100% of the
	bursas in the pre-sampling
	Detection of A3B1 starting at 21 days of age
	nvIBDV A3B1 detected in 100% of the

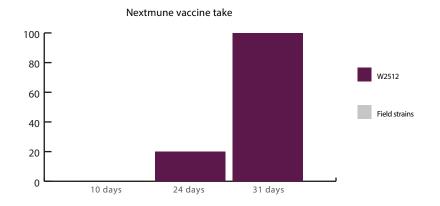


VACCINATION PROGRAM

 Nextmune by In-Ovo application Control group: Conventional drinking water vaccination



RESULTS: Nextmune protection





PERFORMANCE

Variant A3B1

colonizarion









35 EUROS/1000 BIRDS (4,5 FCR diff)



CONCLUSION

The usage of **NEXTMUNE®** Stop the Gumboro Cycle and displaced the nvIBDV A3B1, and as consequence improved the performance with a benefit of 35 euros per 1.000 broilers.



Russia



MATERIAL AND METHODS

SAMPLING PERIOD	2024
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 23, 28 doa;



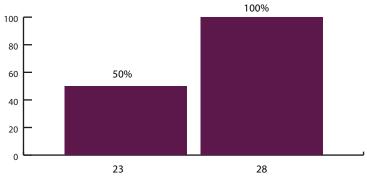
VACCINATION PROGRAM

 Nextmune by In-Ovo application Control group (1 & 2): Conventional drinking water vaccination



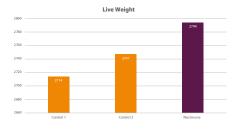
RESULTS: Nextmune protection

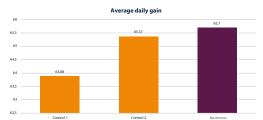
Nextmune vaccine take



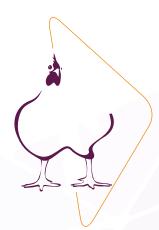


PERFORMANCE









CONCLUSION

The improvement in performance due the usage of **NEXTMUNE®** yielded a benefit of 85 euros per 1.000 broilers.



Brazil





MATERIAL AND METHODS

SAMPLING PERIOD	2024
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 7, 14, 21, 28, 35, 42 doa; Serology at 42 doa.
HISTORICAL	High detection of Brazilian IBDV Serology (IDEXX) at 42 doa variant in the pre-sampling, starting at 21 days of age

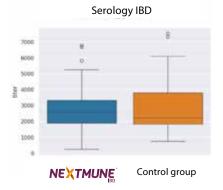


VACCINATION PROGRAM

 Nextmune by In-Ovo application Control group: other immune-complex vaccine



RESULTS:



NEXTMUNE® demonstrated a more uniform IBD titers at 42 days of age.

PCR – 1st Cycle

AGE (WEEKS)	NEXTMUNE	CONTROL 2
1	NEG	NEG
2	NEG	NEG
3	W2512 (50%)	NEG
4	W2512 (100%)	VAR. IBDV (75%)
5	W2512 (100%)	VAR. IBDV (75%)
6	W2512 (100%)	VAR. IBDV (75%)

PCR – 2st Cycle

AGE (WEEKS)	NEXTMUNE	CONTROL 2
1	NEG	NEG
2	NEG	NEG
3	W2512 (100%)	NEG
4	W2512 (100%)	VAR. IBDV (50%)
5	W2512 (100%)	VAR. IBDV (50%)
6	W2512 (100%)	VAR. IBDV (50%)



CONCLUSION

The usage of **NEXTMUNE®** Stop the Gumboro Cycle and displaced the IBDV variant.



Portugal



MATERIAL AND METHODS

SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 24, 28, 32 doa;
HISTORICAL	High detection of Portuguese IBDV variant in the pre-sampling, starting at 14 days of age

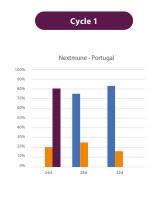


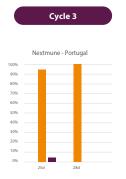
VACCINATION PROGRAM

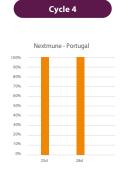
Nextmune by subcutaneous application

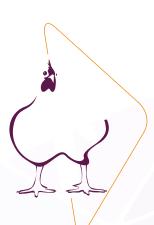












CONCLUSION

The usage of **NEXTMUNE®** Stop the Gumboro Cycle and displaced the Portuguese variant.



Russia



MATERIAL AND METHODS

SAMPLING PERIOD	2023
TARGET	Broiler farm
SAMPLING AGE	IBDV PCR (+sequencing): 22, 28, 39 doa;
	IBD serology at 40 doa.

22 days



39 days

VACCINATION PROGRAM

 Group 1: Nextmune by subcutaneous Group 1: Nextmune by subcutaneous + Field Vaccine at 11 doa.



RESULTS:

100

60

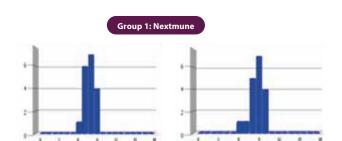
40

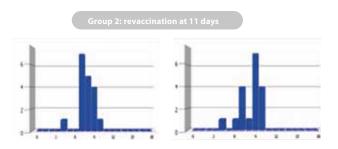
100% 100%

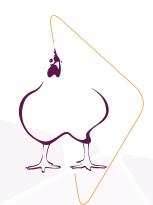
Nextmune vaccine take

28 days









CONCLUSION

The usage of **NEXTMUNE**® applied in the hatchery was enough to protect the flocks. Field revaccination is not needed.



Indonesia



MATERIAL AND METHODS

SAMPLING PERIOD	2023
TARGET	Broiler farm (8 flocks)
SAMPLING AGE	IBDV PCR (+sequencing): 32-33 doa;

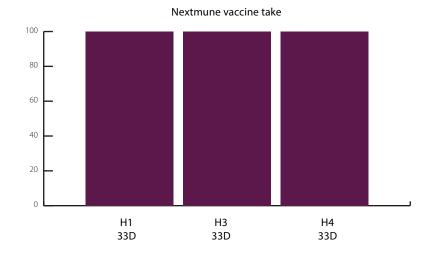


VACCINATION PROGRAM

 Nextmune In-Ovo application Control group: Other immune-complex vaccine + field vaccination at 14 doa

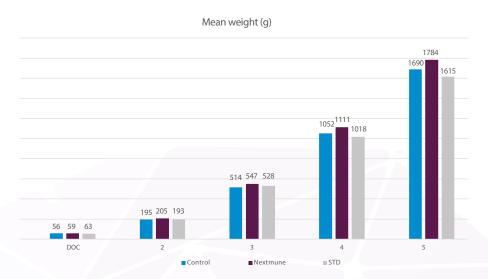


RESULTS: Nextmune protection

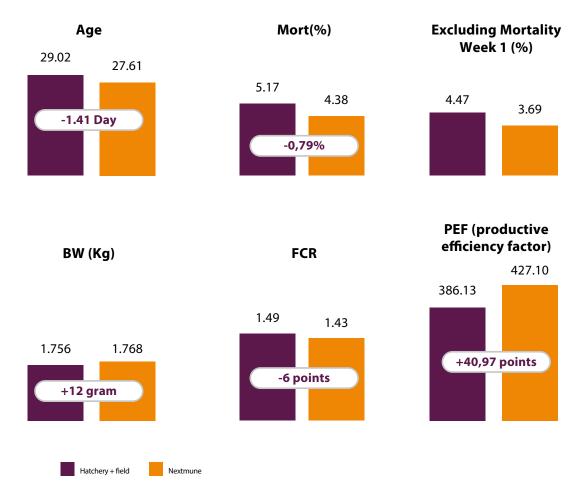




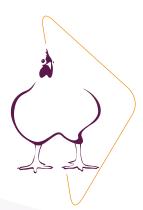
PERFORMANCE:











CONCLUSION

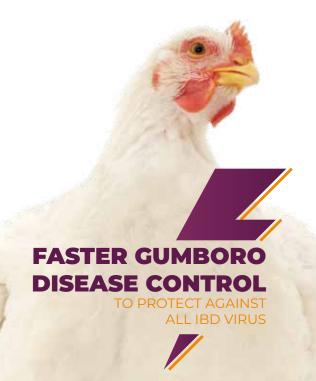
The usage of **NEXTMUNE**® applied in the hatchery was enough to protect the flocks. The simplification of the vaccination program with **Nextmune**® improved significantly the flock performance.





Summary of profitability

COUNTRY	COMPETITOR	ECONOMICAL BENEFIT (EUROS / 1.000 BROILERS)
	VERY INVASIVE STRAIN VACCINE	155 EUROS
6	OTHER IMMUNE-COMPLEX VACCINE	67 EUROS
6	HVT ND IBD	65 EUROS
	CONVENTIONAL FIELD VACCINES	35 EUROS
	CONVENTIONAL FIELD VACCINES	85 EUROS
	OTHER IMMUNE-COMPLEX VACCINE + FIELD REVACCINATION	52 EUROS





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